and *C. quinquefasciatus* Say. J. Parasitol. 37:419–420.

Jakob, W. L., S. A. Daggers, D. B. Francy, J. Mullenix and K. Moseley. 1979. The *Culex pipiens* complex in Memphis, Tennessee. Mosq. Syst. 11(3):179–186.

Jupp, P. G. 1978. *Culex (Culex) pipiens pipiens* Linnaeus and *Culex (Culex) pipiens quinquefasciatus* Say in South Africa: Morphological and reproductive evidence in favour of their status as two species. Mosq. Syst. 10(4):461–473.

Knight, K. L. 1953. Hybridization experiments with *Culex pipiens* and *C. quinquefasciatus* (Diptera:Culicidae). Mosq. News 13:110–115.

Knight, K. L. 1978. Supplement to a catalog of the mosquitoes of the world. Thomas Say Foundation Suppl. to Vol. VI.

McMillan, H. L. 1958. Study of a naturally occurring population intermediate between *Culex p. pipiens* and *C. p. quinquefasciatus*. Am. J. Trop. Med. Hyg. 7:505–571.

Miles, S. J. 1976. Taxonomic significance of assortive mating in a mixed field population of *Culex pipiens australicus, C. p. quinquefasciatus* and *C. globocoxitus*. Syst. Ent. 1:263–270.

Sirivanakarn, S. 1976. Medical entomology studies. III. A revision of the subgenus *Culex* in the Oriental Region (Diptera:Culicidae). Cont. Am. Ent. Inst. 12(2).

Sundararman, S. 1949. Biometrical studies on intergradations in the genitalia of certain populations of *Culex pipiens* and *Culex quinquefasciatus*. Am. J. Hyg. 50:307–314.

Tate, P. and M. Vincent. 1936. The biology of autogenous and anautogenous races of *Culex pipiens* L. (Diptera). Parasitol. 28:115–145.

MALATHION RESISTANCE IN *ANOPHELES STEPHENSI* LISTON IN LAHORE, PAKISTAN

HAMAYUN R. RATHOR AND GHAZALA TOQIR

Pakistan Medical Research Center, International Health Program, University of Maryland, School of Medicine, 6, Birdwood Road, Lahore, Pakistan

ABSTRACT. The Lahore area of Pakistan has been under malathion spraying pressure since 1976 at the rate of two treatments per year, 1 gm/m² per round. Susceptibility tests on *An. stephensi* from Lahore with 5% malathion (1 hour exposure) showed an average survival of 68.95% indicating the presence of resistant individuals. Malathion resistance in *An. stephensi* from Pakistan is being herein reported for the first time. The gene(s) for malathion resistance was present in high frequencies in populations near Lahore. Selection for malathion resistance in the laboratory resulted in a 20 times increase after only three selected generations.

INTRODUCTION

Anopheles stephensi and *An. culicifacies* are major vectors of malaria in Pakistan and neighbouring countries. In Pakistan, DDT was used for mosquito control from 1961 to 1975 (2 cycles/year, 1–2gm/m²). In the early 1960's DDT showed good control of malaria vector, but by the early 1970's it failed to control these species (WHO, 1976). Therefore, in 1975 DDT was partially replaced by BHC, but resistance to BHC also appeared subsequently. Recently Rathor and Toqir (1980) reported that *An. culicifacies* in the Lahore area has become nearly homozygous for DDT resistance, and the frequency of the DDT susceptible gene was so low that an attempt to select for DDT susceptibility in the laboratory did not succeed. In 1976 malathion was introduced in Pakistan for mosquito control, and it is still being used (2 cycles/year, 1 gm/m²). Sumithion (Fenitrothion) has also been used in selected areas of Punjab Province.

Recently malathion resistance in *An. stephensi* has been reported from Iran (Manouchehri *et al.* 1975, 1976a,b; Eshghy 1978) and in *An. culicifacies* from Gujarat, India (Rajagopal, 1977). Organophosphate resistance in anopheline mosquitoes from other parts of the world has also been reported; for example, in *An. albimanus* from El Salvador (Georghiou 1972; Ayad and Georghiou 1979). Thus, the evolution of resistance to organophoshpate insecticide would seem to be expected eventually in any mosquito control program.

In the light of these reports, and the fact that malathion has been used for mosquito control in Pakistan since 1976, susceptibility tests for malathion were carried out on *An. stephensi* from the Lahore region of Punjab Province to monitor the onset of malathion resistance.

MATERIALS AND METHODS

Fed or half gravid females of *An. stephensi* were collected resting in cattle sheds from January to August 1979 at the following study areas of Punjab province, Pakistan:

1) Khano-Harni (KH) twenty miles southeast of Lahore.
2) Shah-di-Khoi (SK) near Punjab University Campus, Lahore.
3) Kot Baghicha (KB) near Balloki Headworks about 30 miles west of Lahore.
4) Kahna Purana (KP) on Ferozpur Road 16 miles from Lahore.
5 Sattoki (ST) 40 miles southeast of Lahore on the Kasur Road.

The adult progeny of the wild-caught females were used for the malathion susceptibility tests. Tested progeny adults (24 hours old) were unbiased subsample of all progeny collected from mass egg laying.

In addition adults from the following colonized strains were used in control tests:

1) KHS: A strain susceptible to 5% malathion papers from one hour exposure. Colonized from Khano-Harni in May 1978.
2) KHR: A strain resistant to 5% malathion papers for one hour exposure. Colonized from Khano-Harni in May 1979.
3) LT: A strain susceptible to 5% malathion papers for one hour exposure. Colonized from the village called Leti, situated on Talagang Mianwali Road, 26 miles west of Talagang Tehsil, Attock District, Punjab Province in 1975.
4) LH: A strain susceptible to 5% malathion papers for one hour exposure. Colonized from Lahore in 1975.

Initially, malathion impregnated papers supplied by WHO were used in the tests. However, since there were delays in delivery of the papers the papers degraded rapidly. Malathion papers were prepared in our laboratory by modification of the method of Georghiou and Metcalf (1961). A one ml 5% malathion solution in acetone (W/V) and 2 ml absolute ethanol gave uniform spreading on No. 1 Whatman filter paper cut to the standard WHO insecticide paper size (12 x 15 cm) placed on a horizontal nail board. Malathion solution was applied spirally to the paper by an all glass 10 ml syringe fitted with a hypodermic needle (B-D Twin-Pak.25). Control papers were prepared by applying 1 ml acetone and 2 ml of absolute ethanol. The papers became dry within 15 to 20 minutes.

Since Georghiou and Metcalf (1961) pointed out that the papers made with an acetone solution of malathion should be used within 1 to 2 hours of preparation to rule out any possibility of malathion degradation, freshly prepared (1–2 hr old) papers were used for the tests. However, our prepared papers were effective for at least two days after preparation (Table 1) if stored at 26°C carefully in sealed plastic boxes when not in use.

BIOASSAY TESTS AND SELECTION. Initially, adult progeny (24 hr old, ♀♀ and ♂♂) were tested using WHO insecticide papers and adult test kits. Susceptibility tests were made using a diagnostic dose of 5% malathion with 1 hr exposure and 24

Table 1. Comparison of efficacy of 5% malathion papers obtained from WHO and the papers prepared in the laboratory.

Colonies		Susceptible						Resistant	
		KHS		LT		LH		KHR	
Paper	Age of papers	Total tested	Mortality (%)	Total tested	Mortality (%)	Total tested	Mortality (%)	Total tested	Mortality (%)
WHO	>3 months	89	96.6	119	96.6	50	98.0	127	18.1
Laboratory made	1–2 hrs	66	95.5	113	100.0	62	100.0	104	24.0
"	1 day	27	100.0	68	100.0			17	35.3
"	2 days	25	100.0	12	91.0	28	100.0	19	31.6
"	3 days			41	100.0				
"	8 days			41	100.0				

hr holding period (WHO, Unpublished Document, 1975, 1976). The tests were carried out in insectary conditions (27 ± 1°C and RH = 75 ± 5%). Controls were run along with all the tests. No mortalities were observed in most control except for the tests in which wild caught females were used. If more than 5% mortality was observed in the controls, the corrected mortality was calculated by Abbott's formula. The details of the control tests without any mortalities were not included in the tables. In addition to the control tests malathion susceptible laboratory strains (LT and LH) were tested at different intervals throughout the experiment to evaluate the efficacy of malathion papers prepared in our laboratory (Table 1). Half gravid wild caught females were tested for susceptibility with the papers prepared in our laboratory. Those wild caught females which survived the 1 hr exposure to 5% malathion paper and the 24 hr holding period were individually isolated in 35 ml glass vials lined with filter paper and flooded with water to a depth of 3 mm for egg laying. The resulting progeny were reared individually and their F_1 and F_2 progeny tested with 5% malathion.

A selection to produce a malathion resistant stock was started with wild-caught females from KH. The wild-caught blood-fed females were exposed to 5% malathion for 1 hr and the surviving females allowed to oviposit. F_1 and subsequent generation up to F_6 were reared *en masse* and selected with 5% and 10% malathion at different exposure times. F_3 and F_4 individuals were not selected to increase the size of the selected strain. The highest selection pressure was 15 hr exposure to 10% malathion.

RESULTS AND DISCUSSION

Initial tests made on the progeny of wild-caught females from four localities (KH, SK, KB, KP) indicated that *An. stephensi* from 2 localities SK and KB were susceptible, but those from KH and KP were resistant to malathion (Table 2). For

Table 4. Percent mortality of wild caught females and the subsequent selected generation (F_1 to F_6) with 5.0% and 10.0% malathion at different exposure periods in 1979.

Selected generation	Dates Tested[1]	Malathion	Exposure time (hours)	Number tested	Corrected % Mortality
WC	May 3 (1)	5	1	134	6.0
F_1	May 15 (1)	5	1	53	0.0
	Feb. 15 (2)	5	2	23	4.35
	May 15 (3)	5	4	38	5.26
	Jun. 15–Jun. 22 (60)	5	15	2053	69.80
F_2	Jun. 13 (1)	5	15	41	65.85
	Jun. 18 (2)	5	7	96	37.5
	Jun. 21 (3)	5	16	126	50.9
F_5	Jul. 24 (1)	5	1	59	13.56
	Jul. 25–Aug. 8 (20)	5	5	835	51.14
F_6	Sep. 3 (1)	5	5	35	0.0
	Sep. 3–Sep. 9 (15)	10	5	175	0.0
	Sep. 8	5	15	21	9.5
	Aug. 8–Sep. 21	10	15	124	35.48

[1] Figures in parenthesis represent the number of replicates.

comparison, the 3 susceptible colonized strains maintained in our laboratory were also subjected to susceptibility tests throughout the experiment. They showed 100% mortality. Susceptibility tests were made to compare the efficacy of laboratory prepared papers to WHO papers (Table 1) on 3 susceptible (KHS, LT, LH) and 1 resistant strain (KHR). No significant difference was observed in percentage mortality obtained by WHO papers and 1–2 hr old laboratory pre-

Table 2. Susceptibility tests on laboratory reared progeny of wild and colonized female *An. stephensi* to 5% malathion in 1979 (1 hr exposure and 24 hours holding period).

Material	Locality	Dates Tested	Number Tested	% Mortality
Field	KH	Jan. 4–May 14	846 (350)[1]	37.8
	SK	Apr. 13–Apr. 14	64 (20)	100.0
	KB	Apr. 20–Apr. 21	138 (70)	97.1
	KP	Apr. 23–Apr. 27	301 (100)	27.9
Colonized Strains	KHS	Apr. 20–Apr. 21	119 (100)	100.0
	LH	May 12–Jun. 18	247 (150)	100.0
	LT	Apr. 8–May 14	254 (150)	100.0

[1] Numbers in parentheses are the numbers of females in the parent generations offered ovipositing media *en masse*.

pared papers, the value of t = 1.99 (0.2>P>0.1). One locality, KH, was selected for further investigation to confirm physiological resistance. When wild-caught, half-gravid females from KH were exposed to 5% malathion, 6 replicates of tests made on different dates showed resistance to malathion, mortality ranged from 20.8 to 35.9% (Table 3). A total of 199 wild females from KH were tested. The average kill with WHO diagnostic dose was 30.1%. Only 71 females out of the surviving 139 produced F_1 progeny which were individually reared and tested; 30% of these families showed complete survival. A total of 4288 (♀♀ and ♂♂) were tested from the 71 families, with an average kill of 13.78%. Only 2 families produced F_2 progeny and were tested with 5% malathion. Six replicate tests of family 1 showed complete survival, while the 6 replicate tests of family 2 gave very low mortality (0.0% to 9.43%). In addition females from ST were also resistant to malathion (36.5% mortality, Table 3).

The above results clearly indicated that *An. stephensi* from the Lahore area, Punjab have become resistant to malathion. The fact that 2 families out of 71 tested showed almost 100% survival in their F_1 and F_2 progeny indicated the presence of individuals homozygous for malathion resistance. Selection in the laboratory resulted in a rapid increase in resistance. The F_6 (Table 4) showed no mortality after an exposure of 5 hr to 10% malathion while a susceptible colongy (LT) showed complete mortality after ½ hr exposure to 5% malathion.

Table 3. Percent mortality in wild-caught fed females of *An. stephensi* exposed to 5% malathion for 1 hr. in 1979.

Locality	Dates Tested	Total Tested	Mortality	Mortality %	Corrected* % Mortality
Kano-Harni	July 11	3	1	33.33 (0)[1]	33.33
"	July 17	24	5	20.83 (0)	20.83
"	July 18	25	7	28.00 (0)	28.00
"	July 22	14	4	28.57 (0)	28.57
"	July 24	39	14	35.90 (0)	35.90
"	July 31	94	29	30.85 (4.35)	30.85
Sattoki	Aug. 2	79	32	40.51 (6.25)	36.54

* Corrected by Abbott's formula.

[1] Figures in parenthesis are the % mortalities in controls.

ACKNOWLEDGMENTS

We thank Drs. R. H. Baker, D. Nalin, R. K. Sakai, W. K. Reisen, and G. Davidson for reviewing the manuscript and offering suggestions. We are thankful to Professor G. P. Georghiou for his advice on the preparation of insecticide papers in our laboratory. Our thanks are due to the members of Entomology and Ecology departments of Pakistan Medical Research Center, for their assistance in collecting and rearing the mosquitoes, and specially Mr. M. Rais for his help in the bioassay tests. This research was supported by Grant No. AI-10049 from the National Institute of Allergy and Infectious Diseases, N.I.H.

References Cited

Ayad, H. and G. P. Georghiou. 1979. Resistance pattern of *An. albimanus W.* following selection by parathion. Mosq. News. 39:121–125.

Eshghy, N. 1978. Tolerance of *An. stephensi* to malathion in the province of Fars, Southern Iran. Mosq. News. 38:580–83.

Georghiou, G. P. 1972. Studies on resistance to carbamate and organophosphorus insec-

ticides in *An. albimanus*. Am. J. Trop. Med. Hyg. 21:791–806.

Georghiou, G. P. and R. L. Metcalf. 1961. A bioassay method and results of laboratory evaluation of insecticides against adult mosquitoes. Mosq. News. 21:328–37.

Manouchehri, A. V., B. Djanbakhsh and F. Roughani. 1976b. Studies on the resistance of *An. stephensi* to malathion in Bandar Abbas, Iran. Mosq. News. 36:220–22.

Manouchehri, A. V., A. Zaini & B. Djanbakhsh. 1976a. Preliminary note on the resistance of *An. stephensi* to malathion in Bander Abbas, Southern Iran. Mosq. News. 36:207–8.

Manouchehri, A. V., A. Zaini and H. Yazdanpanah. 1975. Selection for resistance to malathion in *An. stephensi mysorensis*. Mosq. News. 35:278–280.

Rajagopal, R. 1977. Malathion resistance in *An. culicifacies* in Gujarat. Ind. J. Med. Res. 661:27–28.

Rathor, H. R. and G. Toqir. 1980. Selection for DDT susceptibility in *An. culicifacies* from Lahore, Pakistan. Southeast Asian J. Trop. Med. Pub. Hlth. 11(1):113–118.

W. H. O. 1976. Resistance of vectors and reservoirs of disease to pesticides. World Health Organization Technical Report Series No. 585 88 pp.