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AN ANALYSIS OF INSECTICIDE RESISTANCE IN MALARIA VECTOR MOSQUITOES IN PAKISTAN AND RECOMMENDATIONS FOR FUTURE ACTION

Dr. George P. Georghiou, Consultant to USAID

January 19, 1990 Islamabad, Pakistan

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With best regards,

George P. Georghiou

Proefessor of Entomology

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Dr. Rifaq A. Ismail
Project Manager
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Dear Dr. Ismail:

Since returning from Pakistan last Saturday I have been completing the report of my consultantship. Unfortunately the diskette I had brought with me was not readable by any of our programs, so the report had to be retyped in its entirety. I hope you will find it interesting as well as useful. If questions arise please do not hesitate to let me know.

I greatly appreciated the opportunity to work with you again and to be of service to the project.

With many thanks and best wishes.

Sincerely,

George P. Georghiou
Professor of Entomology

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Enclosure : Report, 51 pp.

TABLE OF CONTENTS

		Page
1.	EXECUTIVE SUMMARY	1
2.	INTRODUCTION	2
2.1	Purpose	2
2.2	Schedule	3
2.3	Documentation Process	3
3.	PROCUREMENT OF INSECTICIDES AND STATUS OF MALARIA	4
4.	INSECTICIDE RESISTANCE	7
4.1	General	7
4.2	Resistance Surveillance in Pakistan	8
4.2.1	Sources of Information	8
4.2.2	Resistance Surveillance within the Malaria Control Program	8
4.2.3	Some Limitations of the Resistance Surveillance Activities	10
4.2.3.1	Aging of Test Papers and the Loss of Toxicity	10
4.2.3.2	Increased Mortality of Test Mosquitoes due to handling	11
4.2.3.3	Influence of Age of Mosquitoes on Susceptibility to Insecticides	11
4.2.4	Relating Susceptibility Test Data to Field Performance of Insecticides	11
4.3	Status of Insecticide Resistance	13
4.3.1	DDT	14
4.3.2	Dieldrin - BHC	15
4.3.3	Malathion	17
4.3.4	Fenitrothion	32
4.3.5	Role of Agricultural Insecticide in Resistance	32
5.	DISCUSSION	40
6.	RECOMMENDATIONS	48
7.	REFERENCES CITED	49
8.	ACKNOWLEDGEMENTS	51

1. EXECUTIVE SUMMARY

The status of insecticide resistance in malaria vector mosquitoes in Pakistan, which was reviewed by the consultant in 1986, was re-evaluated during the present visit in order to assist the Government of Pakistan and the U.S. Agency for International Development in determining the need for any change in the type of insecticide used in the Malaria Control Program.

A high frequency of malathion-resistant *Anopheles stephensi* was found in several districts of the Punjab Province, and lower frequencies in the Sind and NWFP provinces. In many of these districts, the appearance of high frequencies of resistance coincided with greater use of malathion in the Malaria Control Program. *An. culicifacies* continues to be essentially susceptible to malathion, and both species are susceptible to fenitrothion.

In view of the high incidence of malathion resistance in *An. stephensi,* and of newly obtained evidence of a role of this species as a vector of malaria in Pakistan, it is recommended that an alternative insecticide, fenitrothion, be employed selectively by the Malaria Control Program during 1990. It is further recommended that the change to fenitrothion be made only where the following criteria are met, i.e. (a) resistance to malathion, as measured by survival of *An. stephensi* in the WHO diagnostic test, has exceeded 50% on average during the last two years, (b) there is clear evidence that the incidence of malaria transmission significantly exceeds the norm for the district.

Since fenitrothion is more toxic than malathion to non-target organisms, including man, the introduction of this compound must be accompanied not only by an intensification of training of the field staff in its safe use but also by an increase in field supervision during spray operations.

A detailed field trial to evaluate the efficacy of two other alternative chemicals, pirimiphos-methyl (an organophosphate) and lambda cyhalothrin (a pyrethroid) vis-a-vis fenitrothion, is also strongly recommended.

2. INTRODUCTION

2.1. Purpose

The Malaria Control Program of Pakistan (MCP) was reviewed by an external review team (ERT) in 1985 for the purpose of (a) assessing the status of the program and the progress made to meet its objectives; and, (b) formulating recommendations for further improvement of the program in the future (ref. 6a).

One of the issues addressed to in that review was the problem of insecticide resistance in the malaria vectors of the country. On the basis of the data then available, the ERT concluded that "insecticide resistance is a serious and urgent problem, as there are strong indications that malathion is not as effective as in previous years in some districts of the Punjab and Sind provinces". The team identified questions on which additional data were essential in order for specific recommendations to be made on appropriate strategies for countering the resistance problem. To meet that need, the Government of Pakistan (GOP) with the assistance and cooperation of the U.S. Agency for International Development (USAID) invited Dr. George P. Georghiou, Professor of Entomology, University of California, Riverside, California 92521, U.S.A., to serve as a consultant during December 8 to 20, 1986, in order to review and summarize district studies on susceptibility of local vectors to insecticides and the impact of malathion spraying, and to formulate recommendations with emphasis on the selection of appropriate insecticides (6b). A second visit was requested for the period of January 10-20, 1990 in

order to assess subsequent developments in the status of resistance in malaria vector mosquitoes, to evaluate the effectiveness of malathion, and to advise on the need for any change in the type of insecticide that is to be used in the 1990 spray operations.

2.2 Schedule

The consultant was provided initial briefing on January 12, 1990, by the USAID Project Manager, and on January 13, 1990 by the Director, Directorate of Malaria Control (DOMC) of the GOP.

The period from December 11 to 18 was used for collecting and analyzing field data and for discussions with Malaria Control Program staff, USAID officers and the WHO Country Representative.

Oral presentations of the consultant's report were made on December 18, 1990 at separate meetings with the representatives of the DOMC and USAID.

2.3 Documentation Process

The consultant has reviewed all data and other documentation provided by the DOMC and provincial MCPs, concerning malaria control activities though 1989. He also reviewed the 1988 Annual Reports of the Directorate of Malaria Control (4), the NIMRT (13), the Environmental Assessment of Pesticides Report (10), and the Report of Malaria Among Afghan Refugees in Pakistan (3).

Since changes in insecticide susceptibility of an insect population can be detected only through a comparative analysis of data over several years, the consultant has made extensive use of background data that were presented in his 1986 Report (6b). The present Report is essentially an updated and expanded version of the 1986 Report, and can thus stand on its own as a comprehensive account of the insecticide resistance problem in malaria vectors in Pakistan.

3. PROCUREMENT OF INSECTICIDES AND STATUS OF MALARIA

In order to provide background information that is essential in the interpretation of the resistance data, information has been included in this report regarding (a) the types and quantities of insecticides procured by the DOMC and (b) the "slide positivity rate" (SPR) for malaria during 1960-89 (Fig. 1). The SPR data indicate a gradual rise from 0.45 to 3.13 during 1979-86, i.e. a 5.5-fold increase, but a decline to 2.15 and 2.01 in 1987 and 1989, respectively (Table 1).

When viewed on a province by province basis, the SPR data indicate uneven patterns of change (Fig. 2), the highest recent (1987-88) values being found in MWFP, Sind and Baluchistan, and the lowest in Punjab (Table 1, Fig. 2).

Table 1. Slide positivity rate (SPR) for various provinces and the entire country during the period 1977-89.

	SPR in %				
Year	Punjab	NWFP	Sind	Baluchistan	Entire Country
1977	2.15	0.38	2.15	0.81	1.78
1978	0.51	0.11	1.89	0.65	0.62
1979	0.36	0.31	1.05	0.46	0.45
1980	0.50	0.68	0.76	0.82	0.59
1981	1.02	1.93	0.99	0.85	1.29
1982	1.37	1.56	1.33	1.52	1.70
1983	2.00	1.33	2.79	2.75	1.99
1984	2.58	1.46	2.17	1.39	2.27
1985	2.13	3.53	3.06	2.36	2.48
1986	2.49	5.08	3.60	2.19	3.13
1987	1.39	3.84	3.43	2.25	2.16
1988	1.09	4.31	2.92	4.67	2.01
1989	(1.13)*	(2.26)*	(5.24)*	(7.56)*	(2.15)*

*The 1989 data are through August only.

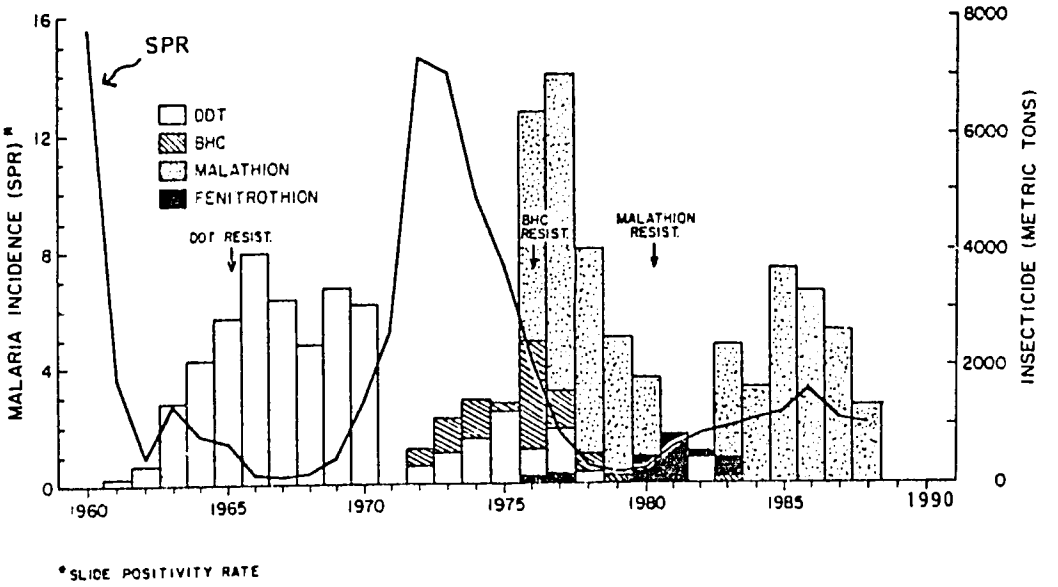

Fig. 1. Types and quantities of insecticides applied annually for malaria vector control in Pakistan during 1961-88; year of first report of resistance to each insecticide in *Anopheles stephensi;* and incidence of malaria based on SPR (% slide positivity rate). (Reproduced by permission from Georghiou, G. P., 1990, An Overview of Insecticide Resistance, in *Fundamental and Practical Approaches to Combatting Resistance,* Moberg, W. k. et al. (eds.). Amer. Chem. Soc., Washington, D.C.

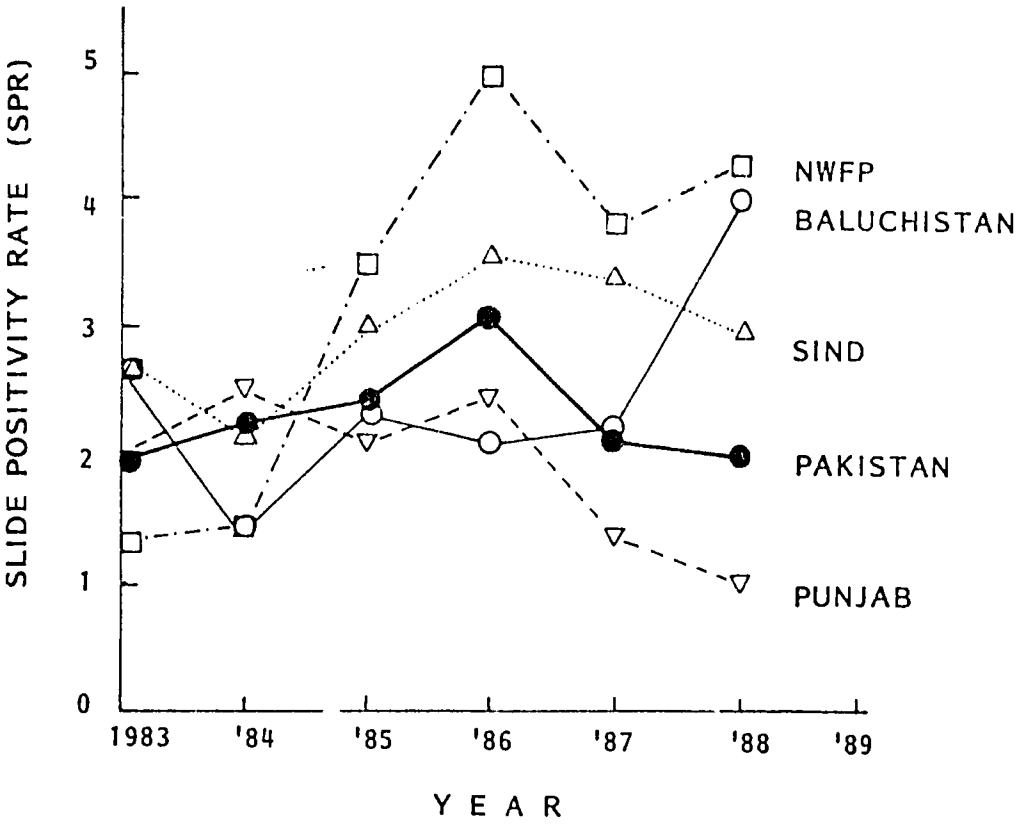

Fig.2 Slide positivity rate (SPR) for Pakistan, and separately for each province, during 1983-88.

4. INSECTICIDE RESISTANCE

4.1 General

Extensive exposure of an insect population to chemical selection pressure over several generations almost invariably results in the development of resistance to that chemical. The ability to detect emerging resistance and to monitor changes in its frequency and intensity, are essential requirements in organized vector control programs. By this means, it is possible not only to appraise the impact of resistance on the level of control that is obtained, but also to identify factors which may directly or indirectly exacerbate the problem, and ultimately to plan control strategies that could minimize its consequences. Such measures might consist of the appropriate choice of an alternative chemical, the timely change to that chemical, the introduction of insecticide usage tactics that could limit the degree of selection pressure, or the inclusion of supplementary, non-chemical measures that may reduce the need for frequent chemical interventions.

Resistance to malathion in *An. stephensi* has been reported earlier from Iran (5, 12), Iraq (11) and India (21). In the last two countries, some fenitrothion resistance was also observed (21). In Pakistan, malathion resistance was first detected near Lahore in 1980 (16), and was found subsequently more widely in the Punjab, as well as in Sind and NWFP Provinces (17).

In *An. culicifacies*, malathion resistance was first found in India in 1973 (15) where it became widespread in the States of Maharashtra and Gujarat (2). Subsequently, multiple organophosphorus insecticide resistance, which included fenitrothion, was reported to be present on a limited scale (2, 9).

In Pakistan, this species generally continues to be susceptible to both malathion and fenitrothion, although the occasional presence of individuals with

reduced susceptibility to these insecticides, as described later in this report (4.3.3), has been detected.

4.2 Resistance surveillance in Pakistan

4.2.1 Sources of Information

There are essentially three sources of information on the occurrence of resistance to insecticides in Pakistan: the resistance surveillance function of the MCP, research projects conducted at the NIMRT, and publications of foreign laboratories that have investigated certain aspects of resistance in the species concerned. This report contains information from all these sources, but the bulk of the data by far is derived from the extensive resistance surveillance records of the MCP.

4.2.2 Resistance Surveillance Within the Malaria Control Program

During the past several years the Malaria Control Program has included resistance surveillance among its activities. This function utilizes the specific test method recommended by WHO, i.e. collection of adult mosquitoes from indoor resting sites, and their bioassay for a definite length of time (60 minutes) on insecticide-impregnated filter paper in special test kits that are provided by WHO (20). Following the 60-min exposure, the mosquitoes are held for an additional period of 23 hours, at the end of which a record is taken of the mortality observed. In most cases, the 60-min exposure test is conducted at or near the collection site. The mosquitoes are subsequently transported to the District headquarters where they are held for the 23-hr observation period.

Treated papers are received from WHO by the DOMC at six-month intervals and are distributed to the Provinces and from there to the Districts as necessary. These papers contain a diagnostic concentration of either DDT (4%), dieldrin (4%), malathion (5%) or fenitrothion (1%). Each of these doses is known to cause 100% mortality of susceptible mosquitoes. Thus, individuals

surviving the test are considered to be resistant. Assuming that adequate numbers are tested, and the tests are repeated seasonally (at the beginning and at the end of the spraying season), it is possible to maintain a fairly accurate index of the relative changes in the frequency of susceptible and resistant individuals in a mosquito population.

The intensity of the resistance surveillance operations of the MCP has varied through the years in each province and district. In general, surveillance has been more extensive and intensive in the Punjab, less so in the MWFP and Sind and least in Baluchistan. A total of 61,956 *An. stephensi* and 54,569 *An. culicifacies* were tested for resistance to malathion during 1981-89 (Table 2) and similarly large numbers were tested with fenitrothion.

Table 2. Number of mosquitoes tested for malathion resistance in Punjab Province during the period 1981-89*.

Year	*An. stephensi*		*An. culicifacies*		Total
1981	1,784	(11)	3,499	(15)	5,283
1982	4,738	(16)	8,248	(19)	12,986
1983	2,981	(14)	4,187	(19)	7,168
1984	13,400	(18)	10,995	(20)	24,395
1985	7,593	(18)	5,120	(22)	12,713
1986	9,170	(19)	5,827	(19)	14,997
1987	9,440	(22)	5,449	(21)	15,433
1988	7,063	(26)	7,303	(24)	14,366
1989	5,787	(22)	3,941	(22)	9,728
Total	61,956		54,569		116,525

*Number of districts in parenthesis.

The high level of resistance surveillance conducted in the Punjab is also evident in the number of districts sampled annually, but the numbers of mosquitoes tested differ considerably from district to district (see Table 7). In

some instances these differences reflected correctly the need for more testing in certain localities due to evidence of resistance (e.g. 1371 *An. stephensi* were tested in Faisalabad and 1334 in Sahiwal in 1986), but not always so (e.g. limited testing was done in D.G. Khan - 335 specimens -, Sheikhupura - 117 specimens -, despite evidence of equally high resistance). There is obviously a need to monitor the results of resistance surveillance as they are obtained, so that they may serve as a guide for placing emphasis where it is most needed.

4.2.3 Some inherent limitations of resistance surveillance

The surveillance of resistance has certain limitations which must be borne in mind in interpreting the results. Some of these limitations are inherent in the test method itself, while others are consequences of the local environment or practices.

4.2.3.1 Aging of test papers and the loss of toxicity

The effective shelf life of malathion- and fenitrothion-treated papers at room temperature is considered to be approximately 6 months, unlike DDT or dieldrin papers which remain active for several years. In Pakistan, fresh malathion and fenitrothion papers are received at 6-month intervals and the old papers are discarded. We have evidence that if the test papers are stored in a refrigerator, their effective use life is considerably extended. Storing papers in a refrigerator should be adopted as a routine measure.

There is no evidence that the observed increased survival of *An. stephensi* in tests conducted in Pakistan is due to loss of activity of the test papers. The fact that many parallel tests performed on *An. culicifacies* have produced complete mortality, provides evidence of adequate activity in these papers.

4.2.3.2 Increased mortality of test mosquitoes due to handling

Injuries and stress suffered by insects due to poor handling, such as excessively vigorous aspirating, exposure to extremes of high temperature and low humidity, and a long waiting period between collecting and testing, often increase the insects' sensitivity to insecticides and cause higher mortality than would have occurred under normal circumstances. Thus, human error or environmental stress by producing higher mortalities would tend to conceal rather than enhance the evidence for resistance.

4.2.3.3 Influence of age of mosquitoes on susceptibility to insecticides

Recent studies suggest that the activity of carboxylesterase, the metabolic enzyme that is responsible for malathion resistance in *An. stephensi* (7, 9), is highest in newly emerged adults, declining gradually as the mosquito ages (18). Similar results were obtained in our laboratory with non-specific esterase. This lowering of enzyme activity results in a reduction in the degree of resistance, as assessed by the WHO test. Thus, if the test sample contains a disproportionate number of older mosquitoes, the frequency of resistance may be underestimated by the test. This is an uncontrolled variable which must be considered in interpreting the data.

4.2.4 Relating susceptibility test data to field performance of insecticides

A question of paramount importance is whether mosquitoes that survived the diagnostic dose of 5% malathion in the WHO test are also able to survive the malathion residue on sprayed wall surfaces. The discriminating dosage (5% malathion) and length of exposure (60-min) used to detect resistance are chosen to ensure reliable killing of all susceptible insects and not to simulate

the exposure which occurs under field conditions. In the absence of data from specifically designed research (such as parallel, simultaneous bioassays of field mosquitoes in WHO tubes as well as on wall surfaces), this question can be answered only from indirect evidence.

It would appear from vector density data obtained during 1985 (6a), that adult mosquitoes are almost entirely absent from sprayed premises for several days post-treatment. In some districts with moderate resistance in 1984, e.g. Bahawalnagar (20% average survival in WHO susceptibility tests) the first post-treatment vector density count in animal sheds showed 4.3 *An. stephensi* and 1.5 *An. culicifacies* per sprayed room vs. 24.3 and 11, respectively, per unsprayed room; i.e. almost the expected 20% survival rate for *An. stephensi* (24.3 x 0.2 = 4.86) and less for the still susceptible species *An. culicifacies.* However, data from other districts with equally high or higher resistance, e.g. Faisalabad and D. G. Khan, show absence of mosquitoes in the sprayed sheds. Whether this is due to the known low repellency for malathion, or to practical difficulties in obtaining accurate counts when density is very low remains a mute question.

Of interest are data obtained by Dr. I. H. Shah in 1984 in a field experiment with malathion and fenitrothion. Susceptibility tests with malathion on the local *An. stephensi* (301 mosquitoes) showed an average mortality of 87.2%. Bioassay of malathion residues on the more typical sprayed surfaces, using reared mosquitoes, showed the following mortality levels:

	Percent Mortality Post-Spray (Days)				
Surface	2	12	24	31	37
Unlimed kacha	93.2	71.7	41.9	24.1	15.6
Limed kacha	98.0	97.9	83.3	60.0	3.7
Brick	93.3	51.4	48.4	35.4	11.7
Avg.	94.8	73.7	57.9	39.8	10.3

It is evident that a proportion of *An. stephensi* are able to survive contact with malathion on the common sprayed surfaces. The survival rate increases as the residue ages. It may, therefore, be assumed that at least some, if not all, mosquitoes surviving the diagnostic dose of 5% malathion in the confines of the WHO test tubes are also able to survive exposure to freshly sprayed malathion residues. Thus, the results of WHO susceptibility tests with malathion constitute a useful indicator of the presence and frequency of effective resistance in the local mosquito population.

4.3 Status of Insecticide Resistance

The following discussion is based on susceptibility records provided by the provinces concerning the two major vector species in Pakistan, *An. stephensi* and *An. culicifacies*, and the insecticides DDT, dieldrin, malathion and fenitrothion. Although emphasis has been placed on the results of tests conducted with malathion and fenitrothion since 1986, all data available since 1981 were collated and examined in order to discern possible trends in susceptibility. As recommended by the ERT in 1985, testing with DDT and dieldrin was reduced considerably whereas testing with malathion and fenitrothion was increased. The analysis of data suffers from the randomness of the collection sites, the lack of collections from the same site at frequent intervals post-treatment and from shifts in the areas treated from year to year. Hence the data from all localities of a district were pooled and are examined with the district as the smallest unit. Also included are limited data on susceptibility to insecticides in *An. stephensi* and *An. culicifacies* in Afghan refugee camps.

4.3.1 DDT

Susceptibility tests which were conducted with DDT in the Punjab during 1986 (Table 3), and in NWFP during 1980-86 (Table 4), indicate that both vector species continue to be highly resistant to this insecticide. The yearly mortality values obtained in NWFP (Table 4), reveal no significant change since 1980: mortality fluctuates within the range of 40 and 64% and averages 52.2 for *An. stephensi* and 53.9% for *An. culicifacies*. It is apparent that there is no prospect that DDT will be of use in anopheline mosquito control in Pakistan in the foreseeable future.

Table 3. Results of susceptibility tests with DDT in the Punjab Province against *An. stephensi* and *An. culicifacies* during 1986*.

District	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
Sheikhupura	-	-	-	100	24-44	31
Kasur	50	-	36	-	-	-
Jhang	542	42-55	46.3	250	50-57	54
Sahiwal	570	52-60	55.3	-	-	-
Behawalnagar	67	38-40	38.8	-	-	-
Vehari	-	-	-	3	60	5
Bahawalpur	-	-	-	250	30-50	40
R. Y. Khan	101	30-31	31	-	-	-
(Total) Range	(1330)	36-60	41.5	(603)	24-60	32.5

*WHO test method: 4% DDT, 60 min exposure.
Compiled from data provided by the Directorate of Health Services, Punjab.

Table 4. Results of susceptibility tests with DDT in MWFP during 1981-86 against *An. stephensi* and *An. culicifacies**.

	An. stephensi				*An. culicifacies*			
	No.	Adults	% Mortality		No.	Adults	% Mortality	
Year	Tests	Tested	Range	Average	Tests	Tested	Range	Average
1980	65	2050	0-85	42.6	8	272	40-60	53.0
1981	50	1565	23-75	44.0	11	322	33-47	40.2
1982	50	1679	28-91	51.6	4	153	53-93	64.3
1983	18	679	43-100	57.4	2	72	44-56	50.0
1984	13	662	40-90	59.5	1	40	40-90	59.5
1985	4	193	54-76	60.0	0	-	-	-
1986	11	463	20-70	50.1	5	228	52-83	56.2

*WHO test method: 4% DDT, 60 min exposure.
Compiled from data provided by Directorate of Health Services, MWFP.

4.3.2 Dieldrin-BHC

As with DDT, tests with dieldrin in the Punjab (Table 5) and MWFP (Table 6) show no definite trend toward reduced resistance in either of the anopheline species. The results of 17 tests with *An. culicifacies* and 22 tests with *An. stephensi* in the Punjab conducted in 1986, were classified as "resistant" by WHO criteria (i.e. showing less than 80% mortality), in 100% and 94% of the cases, respectively (Table 5). In NWFP, the average mortality cause in *An. stephensi* by the diagnostic dose of dieldrin was 55.7% in 1980 vs. 50.8% in 1986 (Table 6).

Table 5. Results of susceptibility tests with dieldrin in the Punjab Province against *An. stephensi* and *An. culicifacies* during 1986*.

District	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
Sheikhupura	-	-	-	125	48-72	64.8
Kasur	-	-	-	51	32-39	35.3
Jhang	564	54-71	62.1	123	62-70	64.2
Sahiwal	50	36-44	40	-	-	-
Behawalnagar	65	28-38	33.8	-	-	-
Sargodha	50	36-56	46	100	44-64	52
Vehari	-	-	-	81	-	91.3
Bahawalpur	-	-	-	200	36-40	38
R. Y. Khan	101	33-36	35	-	-	-
(Total) Range	(830)	28-71	43.4	(680)	32-72	57.6

*WHO test method: 4% dieldrin, 60 min exposure.
Compiled from data provided by Directorate of Health Services, Punjab.

Table 6. Results of susceptibility tests with dieldrin in MWFP during 1980-86 against *An. stephensi**.

Year	No. of Tests	Adults Tested	Percentage of Mortality Range	Percentage of Mortality Average
1980	36	1000	9-95	55.8
1981	10	258	29-83	63-5
1982	12	395	30-70	50.8
1983	6	243	43-55	48.1
1984	1	40	-	52.5
1985	0	-	-	-
1986	5	140	35-58	50.8

*WHO test method: 4% dieldrin, 60 min exposure. Compiled from data provided by Directorate of Health Services, MWFP.

4.3.3 Malathion

The results obtained with malathion are presented separately for the various districts of Punjab (Table 7), MWFP (Table 8), Sind (Table 9), and Baluchistan (Table 10). The data from each district have been tabulated as to show the range and average mortality obtained in the various tests in each year. The data thus reflect the changes that have occurred through the years in each district.

SEE FOLLOWING PAGE

Table 7. Results of susceptibility tests with malathion in the Punjab Province during 1981-89 against *An. stephensi* and *An. culicifacies**.

District	Year	*An. stephensi* Adults Tested	*An. stephensi* % Mortality Range	*An. stephensi* % Mortality Average	*An. culicifacies* Adults Tested	*An. culicifacies* % Mortality Range	*An. culicifacies* % Mortality Average
1. Lahore	1981	-	-	-	157	-	100
	1982	448	72-88	80.5	1277	76-100	95.8
	1983	-	-	-	60	83-100	92.0
	1984	601	76-94	86.6	155	86-100	97.2
	1985	150	72-85	79.8	70	-	100
	1986	402	18-71	36.6	-	-	-
	1987	820	29-77	46.1	-	-	-
	1988	333	16-33	24.0	-	-	-
	1989	94	64-67	64.9	180	-	100
2. Kasur	1981	-	-	-	40	-	100
	1982	90	-	100	-	-	-
	1983	20	-	100	105	-	100
	1984	-	-	-	-	-	-
	1985	117	76-84	81.1	287	-	100
	1986	742	47-90	60.9	52	-	100
	1987	1156	31-76	59.1	163	-	100
	1988	555	16-68	47.5	416	96-100	97.8
	1989	217	32-64	45.6	120	96-100	99.1
3. Sheikhupura	1981	-	-	-	-	-	-
	1982	-	-	-	404	-	100
	1983	-	-	-	127	-	100
	1984	74	-	84	319	-	100
	1985	-	-	-	414	-	100
	1986	117	23-81	56.4	577	-	100
	1987	168	22-50	43.4	450	-	100
	1988	150	30-72	47.3	1267	95-100	99.6
	1989	70	52-68	58.5	442	98-100	99.7
4. Okara	1983	-	-	-	148	-	100
	1984	190	59-76	72.5	762	-	100
	1985	1160	47-100	61.9	-	-	-
	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	227	20-66	39.2	75	-	100
	1989	675	38-56	45.9	43	-	100
5. Gujranwala	1981	-	-	-	70	80-88	84
	1982	85	71-89	86.6	676	-	100
	1983	390	96-100	99.0	-	-	-
	1984	100	-	95.0	400	-	100
	1985	49	-	57.0	643	-	100
	1986	-	-	-	769	-	100

Table 7. Continued.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
5. Gujranwala (cont'd.)	1987	95	43-51	49.5	470	-	100
	1988	161	54-75	68.1	397	98-100	99.5
	1989	-	-	-	353	-	100
6. Gujrat	1981	20	-	-	50	-	-
	1982	-	-	-	23	-	-
	1983	-	-	-	-	-	-
	1984	-	-	-	147	-	-
	1985	-	-	-	-	-	-
	1986	19	-	90.0	42	86-100	95.2
	1987	25	-	68.0	128	69-100	87.5
	1988	55	-	85.5	783	62-100	91.1
	1989	35	75-93	82.8	361	94-100	98.6
7. Sialkot	1981	-	-	-	25	-	100
	1982	-	-	-	50	-	100
	1983	-	-	-	388	96-100	99
	1984	-	-	-	945	-	100
	1985	-	-	-	151	96-100	100
	1986	40	-	100	330	-	100
	1987	-	-	-	345	-	100
	1988	-	-	-	175	-	100
	1989	-	-	-	-	-	-
8. Rawalpindi	1981	155	92-100	99.2	110	90-100	97
	1982	85	-	100	55	-	100
	1983	385	90-100	96.3	15	-	100
	1984	245	-	100	270	96-100	99.7
	1985	-	-	-	140	90-100	98.6
	1986	200	86-95	92.5	100	-	100
	1987	-	-	-	20	-	100
	1988	60	30-50	36.6	40	90-95	92.5
	1989	45	20-80	46.6	40	90-95	92.5
9. Attock	1981	140	88-100	96.6	227	85-100	98.6
	1982	143	-	100	174	-	100
	1983	60	-	100	45	-	100
	1984	60	-	100	135	-	100
	1985	40	-	100	95	-	100
	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	-	-	-	-	-	-
	1989	35	-	100	45	-	100

Table 7. Continued.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
10. Jehlum	1983	225	96-100	99.4	250	96-100	99.1
	19841	85	-	100	60	-	100
	1985	75	-	100	120	-	100
	1986	60	-	100	60	-	100
	1987	35	-	100	65	-	100
	1988	60	-	100	35	-	100
	1989	60	-	100	20	-	100
11. Sargodha	1981	160	83-93	88.9	-	-	-
	1982	397	88-100	98.6	45	-	100
	1983	356	88-100	94.9	50	-	100
	1984	804	65-98	81.5	498	89-100	96.2
	1985	226	72-100	82.5	226	90-100	96.5
	1986	403	50-100	68.2	473	-	100
	1987	146	48-64	52.7	44	-	100
	1988	170	32-64	56.4	90	85-100	91.1
	1989	103	48.60	51.4	147	98-100	99.3
12. Khushab	1983	50	-	100	-	-	-
	1984	-	-	-	-	-	-
	1985	126	86-100	95.3	51	-	100
	1986	283	55-65	56.9	66	-	100
	1987	201	19-32	25.3	-	-	-
	1988	100	44-56	50.0	21	-	100
	1989	145	33-44	37.2	18	-	100
13. Mianwali	1981	50	93-100	97.7	30	-	100
	1982	75	92-100	97.3	48	95-100	97.5
	1983	-	-	-	-	-	-
	1984	-	-	-	-	-	-
	1985	-	-	-	52	-	100
	1986	-	-	-	-	-	-
	1987	220	55-100	91.0	125	-	100
	1988	100	40-80	64.0	40	80-90	85.0
	1989	150	22-100	73.3	95	-	100
14. Faisalabad	1981	149	72-93	84.2	51	-	100
	1982	1920	31-100	80.0	377	79-100	95.9
	1983	314	80-100	90.2	281	88-100	96.0
	1984	4021	58-94	72.3	1247	66-100	96.8
	1985	1094	50-100	70.7	651	99-100	100
	1986	1371	44-74	52.4	398	-	100
	1987	801	31-74	42.7	255	94-100	99.6
	1988	473	20-100	39.7	375	96-100	99.7
	1989	344	32-64	67.7	147	92-100	98.6

Table 7. Continued.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
15. Jhang	1981	84	-	100	446	96-100	99.8
	1982	44	-	100	241	93-100	98
	1983	81	-	100	220	-	100
	1984	710	83-95	86.6	811	83-100	99.6
	1985	130	-	100	237	-	100
	1986	2367	80-97	85.8	1664	88-100	94.4
	1987	1273	38-80	63.4	1064	88-100	95.6
	1988	461	13-40	27.9	457	86-99	93.6
	1989	-	-	-	-	-	-
16. Multan	1981	60	98-100	99.0	85	-	100
	1982	25	-	96.0	466	86-100	96.4
	1983	-	-	-	192	-	100
	1984	117	86-100	94.5	304	95-100	98.6
	1985	75	90-100	96.3	501	85-100	95.6
	1986	115	75-96	86.1	154	-	100
	1987	200	80-100	96.5	119	-	100
	1988	156	55-78	67.3	384	-	100
	1989	144	75-100	85.9	300	-	100
17. Vehari	1981	-	-	-	353	-	100
	1982	46	-	100	803	-	100
	1983	-	-	-	413	-	100
	1984	124	-	100	221	-	100
	1985	-	-	-	96	97-100	99.4
	1986	205	69-75	72.7	167	-	100
	1987	130	-	100	168	-	100
	1988	94	31-61	44.6	510	-	100
	1989	265	60-75	70.1	291	72-100	90.3
18. Sahiwal	1981	250	98-100	99.3	987	-	100
	1982	290	86-87	84.4	2087	-	100
	1983	80	-	90	260	-	100
	1984	1174	52-92	72.4	1879	-	100
	1985	2344	36-100	62.1	412	-	100
	1986	1334	58-77	65.9	193	-	100
	1987	1757	56-72	65.1	111	92-100	98.1
	1988	1622	30-71	55.1	96	-	100
	1989	1258	38-75	51.1	22	-	100
19. Bahawalpur	1982	-	-	-	193	-	100
	1983	-	-	-	66	-	100
	1984	453	76-85	80.2	1233	-	100
	1985	410	20-100	60.6	200	-	100
	1986	-	-	-	210	-	100

Table 7. Continued.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
19. Bahawalpur (cont'd.)	1987	300	33-44	38.6	645	-	100
	1988	530	16-33	21.3	470	93-100	97.8
	1989	344	19-90	36.6	1016	77-100	89.7
20. Bahawalnagar	1982	341	74-90	81.9	65	88-98	92.8
	1983	625	80-100	91.7	451	75-98	89.3
	1984	827	66-95	80.8	426	92-100	97.3
	1985	229	60-76	65.5	251	-	100
	1986	833	41-70	53.5	160	-	100
	1987	585	40-56	49.4	290	-	100
	1988	611	24-58	37.4	155	-	100
	1989	595	32-38	33.6	70	-	100
21. R.Y. Khan	1982	385	96-100	98.4	571	98-100	99.8
	1983	158	94-100	96.1	515	91-100	96.3
	1984	1495	60-94	82.4	361	96-100	98.0
	1985	392	82-94	89.1	205	92-96	94.2
	1986	101	90-96	93.1	-	-	-
	1987	279	56-77	70.6	102	-	100
	1988	252	72-95	78.9	152	94-98	94.1
	1989	203	42-92	59.1	-	-	-
22. D.G. Khan	1981	158	57-93	74.9	108	-	100
	1982	249	64-87	77.7	452	-	100
	1983	72	51-84	72.5	496	-	100
	1984	1395	60-93	80.7	722	-	100
	1985	226	54-73	64.2	218	92-100	99.1
	1986	335	27-73	46.6	67	-	100
	1987	271	28-55	31.3	76	-	100
	1988	269	15-55	31.6	91	94-100	98.9
	1989	451	0-41	21.5	158	93-100	97.5
23. Muzaffargarh	1981	558	86-100	98	760	95-100	99.5
	1982	115	90-100	93.8	241	90-100	97.1
	1983	165	96-100	97.7	105	95-100	99.0
	1984	825	80-100	90.7	100	96-100	99.0
	1985	700	88-100	94.7	50	-	100
	1986	-	-	-	-	-	-
	1987	200	90-96	94.0	-	-	-
	1988	75	91-96	94.6	-	-	-
	1989	377	56-95	80.1	23	-	100
24. Layyah	1985	50	-	96	50	-	100
	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-

Table 7. Continued.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
24. Layyah	1988	101	65-68	66.3	301	98-100	98.6
(cont'd.)	1989	-	-	-	-	-	-
25. Khanewal	1986	133	65-80	73.7	191	-	100
	1987	260	64-100	94.2	157	-	100
	1988	20	-	65.0	229	-	100
	1989	22	-	100	40	-	100
26. Rajanpur	1986	110	25-85	62.7	155	-	100
	1987	200	15-55	49.0	630	-	100
	1988	170	0-25	8.2	40	-	100
	1989	-	-	-	40	-	100
27. T.T. Singh	1986	-	-	-	-	-	-
	1987	318	71-78	75.1	22	-	100
	1988	132	84-95	87.8	42	-	100
	1989	150	98-100	99.3	-	-	-
28. Bhakar	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	126	54-76	65.1	-	-	-
	1989	-	-	-	-	-	-

*Compiled from data provided by the Directorate of Health Services, Punjab.

Table 8. Results of susceptibility tests with malathion during 1981-89 in selected districts of NWFP against *An. stephensi*.

District	Year	Adults Tested	Percentage Mortality Range	Percentage Mortality Average
Kohat	1980	525	-	100
	1981	75	-	100
	1982	100	-	100
	1983	75	85-93	89.7
	1984	200	77-100	89.0
	1985	75	81-84	82.3
	1986	100	53-82	67.5
	1989	320	70-98	85.0
Bannu	1980	150	-	100
	1982	200	80-90	85.6
	1983	150	92-100	95.6
	1984	375	86-100	95.0
	1985	150	92-100	96.6
	1986	325	68-78	73.0
	1987	365	42-100	85.2
	1988	406	93-100	96.8
	1989	397	29-100	82.1
D.I. Khan	1980	150	-	100
	1981	25	-	100
	1982	25	-	87.8
	1983	75	-	100
	1984	300	78-89	83.5
	1985	125	83-92	87.4
	1986	300	48-67	57.5
	1987	960	48-58	57.5
	1988	380	53-56	53.7
	1989	546	50-54	51.9
Peshawar	1987	145	86-93	84.1
	1989	148	57-80	67.5
Swat	1987	100	72-88	81.0
	1988	75	76-88	78.6
	1989	45	88-100	93.3
Abbottabad	1987	38	-	100
	1988	20	-	100
	1989	30	-	100
Mardan	1987	359	-	100
	1989	50	-	100

*Compiled from data provided by Directorate of Health Services, MWFP.

Table 9. Results of susceptibility tests with malathion in the Sind Province during 1984-89 against *S. stephensi* and *An. culicifacies*.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
Sukkur	1984	40	-	90.0	40	-	100
	1985	151	31.1-50	43.5	25	-	100
	1987	100	50-100	80.0	120	-	100
	1988	40	40-45	42.5	240	-	100
	1989	80	35-60	43.7	160	-	100
Nawab Shah	1984	25	-	100	20	-	100
	1985	133	33.3-80	51.8	20	-	100
	1988	530	52-80	61.3	213	-	100
	1989	92	36-46	40.2	38	-	100
Mirpurkhas	1984	219	-	97.8	524	-	97.5
	1985	145	95-100	97.2	264	96-100	97.1
	1986	227	96-100	94.6	479	-	98.5
	1987	50	-	90.0	20	-	75.0
	1988	30	-	100	44	-	100
Karachi	1985	115	95-100	99.0	25	-	100
	1986	40	-	95.0	20	-	90.6
Shikarpur	1985	82	30-45	35.5	61	-	100
Hyderabad	1985	40	-	100	85	-	100
	1986	20	-	100	40	-	100
	1987	20	-	100	80	-	100
	1989	20	-	95	20	-	100
Badin	1985	-	-	-	75	-	100
Khairpur	1985	398	52-72	59.2	47	-	100
	1986	100	56-71	64	25	-	100
Sanghar	1985	178	25.7-40	34.5	-	-	-
Dadu	1985	-	-	-	25	-	96
Larkana	1985	-	-	-	50	-	100
	1989	-	-	-	80	-	100
Jacobabad	1986	-	-	-	45	80-100	98.5

*Compiled from data provided by Directorate of Health Services, Sind.

Table 10. Results of susceptibility tests with malathion in the Buluchistan Province during 1984-89 against *An. stephensi* and *An. culicifacies*.

District	Year	*An. stephensi* Adults Tested	*An. stephensi* % Mortality Range	*An. stephensi* % Mortality Average	*An. culicifacies* Adults Tested	*An. culicifacies* % Mortality Range	*An. culicifacies* % Mortality Average
Loralai	1984	-	-	-	22	-	100
Sibi	1984	46	65-84.6	74.8	141	83.5-100	92.9
	1985	51	92	92	51	-	100
	1986	28	-	89.2	221	92-100	98.7
	1987	23	-	91.3	27	71-100	100
	1988	58	80-100	89.6	113	71-100	85.8
Nasseerabad	1985	75	96-96.9	95	53	96-100	98
	1987	-	-	-	83	97-100	98.8
	1989	-	-	-	55	-	100
Pishin	1985	50	-	60	104	96-100	98
	1986	50	96-100	98	195	96-100	99
	1987	-	-	-	258	96-100	99.6
	1988	-	-	-	241	97-100	99.5
	1989	-	-	-	177	-	100
Khuzdar	1986	98	88-96	93.8	50	-	92
	1987	25	-	88	48	-	100
Zhob	1986	-	-	-	48	-	100
	1987	-	-	-	26	-	96
Kichhi	1986	-	-	-	55	-	100
	1989	-	-	-	30	-	96.6
Quetta	1987	25	-	100	128	88-100	96.8
	1988	26	-	92.3	195	83-100	96.4
	1989	100	80-100	87.7	260	88-100	97.6

*Compiled from data provided by Directorate of Health Services, Buluchistan.

(a) *Anopheles stephensi*

There is clear evidence of resistance to malathion in *An. stephensi* in several districts of the Punjab, Sind and NWFP, as well as in Afghan refugee camps (Table 11). If an average mortality of near 50% or less during the latest two years of testing is used as a working basis for defining the significance of resistance operationally, the districts listed in Table 12 may be considered as confronted with a malathion resistance problem. Districts with inadequate data have not been included, although in some of these districts resistance is also evident. More data are necessary from the refugee camps as well as from areas of Sind, NWFP and Buluchistan.

The data for the Punjab districts (Table 7) are amenable to closer scrutiny since in many instances they are derived from large numbers of insects and often represent several consecutive years of testing. The most pronounced decline in susceptibility to malathion has obviously occurred during 1983-87, coincident with the use of the largest quantities of malathion in the malaria control program (Table 13, Fig. 3). For the 14 "problem districts" of Punjab identified in Table 13, the data for the last four years have been collated in Table 14 in order to provide quantitative comparisons between malathion and fenitrothion resistance in the two vector species.

(b) *Anopheles culicifacies*

Field populations of *An. culicifacies* continue to demonstrate susceptibility to malathion throughout the country, but in a small number of instances, less than complete kill has been obtained in the WHO resistance test (see Jhang, Bahuwalnagar Sargodha, etc.) (Table 7). The MCP Entomologist in Punjab also noted that unlike in earlier years, knockdown of *An. culicifacies* in the WHO test now occurs more slowly. Especially frequent survival of *An. culicifacies* has been observed in WHO tests with malathion in the Jhang District. Here, overall

Table 11. Results of susceptibility tests in Afghan refugee camps - 1989*.

Test Month	Locality	District or Agency	Insecticide	Species Tested	Number Exposed	% Mortality
Sept.	Deja Ghundi	S. Waziristan	Malathion 5%	*An. culicifacies*	15	86.7
	Azam Warsack	"	"	"	16	86.1
	"	"	Permethrin 0.25%	"	20	100.0
	"	"	DDT 4%	*An. stephensi*	16	42.1
	"	"	Malathion 5%	"	19	81.7
	"	"	Permethrin 0.25%	"	18	93.5
Oct.	Kachi an	Y. Gh. Mohmand	DDT 4%	"	21	28.0
	"	"	Malathion 5%	"	105	29.6
	"	"	Permethrin 0.25%	"	33	100.0
	"	"	Bendiocarb 0.1%	"	62	87.1
	Khugiani	"	DDT 4%	"	40	20.0
	"	"	Malathion 5%	"	60	33.3
	"	"	Permethrin 0.25%	"	49	100.0
	Darsamad	Kohat, NWFP	Malathion 5%	"	48	18.7

*Data from ref. 3.

Table 12. Ranking of 14 high resistance districts of Punjab, Sind and NWFP, and areas under the control of the U.N. High Commissioner for Refugees (UNHCR), in decreasing order of resistance to malathion observed in *An. stephensi* during the latest two years of tests.

District		Average Mortality (%) W.H.O. Test
Kohat	UNHCR	18.7
D. G. Khan	Punjab	26.6
Rajanpur	Punjab	28.6
Bahawalpur	Punjab	29.0
Y. Gh. Mohamand	UNHCR	31.5
Singhar	Sind	34.5
Bahawalnagar	Punjab	35.5
Rawalpindi	Punjab	41.6
Okara	Punjab	42.5
Sukkur	Sind	43.1
Khushab	Punjab	43.6
Lahore	Punjab	44.4
Jhang	Punjab	45.7
Kasur	Punjab	46.6
Sheikhupura	Punjab	52.9
Sahiwal	Punjab	53.6
Faisalabad	Punjab	53.7
Sargodha	Punjab	53.9

Table 13. Quantities of malathion and fenitrothion applied in Punjab Province during 1980-89.

Year	Quantity (M. Tons) Malathion (50%)	Fenitrothion (40%)
1980	1062.9	391.5
1981	156.9	391.5
1982	106.9	426.3
1983	1222.0	346.4
1984	1430.4	40.0
1985	2195.0	-
1986	2310.8	-
1987	1296.0	-
1988	619.2	-
1989	549.5*	-

*Total quantity available for use.

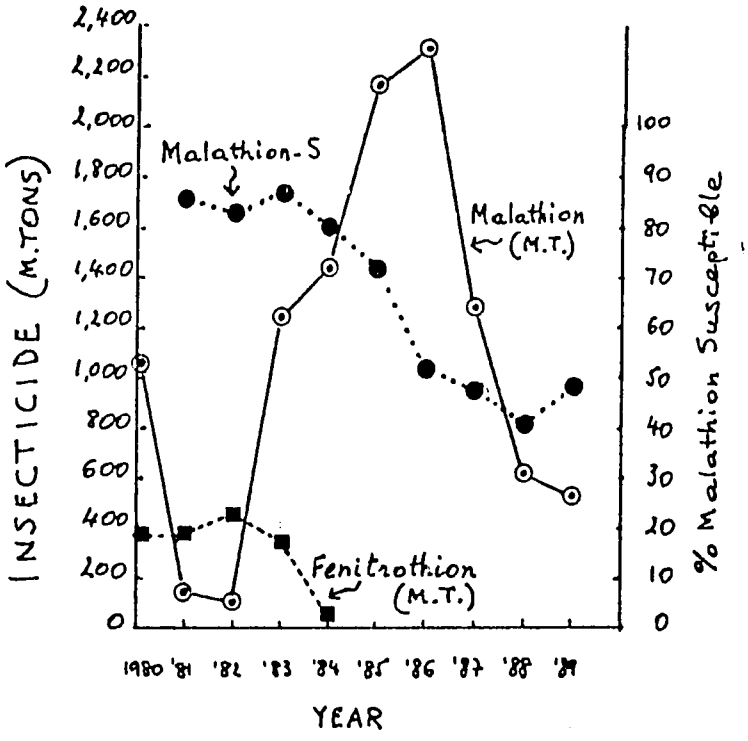

Fig. 3. Quantities of malathion and fenitrothion used in malaria control in the Punjab province during 1980-89, and frequency (%) of malathion susceptible *Anopheles stephensi.*

mortality during 1989 was 94.4%, some mosquitoes having survived in 50% jof the tests conducted. As had been observed in earlier years with DDT and BHC, it is now also evident with malathion, that, *An culicifacies* demonstrates a slower propensity than *An. stephensi* for development of resistance. However, it must be noted that a malathion-resistant strain of *An. culicifacies* has been selected in the laboratory at NIMRT (I. Rathor, pers. comm.).

4.3.4 Fenitrothion

To date, all susceptibility tests with fenitrothion against both anopheline species have been essentially negative, cases of survival to the diagnostic dose of 1% fenitrothion having been extremely low (Tables 14, 15). Among several hundred specimens tested in the Punjab during 1986-89, only rare individuals of *An. culicifacies* (in Gujrat, Sargodha, and Vehari) survived the test. In Faisalabad, 4 specimens of *An. stephensi* among 300 tested in May 1984, survived; however, follow-up tests at the same site in September failed to detect any resistant individuals. In Bannu, NWFP, individual *An. stephensi* were found to have survived the diagnostic dose of fenitrothion in two of the tests conducted in 1984. In none of the tests conducted in 1986 were there any survivors. It is concluded that fenitrothion continues to be active against both vector species, despite the high resistance to malathion found in *An. stephensi.*

4.3.5 Role of agricultural insecticides in resistance

There is clear evidence from different countries that insecticide resistance in mosquitoes is most serious in agricultural areas that receive heavy and frequent applications of insecticides. Such use causes contamination of mosquito breeding sites or direct exposure of adult mosquitoes to insecticide sprays, leading to enhanced selection pressure toward resistance. The crop most frequently implicated is cotton, but rice, sugar cane, and corn are also

Table 14. Comparative susceptibility in *An. stephensi* and *An. culicifacies* to malathion and fenitrothion during 1986-89 in 14 districts of Punjab Province in which *An. stephensi* demonstrates high resistance to malathion.

District	*An. stephensi* Malathion					*An. stephensi* Fenitrothion					*An. culicifacies* Malathion					*An. culicifacies* Fenitrothion				
	86	87	88	89	Avg.	86	87	88	89	Avg.	86	87	88	89	Avg.	86	87	88	89	Avg.
Lahore	36.6	46.1	24.0	64.9	42.9	100	100	100	100	100	-	-	-	100	100	-	-	-	100	100
Kasur	60.9	59.1	47.5	45.6	53.3	100	100	100	100	100	100	100	97.8	99.1	99.2	100	100	100	100	100
Sheikhupura	56.4	43.4	47.3	58.5	51.4	100	-	100	-	100	100	100	99.6	99.7	99.8	100	100	98.5	100	99.6
Okara	-	-	39.2	45.9	42.6	-	-	100	100	100	-	-	100	100	100	-	-	-	-	-
Rawalpindi	92.5	-	36.6	46.6	58.6	100	100	100	-	100	100	100	92.5	92.5	96.3	100	100	97.1	-	99.0
Sargodha	68.2	52.7	56.4	51.4	57.2	100	100	100	100	100	100	100	91.1	99.3	97.6	100	-	84.	99.2	94.4
Khushab	56.9	25.3	50.0	37.2	42.4	100	100	100	100	100	100	-	100	100	100	100	-	-	-	100
Faisalabad	62.4	42.7	39.7	67.7	53.1	100	100	100	100	100	100	99.6	99.7	98.6	99.5	100	100	100	100	100
Jhang	85.8	63.4	27.9	-	59.0	100	97.6	100	-	99.2	94.4	95.6	93.6	-	94.5	100	98.8	98.3	-	99
Bahawalpur	-	38.6	21.3	36.6	32.2	-	100	100	100	100	100	100	97.8	89.7	96.9	100	100	100	100	100
Sahiwal	65.9	65.1	55.1	52.1	59.6	100	100	100	100	100	100	98.1	100	100	99.5	100	100	96.2	-	98.7
Bahawalnagar	53.5	49.4	37.4	33.6	43.5	100	100	99.5	100	99.9	100	100	100	100	100	100	100	-	100	100
D.G. Khan	46.6	31.3	31.6	21.5	32.3	100	100	100	100	100	100	100	98.9	97.5	99.1	100	100	100	100	100
Rajanpur	62.7	49.0	8.2	-	40.0	-	-	-	-	-	100	100	100	100	100	-	-	-	-	-
Average	62.4	47.2	37.3	46.8	47.8	100	99.8	99.9	100	99.9	99.5	99.4	97.8	98.2	98.7	100	99.9	97.1	99.9	99.2

Table 15. Results of susceptibility tests with fenitrothion in the Punjab Province during 1986-89 against *An. stephensi* and *An. culicifacies*.*

District	Year	*An. stephensi* Adults Tested	*An. stephensi* % Mortality Range	*An. stephensi* % Mortality Average	*An. culicifacies* Adults Tested	*An. culicifacies* % Mortality Range	*An. culicifacies* % Mortality Average
1. Lahore	1986	-	-	100	-	-	-
	1987	-	-	100	-	-	-
	1988	279	-	100	-	-	-
	1989	53	-	100	101	-	100
2. Kasur	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	507	-	100	292	-	100
	1989	298	-	100	136	-	100
3. Sheikhupura	1986	-	-	100	-	-	100
	1987	-	-	-	-	-	100
	1988	48	-	100	953	91-100	98.5
	1989	-	-	-	382	-	100
4. Okara	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	163	-	100	-	-	-
	1989	412	-	100	-	-	-
5. Gujranwala	1986	-	-	-	-	-	100
	1987	-	-	-	-	-	-
	1988	-	-	-	-	-	100
	1989	-	-	-	106	-	100
6. Gujrat	1986	-	-	100	-	-	100
	1987	-	-	-	38	-	94.7
	1988	-	-	-	399	84-100	95.9
	1989	15	-	100	234	96-100	98.7
7. Sialkot	1986	-	-	-	-	-	100
	1987	-	-	-	-	-	100
	1988	25	-	100	50	-	100
	1989	-	-	-	50	-	100
8. Rawalpindi	1986	-	-	100	-	-	100
	1987	-	-	-	20	-	100
	1988	20	-	100	35	95-100	97.1
	1989	-	-	-	-	-	-

Table 15. Continued.

District	Year	*An. stephensi* Adults Tested	*An. stephensi* % Mortality Range	*An. stephensi* % Mortality Average	*An. culicifacies* Adults Tested	*An. culicifacies* % Mortality Range	*An. culicifacies* % Mortality Average
9. Attock	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	-	-	-	-	-	-
	1989	-	-	-	-	-	-
10. Jhelum	1986	-	-	-	-	-	-
	1987	-	-	100	-	-	100
	1988	85	-	100	100	-	100
	1989	-	-	-	-	-	-
11. Sargodha	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	-
	1988	69	-	100	25	-	84
	1989	71	-	100	125	98-100	99.2
12. Khushab	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	-
	1988	98	-	100	-	-	-
	1989	100	-	100	-	-	-
13. Mianwali	1986	-	-	-	-	-	-
	1987	-	-	100	-	-	100
	1988	55	93-100	96.4	60	95-100	96.7
	1989	50	-	100	95	-	100
14. Faisalabad	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	272	-	100	250	-	100
	1989	50	-	100	95	-	100
15. Jhang	1986	-	-	100	-	-	100
	1987	383	-	97.6	445	-	98.3
	1988	233	-	100	246	95-100	98.3
	1989	-	-	-	-	-	-
16. Multan	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	114	-	100	329	-	100
	1989	-	-	-	156	-	100
17. Vehari	1986	-	-	-	-	-	100
	1987	-	-	100	-	-	100
	1988	21	-	100	414	92-100	99.5
	1989	90	-	100	115	68-100	85.2

Table 15. Continued.

District	Year	*An. stephensi* Adults Tested	*An. stephensi* % Mortality Range	*An. stephensi* % Mortality Average	*An. culicifacies* Adults Tested	*An. culicifacies* % Mortality Range	*An. culicifacies* % Mortality Average
18. Sahiwal	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	1219	-	100	105	84-100	92.2
	1989	390	-	100	-	-	-
19. Bahawalpur	1986	-	-	-	-	-	100
	1987	-	-	100	-	-	100
	1988	380	-	100	375	-	100
	1989	247	-	100	790	-	100
20. Bahawalnagar	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	234	93-100	99.5	-	-	-
	1989	275	-	100	65	-	100
21. R.Y. Khan	1986	-	-	100	-	-	-
	1987	-	-	100	-	-	100
	1988	255	-	100	101	-	100
	1989	203	-	100	-	-	-
22. D.G. Khan	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	226	-	100	95	-	100
	1989	353	-	100	128	-	100
23. Muzaffargarh	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	-	-	-	-	-	-
	1989	145	-	100	-	-	-
24. Layyah	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	-	-	-	228	-	100
	1989	-	-	-	-	-	-
25. Khanewal	1986	-	-	100	-	-	100
	1987	-	-	100	-	-	100
	1988	20	-	100	193	-	100
	1989	-	-	-	38	-	100
26. Rajanpur	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	-	-	-	-	-	-
	1989	-	-	-	-	-	-

Table 15. Continued.

District	Year	*An. stephensi* Adults Tested	% Mortality Range	% Mortality Average	*An. culicifacies* Adults Tested	% Mortality Range	% Mortality Average
27. T.T. Singh	1986	-	-	-	-	-	-
	1987	-	-	100	-	-	-
	1988	-	-	-	-	-	-
	1989	-	-	-	-	-	-
28. Bhakar	1986	-	-	-	-	-	-
	1987	-	-	-	-	-	-
	1988	49	-	100	-	-	-
	1989	-	-	-	-	-	-

*Compiled from data provided by the Directorate of Health Services, Punjab.

often suggested. The most serious cases of such resistance are those of *An. albimanus* in Central America and *An. sacharovi* in Turkey.

The data on the geographic distribution or malathion resistance in *An. stephensi* in Pakistan show a general correlation with the distribution of cotton cultivation (Fig. 4). In 1984, Pakistan imported some 9,189 metric tons of formulated insecticides, 57.1% of which were organophosphates (Table 16). By 1988 the quantity of insecticides imported had increased 1.77-fold (1) consisting of:

organophosphates	47.86%
Synthetic pyrethroids (and their O-P combinations)	39.29%
chlorinated hydrocarbons	9.69%
carbamates	3.10%

It is understood that cotton crops receive the largest share of these insecticides. The potentially unfavorable effects of such heavy use of insecticides on the susceptibility of mosquitoes must be borne in mind in the planning of mosquito control activities.

Table 16. Types and quantities of insecticides imported in Pakistan during 1984*.

Type	Name	Quantity* (metric tons)	%
Organophosphates	Phorate	2,010.200	
	Disulfoton	515.400	
	Dimethoate	498.800	
	Triazophos	286.360	
	Monocrotophos	282.578	
	Fenitrothion	235.500	
	Diazinon	203.295	
	Parathion methyl	160.185	
	Azinphosmethyl	131.526	
	Malathion	123.500	
	Chlorpyrifos	117.775	
	Other	679.653	

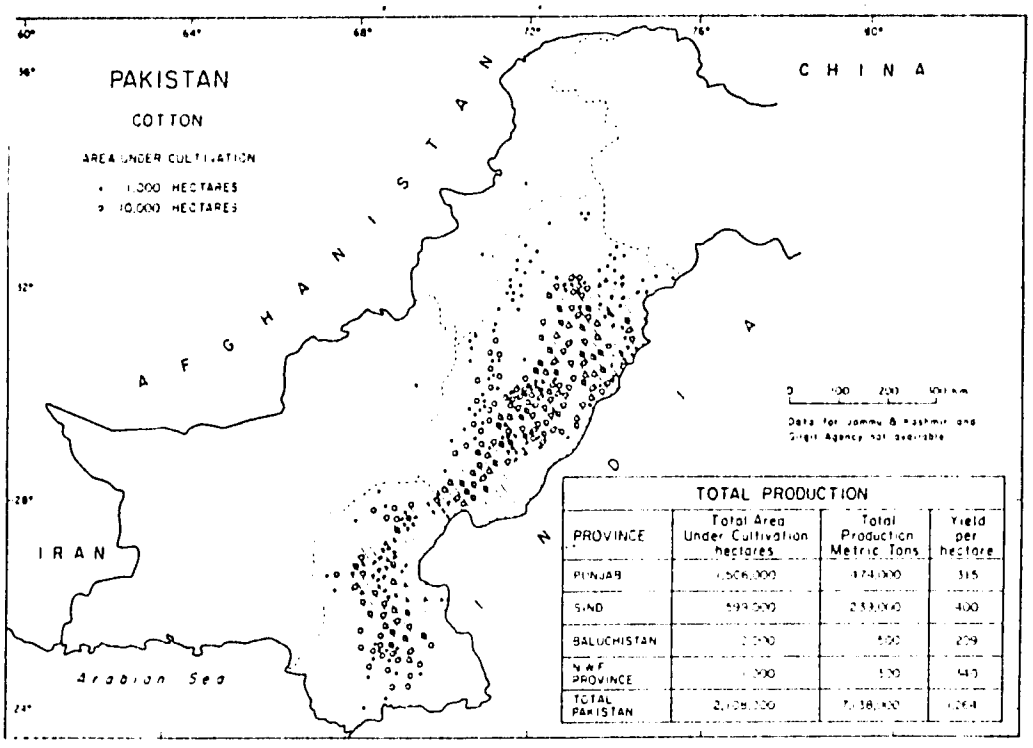

Fig. 4. Geographical distribution of cotton cultivation and malathion resistance in *Anopheles stephensi* in Pakistan, 1986. (Shaded area: malathion resistance; circles and dots, area under cotton cultivation.) From: Georghiou, G. P., The Effect of Agrochemicals on Vector Populations, in *Pesticide Resistance in Arthropods*, Roush, R. and Tabashnik, B. (eds.). Chapman and Hall, New York (in press).

Table 16 Continued.

Type	Name	Quantity* (metric tons)	%
	Total	5,244.752	57.1%
Carbamates	Carbaryl	649.132	
	Cartap	596.812	
	Carbofuran	326.404	
	Other	20.980	
	Total	1,593.328	17.3%
Organochlorines	BHC	522.155	
	Endosulfan	256.710	
	Endrin	219.482	
	DDT	153.302	
	Dieldrin	38.521	
	Heptachlor	9.940	
	Total	1,200.110	13.1%
Pyrethroids	Cypermethin**	627.594	
	Cypermethrin	107.452	
	Fenvalerate	269.515	
	Deltamethrin	71.980	
	Cyfluthrin	38.641	
	Permethrin	35.676	
	Total	1,150.858	12.5%
	GRAND TOTAL	9,189.047	

*Compiled from data provided by Dr. U. K. Baloch, Agricultural Research Council, Islamabad.
**Includes profenofos (organophosphate).

5. DISCUSSION

5.1 In view of the evidence for the presence of high resistance to malathion in *An. stephensi* in certain districts of the country, the question is raised whether malathion is still the most appropriate chemical for use in the malaria control program.

Among the very few available chemicals that are suitable as alternatives to DDT for indoor residual application, malathion has traditionally been considered as the chemical of choice due to its low mammalian toxicity, relatively low cost compared to other, non-chlorinated insecticides, and most importantly, due to the unique mechanism of resistance by which insects may eventually become resistant to it. This mechanism, based on the enzyme carboxylesterase (7), is highly specific for malathion, thus insects becoming resistant to it by this means are not cross-resistant to other insecticides.

The absence of cross-resistance to other insecticides in malathion-resistant *An. stephensi* has been demonstrated with fenitrothion using adult mosquitoes (8) and more recently with a larger series of insecticides tested against larvae (19). The absence of cross-resistance is apparent in the results shown in Table 17.

Table 17. Relative toxicity of insecticides to larvae of malathion-susceptible and -resistant *An stephensi* (19).

Insecticide	Susceptible LC50 (ppm)*	Malathion-Resistant LC50 (ppm)*	Resistance Level
Malathion	0.18	1.6	8.7
Phenthoate	0.045	0.303	6.7
Fenitrothion	0.027	0.035	1.3
Bendiocarb	1.7	1.5	0.91
Carbaryl	0.72	0.515	0.7
Permethrin	0.022	0.011	0.5

*Concentration in parts per million needed to kill 50% of larvae.

Most other organophosphate insecticides (fenitrothion, temephos, chlorpyrifos, fenthion, etc.) are resisted by insects primarily through broadly acting enzymes, referred to as phosphotriesterases (non-specific esterases), which impart cross-resistance at varying levels to several organophosphate insecticides.

It must be pointed out that when anopheline mosquitoes are exposed to multiple types of insecticides, as in agricultural environments, broadly acting resistance mechanisms such as phosphotriesterases and insensitive acetylcholinesterases may be selected, resulting in broad spectrum resistance of (viz. *An. albimanus* in Central America, *An. sacharovi* in S. E. Turkey, etc.). This is an insidious risk which may eventually be expressed in the heavily sprayed agricultural areas of this country as well.

Has resistance in *An. stephensi* in certain districts of Pakistan reached a level at which the use of malathion is no longer efficacious? There is no doubt that significant proportions of *An. stephensi* populations in several ares are malathion-resistant (see section 4.3.3). The question of whether the observed high levels of malaria in a number of localities during the past few years are associated with more rapid re-attainment of critical densities by the vector problems in sprayed areas, although plausible, can be answered only through systematically obtained entomological and epidemiological data from sprayed and non-sprayed areas.

The evidence of high resistance to malathion in *An. stephensi* and the added evidence of a role of this species in the transmission of malaria in the country (14), leads this consultant to the conclusion that a second, alternative insecticide must be made available for use in districts in which resistance is most pronounced. This opinion is based on two principal assumptions:

a. that suppression of *An. stephensi* is essential in malaria control.

Though the vectorial efficiency of this species may be low, it most likely is enhanced by its ability to attain high population densities and,

b. that *An. stephensi* develops resistance more rapidly than *An. culicifacies*.

Past experience with DDT and dieldrin/BHC has shown that *An. stephensi* posseses greater propensity for development of resistance than *An. culicifacies*, the primary vector of malaria in the country. *An. stephensi* has preceded *An. culicifacies* in developing resistance to DDT and dieldrin and has also done so with malathion. The reasons for this difference are unknown; they undoubtedly stem from differences in the ecology, feeding behavior, mobility, dispersal characteristics, and other factors which influence the extent exposure of the species to chemical selection. Though both species have been shown to resist malathion by the same enzyme, carboxylesterase, there may be differences in the initial frequency of the enzyme in natural populations of each species.

Regardless of the reasons for these differences, a change in the susceptibility of field populations of *An. stephensi* can serve as an indicator of selection in progress which may also affect, albeit more slowly, the sympatric *An. culicifacies*. Fortunately, the latter continues to exhibit susceptibility to malathion: resistance tests in 14 districts of Punjab in which *An. stephensi* is highly malathion-resistant have produced almost complete mortality in *An. culicifacies*, e. g. 99.5% (1986), 99.4% (1987), 97.8% (1988), and 98.2% (1989) (see Table 14).

5.2 Indicators of Resistance in *An stephensi*

There are several lines of evidence for high level, genetic resistance to malathion in natural populations of *An. stephensi*. Some of this evidence is direct, while other is derived deductively from the available data.

5.2.1 A larger proportion of adult mosquitoes survive the WHO susceptibility test than previously.

Since 1983 there has been a generally consistent pattern of increased survival of mosquitoes in the WHO susceptibility test. This "diagnostic test" is designed to kill all susceptible individuals. Thus, the number of survivors

indicates the frequency of resistant mosquitoes in a population. In districts of the Punjab for which susceptibility data are available since 1981, the average mortality in the WHO test has dropped steadily from 87% in 1981 to 40% in 1988 (Fig. 3). The 1988/89 results from some districts show considerably lower mortality than in previous years (Table 7). Thus,

		Avg.	Range
Rajanpur	(1988)	8.2%	0-25%
Lahore	(1988)	24.0%	16-33%
Jhang	(1988)	27.9%	13-40%
D. G. Khan	(1989)	21.5%	0-41%

At such low frequency of susceptibles, there must exist in the population a significantly high proportion of heterozygous and homozygous resistant individuals. By applying the Hardy-Weinberg formula ($p^2 + 2pq + q^2 = 1$) to the Lahore data, the following results are obtained:

$$\underline{S} \text{ gene frequency} = \sqrt{24} = 0.49$$

$$\underline{R} \text{ gene frequency} = 1 - \underline{S} = \underline{0.51}$$

$$1.00$$

thus, the genotypic frequencies are:

$$\underline{SS} = 24$$

$$\underline{RS} = 50$$

$$\underline{RR} = 26$$

Clearly, a 26% frequency of homozygous resistant individuals (+ 50% heterzygous resistant) is high enough to be cause for concern.

5.2.2 Survival is due to inheritable, genetic resistance

It may be asked whether the mosquitoes surviving the WHO test do so because they are simply more vigorous, due to nutritional or other, non-genetic reasons.

The genetic basis of malathion resistance in *An. stephensi* is beyond doubt: it has been studied and confirmed in laboratory experiments following further selection and "purification" of resistance to homozygosity (7, 19).

Evidence obtained from field populations (Javed I. Malik, Lahore DOMC Entomologist) is consistent with genetic inheritance of resistance: survivors of WHO tests were allowed to oviposit in the laboratory, their offspring reared to the adult stage and then tested by the same method. The tests showed that resistance had been inherited by the progeny and that a higher percentage of the progeny than of the parents survived the treatment (Table 18).

Table 18. Confirmation of resistance by comparison of malathion susceptibility of field-collected (parental) *An. stephensi* and of the F-1 progeny of their survivors tested in W.H.O. tubes (4% malathion, 1 hour exp.).

		Parental		F1	
District	Year	No. Tested	% Kill	No. Tested	% Kill
Lahore	1986	377	42.7	210	49.8
	1987	609	40.1	196	50.1
	1988	333	24.0	166	32.5
Kasur	1986	276	57.0	298	54.0
	1987	774	57.2	702	49.1
	1988	126	34.9	115	19.1
Faisalabad	1986	713	67.0	559	48.5
	1987	101	40.5	52	34.6
	1988	176	31.8	104	44.2
Sahiwal	1986	205	67.6	194	43.9
	1987	124	54.3	135	40.7
	1988	392	51.5	414	41.3

Table 18. Continued.

District	Year	Parental No. Tested	Parental % Kill	F1 No. Tested	F1 % Kill
Bahawalnagar	1986	333	60.6	270	10.6
	1987	-	-	-	-
	1988	101	35.6	191	14.6
Sargodha	1986	251	63.0	149	62.0
Khushab	1986	155	57.6	52	71.2
Jhang	1987	456	60.3	537	20.1
Rajanpur	1987	110	54.5	240	33.3
Okara	1988	152	32.2	50	56.0
Vehari	1988	94	44.6	107	14.0
Bhakar	1988	26	69.2	50	48.0
Layyah	1988	101	66.3	50	40.0

Yearly Average Kill for All Districts:

	Parental	F-1
1986	59.4	48.6
1987	53.0	38.0
1988	43.4	34.4

5.2.3 Can survivors of the WHO test also survive the field dosage of malathion?

Since there is clear evidence of increasing survival rate in the WHO test as described in 5.2.1 above, the resistance gene must undoubtedly confer some effective protection, otherwise there would never have been a selection pressure to raise the frequency of this gene.

It is not known whether only resistant RR individuals, or heterozygotes RS as well, are able to survive the full dose of malathion applied. Since there is almost complete suppression of numbers of mosquitoes found inside houses immediately post-treatment, it is likely that at least the heterozygotes are killed. As the insecticide residue ages, newly arriving RR and RS individuals are able

to survive, and so they are selected preferentially over susceptible SS individuals. As the frequency of the resistance gene rises in successive generations there is also a corresponding increase in the numbers of mosquitoes surviving the WHO test.

5.3 Fenitrothion as a substitute for malathion

It is evident from numerous susceptibility tests conducted throughout the country that the toxicity of fenitrothion to *An. stephensi* and *An. culicifacies* is unaffected by the presence of malathion resistance (Table 14). The Pakistan Malaria Control Program has had previous experience in the use of fenitrothion, thus phasing this insecticide into the control program should not present insurmountable difficulties.

It is highly important that the transition to fenitrothion be gradual and that it be made only where there is very clear evidence of substantial resistance to malathion (see Recommendations). Where still effective, malathion should continue to be used, in view of its distinct advantages (see 5.1). The criteria for change must include consideration of (a) the WHO susceptibility test results; (b) increases in malaria incidence; (c) safety; (d) post-treatment mosquito counts; and (e) affordability.

The area deserving consideration for treatment with fenitrothion consists of those districts of the Punjab Province in which the *An. stephensi* mortality rate in WHO tests conducted during the last two consecutive years has averaged 50% or less. The districts qualifying on the basis of this criterion alone are listed in Table 12. When the additional criterion of increased malaria incidence is also applied, the areas qualifying for fenitrothion use are reduced further.

6. RECOMMENDATIONS

6.1. The insecticide fenitrothion may be introduced selectively in the Malaria Control Program on a district by district basis where there is clear evidence that high malathion resistance in *An stephensi* is prevalent.

6.2. The criteria for replacement of malathion will be (a) 50% or less average mortality of *An. stephensi* in susceptibility tests conducted over a defined area during the last two years according to the standard WHO procedure, and (b) an unacceptably high level of malaria transmission relative to the norm for the area.

6.3. The replacement of malathion by fenitrothion in 1990 should be considered only for districts of the Punjab Province, in view of their ability to conduct spray operations with relative efficiency.

6.4 The introduction of fenitrothion should be accompanied by research, to be conducted in Pakistan or in foreign laboratories, in order to determine through accelerated selection pressure on representative colonies of *An. stephensi* and *An. culicifacies*, the impact of exposure to fenitrothion on (a) the speed of development of resistance and (b) the spectrum of cross-resistance.

6.5 A third line of chemicals, suitable for indoor use, should be identified and included in the susceptibility tests conducted in the districts that are under fenitrothion treatment. Candidates for consideration in this category are the organophosphate pirimiphos methyl (Actellic), and a pyrethroid (lambda cyhalothrin). Malathion should continue to be included in the susceptibility tests and should be returned to operational use in fenitrothion localities when these tests show re-attainment of adequate malathion susceptibility.

6.6 It is strongly recommended that a detailed field trial on the efficacy of pirimiphos-methyl and of lambda cyhalothrin be conducted during 1990 or 1991 by the the NIMRT.

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