

Odum, E. P. and Odum, H. T. 1959. Fundamentals of ecology. 2nd ed. Philadelphia, Saunders. xvii+546 pp.

Snedecor, G. W. and Cochran, W. G. 1967. Statistical methods. 6th ed. Ames, Iowa State Univ. Press. xvi+593 pp.

1266 9

THE PRESENT STATUS OF INSECTICIDE RESISTANCE IN VECTOR ANOPHELINE MOSQUITOES IN IRAN, 1974¹

B. JANBAKHSI, N. ESHGHY AND A. V. MANOUCHEHRI

Vector Control Unit, Department of Environmental Health, School of Public Health and Institute of Public Health Research, University of Teheran, P.O. Box 1310, Teheran, Iran

ABSTRACT. Susceptibility levels of adult vector anopheline mosquitoes in Iran were studied in field surveys during 1970-1974. Tests were carried out by WHO standard methods.

The development of physiological resistance to DDT and dieldrin in *A. stephensi* is probably the most serious technical problem encountered in the malaria eradication campaigns in southern Iran. Resistance to DDT was observed in 1957 and to dieldrin in 1960. In later tests throughout the country resistance to both insecticides was confirmed. In southern Iran malathion was applied since 1968, but no resistance was observed up to this date.

A. fluviatilis and *A. superpictus* are still sus-

ceptible to DDT, despite widespread residual spraying. Tests showed no resistance to dieldrin.

After DDT applications for 10-14 years tolerance in *A. maculipennis* was confirmed in the Caspian littoral, in northwest Azarbaijan, and in the central plain of Isfahan.

Studies on *A. sacharovi* showed that this species is resistant to DDT in Moghanshahr, Azarbaijan province in the northwest and tolerant in the Izeh area of Khuzistan province in the southwest. This species is susceptible to dieldrin and malathion.

Resistance of *A. culicifacies* to DDT and susceptibility to dieldrin and malathion were shown through tests in Baluchestan in May 1973.

INTRODUCTION

Insecticide resistance in arthropods has created technical problems in many parts of the world. In Iran, *Anopheles stephensi* Liston has developed resistance to DDT and dieldrin (Mofidi and Samimi 1960), and *A. culicifacies* Giles and *A. sacharovi* Favr. to DDT (Zaini and Manouchehri 1973).

As the emergence of insecticide resistance has great impact on malaria control, an evaluation of the susceptibility level of anopheline vectors has been regularly carried out by members of the Vector Control Unit, Department of Environmental

Health, School of Public Health and Institute of Public Health Research.

The known malaria vectors in Iran are *A. stephensi*, *A. fluviatilis* James, *A. culicifacies* and *A. dithali* Patton in the southern part of the country and *A. maculipennis* Meigen in the northern part. *A. superpictus* Grassi is widely distributed, except on the Caspian littoral and the coast of the Oman Sea and Perisan Gulf, and *A. sacharovi* is relatively scattered in the northwest, central and southwest parts of the country.

MATERIAL AND METHODS

The method used in testing was that developed by the World Health Organization for evaluating the susceptibility of a field population of adult anophelines WHO (1970). Paper impregnated with DDT in Risella oil at concentrations of

¹ This study was supported in part by the funds of the School of Public Health and Institute of Public Health Research, University of Teheran, and partly by the Public Health Research Project of the Ministry of Health and Plan Organization.

0.5, 1.0, 2.0 and 4.0%, malathion-impregnated paper at concentrations of 0.5, 3.2 and 5.0% and dieldrin-impregnated paper at concentrations of 0.2, 0.4, 0.8, 1.6 and 4.0% were provided by WHO. For the controls, paper impregnated with Risella oil alone was used. All observed mortality was corrected by Abbott's formula when necessary. LC₅₀'s were estimated by plotting the dosage-mortality lines.

Since 1968, all areas south of the Zagros mountain range went under a malathion and DDT spraying combination. In the regions where *A. stephensi* is the sole vector, only malathion spraying was done, while in the areas where *A. stephensi* is associated with other vectors such as *A. fluviatilis*, *A. thali* and *A. superpictus*, one or two rounds of malathion followed by one or two rounds of DDT were performed.

RESULTS AND DISCUSSION

A. stephensi. Before the development of resistance, the LC₅₀ of DDT was 0.9% to 1.1% and 0.08% to 0.32% for dieldrin. At the present time, *A. stephensi* is resistant to both DDT and dieldrin.

The average mortality obtained with exposure to 4% DDT for 1 hr was between zero and 31% in the villages of Gawshakhi and Chelow in the Mamasani and Minab areas, respectively, of southern Iran. Prolonged exposure to 4% DDT for 4 hr in the village of Gawshakhi did not give more than 13% mortality.

Susceptibility tests with 4% dieldrin papers and 1 hr exposure showed 20.3%, 36.9% and 87.3% mortality in the villages of Dehmir, Tolkharaiki and Chelow in the Iranshahr, Borazjan and Minab areas, respectively, of southern Iran in 1970-74.

There is no evidence at present of development of resistance to malathion. The test on 3.2% malathion paper showed 100% mortality for 1 hr exposure and the LC₅₀ was 0.81% in the village of Chelow (Minab) before the application of malathion in the south of Iran in 1964 (Shahgudian et al. 1965).

According to recent records obtained from different localities in Iran during the years 1970-74, *A. stephensi* was found susceptible to malathion in areas where spraying with this insecticide is in progress (Eshghy et al. 1970). Tests made in March 1971 in the village of Chelow in the Minab area, where malathion spraying had been done for 17 rounds, indicated 100% mortality with 3.2% malathion paper and 1 hr exposure. In tests performed in 1973 in several villages in southern Iran, the mortality with 3.2% malathion paper and 1 hr exposure was between 94% and 100%.

With regard to the 0.1% and 1.0% (OMS-43) papers, the range of mortality after 1 hr exposure, followed by a 24-hour recovery period, was observed in the villages of Bakesh-dodangeh and Shirspary (in the Mamasani and Kazeroun areas of southern Iran) to be between 0.7% to 100% and 5.8% to 100% respectively.

A. fluviatilis. The base-line values of LC₅₀ for DDT and dieldrin were 0.36% and 0.15% respectively, being performed in the unsprayed village of Selbiz in the Kazeroun area in October 1960 (Eshghy and Ghiasseddin 1966).

Susceptibility tests carried out on *A. fluviatilis* obtained 100% mortality for 1 hr exposure to 3.2% malathion paper, and the LC₅₀ was 0.81% in the village of Chelow in the Minab area before the application of malathion in October 1964 (Shahgudian et al. 1965). Since 1950, despite several residual applications of DDT and subsequently of dieldrin and malathion, this species remained susceptible to these insecticides. LC₅₀ values of 1.1% DDT and 0.14% dieldrin were found in the village of Chelow (Minab) in February 1971. The mortality to 5% malathion paper after 0.5 hr exposure followed by a 24 hr recovery period was 100% in the village of Chelow (Minab) in February 1971.

A. superpictus. The LC₅₀ of DDT in the unsprayed village of Hadjiabad in the Sabzevar area in northeastern Iran was 0.72% in November 1955. In susceptibil-

ity tests performed on *A. superpictus* in the untreated villages of Beheshtabad and Dopolan at Ardal in Isfahan province, the LC₅₀ of DDT was 0.68% and the LC₅₀ of dieldrin 0.1% in August 1957 (Mofidi and Samimi 1960).

DDT has been applied as an adulticide for 15–20 years and, subsequently, dieldrin and malathion were used on the southern slopes and foothills of the Zagros range. *A. superpictus* has remained susceptible to these insecticides.

In treated areas, the LC₅₀ of DDT was found to be 1.15% in Junaghan (Shakr-kord) in August 1970 and 1.3% in Piran-shahr Rezaich in July 1971. Studies showed the LC₅₀ of DDT to be between 0.8–1.5% in DDT-treated areas. The LC₅₀ of dieldrin was observed to be between 0.18–0.19% in Saghez and Gazaneh (Sanandaj) in August 1971.

A. maculipennis. In order to obtain the base-line LC₅₀ levels of *A. maculipennis*, tests of adults for DDT susceptibility were carried out in the unsprayed village of Ateshgah in Isfahan province in October 1955, and the LC₅₀ of DDT was found to be 1.6%. In following susceptibility tests conducted on *A. maculipennis* in Isfahan city along the Zayanderoud River where DDT spray treatment had not been applied, the LC₅₀ of DDT was observed to be 2.6% in September 1956. Tests performed in the untreated village of Beheshtabad in the Ardal area of the Isfahan region showed the LC₅₀ of DDT to be 1.45% in August 1957 (Mofidi et al. 1958).

In the village of Baghkoumeh, which had been sprayed for 4 years, the LC₅₀ of DDT was observed to be 0.8% in September 1955. Tests were also carried out in September 1956 in the villages of Baghkoumeh and Sahlavan (Isfahan area), which had been sprayed for 5 consecutive years with DDT, and the LC₅₀ of DDT obtained was 3.2–3.6% and of dieldrin 0.2%.

DDT tolerance increased greatly in autumn, when fat bodies were observed in *A. maculipennis*. In the unsprayed city of

Isfahan, the LC₅₀ of DDT increased from 1% in August to 6% in October 1957.

Tests made in 1970–72 in the provinces of Isfahan, Khorassan, Mazandaran, Guilan and Azerbaijan in the central, north-eastern, north and northwestern parts of the country, showed the LC₅₀ of DDT to lie between 1.8–3.9%. The LC₅₀ of DDT was found in several villages in Isfahan province to be between 2.8–3.7% in August 1970, and 2.1–3.8% in July 1971. In tests performed in the provinces of Mazandaran and Guilan in the Caspian littoral, the LC₅₀ of DDT was found to be between 2.1 to 3.4% in July–August 1970. The LC₅₀ of DDT in the Azerbaijan area, northwestern Iran, was between 2.3–3.6% in August 1970 and the LC₅₀ of DDT in several villages in Khorassan province, northeastern Iran, was between 2.1–2.2% in July 1972.

The LC₅₀ of dieldrin in the villages of Ahmadabad and Siahcheshmeh in the provinces of Isfahan and Azerbaijan was found to be 0.13% and 0.31% respectively, in July 1971. Also, the LC₅₀ of dieldrin in several villages in Mazandaran and Guilan provinces was between 0.12–0.18% in August 1972 (Eshghy et al. 1970, Manouchehri et al. 1972, and Zaini et al. 1971).

A comparison between areas that had been sprayed for several years with DDT showed that *A. maculipennis* has not yet developed resistance to DDT and is susceptible to dieldrin. This species showed tolerance to DDT in Isfahan, Guilan and Mazandaran.

A. sacharovi. Susceptibility tests carried out in early September 1957, in the village of Dopolan, in the unsprayed area of Ardal in Isfahan province, revealed that the LC₅₀ of DDT and dieldrin for adult *A. sacharovi* was about 0.5% and 0.1% respectively (Mofidi and Samimi 1960).

The LC₅₀ of DDT in the unsprayed village of Ghantarghalou in the Shiraz area was 1.1% in September 1956, and complete mortality was obtained with 4.0% DDT. It should be remembered that the surrounding villages of the Ghan-

Table 1. Results of DDT susceptibility tests on *A. sacharovi* adults in Khuzestan Province, S.W. Iran, 1957-1973.

Area and Location	Date	Spraying Cycles	Exposure Time-hrs	Cont.	% Mortality after 24 hours					LC50
					0.25	0.5	1.0	2.0	4.0	
Izeh	07/47	4x DDT	1	14 (108)	41 (108)	68 (108)	82 (108)	100 (36)	0.6%	
"	08/57	"	1	9 (108)	50 (108)	72 (108)	82 (108)	..	0.5%	
"	10/57	"	1	9 (147)	..	71 (159)	86 (165)	98 (194)	0.7%	
Takab-Bandan Izeh	07/69	4x DDT 2x Mal	1	0 (154)	8.4 (95)	43.5 (150)	68.5 (156)	90.6 (166)	1.1%	
"	06/70	9x DDT 2x Mal	1	0 (225)	5.5 (199)	36.6 (202)	61.9 (213)	93.2 (222)	1.4%	
Bardboran Izeh	06/71	10x DDT 3x Mal	2	0 (100)	34.3 (99)	42.2 (97)	72.5 (102)	97 (101)	..	
Takab-Bandan Izeh	06/71	"	1	0 (201)	13.4 (146)	32.5 (200)	60.4 (197)	93.5 (203)	1.4%	
Bardboran Izeh	06/72	12x DDT 4x Mal	1	0 (100)	2 (100)	4 (100)	31 (100)	68 (100)	3.6%	
"	06/73	"	1	0 (199)	5.6 (198)	10.6 (198)	31 (202)	53.7 (199)	4 %	
"	06/73	"	2	0 (173)	60 (173)	90 (176)	..	

Figures in parentheses represent the numbers of mosquitoes tested at each concentration.
Mal=Malathion

Table 2. Results of DDT susceptibility tests on *A. culicifacies* adults in Baluchestan Province, S.E.-Iran, 1959-1973.

Area and Location	Date	Spraying Cycles	Exposure Time-hrs	% Mortality after 24 hours						LC50
				Cont.	0.25	0.5	1.0	2.0	4.0	
Dadın, Zabol	10/59	not sprayed	1	7 (13)	..	94 (24)	100 (26)	100 (45)
Daman & Piratch, Iranshahr	07/63		1	2.7 (74)	30 (53)	42 (64)	..	96 (71)	100 (30)	0.55
Kheirabad & Koottch Iranshahr	07/63		1	0 (12)	100 (3)	100 (19)	..
Kheirabad, Iranshahr	04/73	22 X DDT 6 X DL 8 X Mal	1	0 (33)	..	0 (14)	0 (13)	8.8 (34)	38.7 (31)	>4
Said Abad, Iranshahr	04/73	"	1	0 (26)	..	0 (16)	0 (14)	4.7 (21)	46.1 (26)	>4
Hit, Nick-Shahr	05/73	23 X DDT 6 X DL 8 X Mal	1	2.1 (93)	13.6 (95)	25.2 (91)	43.3 (90)	>4
Hit, Nick-Shahr	"	"	4	0 (91)	70.1 (87)	..

Figures in parentheses represent the numbers of mosquitoes tested at each concentration.
Mal=Malathion DL=Dieldrin

targhalou area have been sprayed with DDT for the last 4 years. Tests performed in December 1956, in the villages of Kalduzakh and Bone-Esfandiari in the Izeh area in Khuzestan province, on *A. sacharovi* which were in the pre-hibernation period and all with fat bodies, gave an LC₅₀ of 1.5%. In similar tests during the activity season on blood-fed *A. sacharovi* conducted in the same village in July, August and October 1957, the LC₅₀ of DDT was 0.75%, 0.6% and 0.7% respectively.

In the villages of Polabguineh and Dadin-Bala in the Kazeroun region of southern Iran, which had been treated with DDT for 5 years, the mortality on 4.0% DDT paper was 35-40%. These tests were made in early October 1956, when the weather was still hot and there was no sign of hibernation, (Brown and Pal 1971).

Tests made in August 1970, in the village of Mohammad-abad in the Moghan-shahr area (northwest) indicated zero mortality on 4.0% DDT paper, and when the test exposure period was extended to 4 hours, the mortality was observed to be nil on 4.0% DDT paper. Other tests made in the villages of Diktash and Goltapeh in the Moghan-shahr area in July 1971, showed zero mortality with 1 and 4-hr exposure to 4.0% DDT paper. It may be concluded that strong DDT resistance had developed in *A. sacharovi* in the Moghan-shahr district in the northwest of the country where DDT residual house spraying has been applied for the last 11 years by the Malaria Eradication Program.

Tests carried out in June 1970-73, in the villages of Takabbandan and Bordboran in the Izeh area, southwestern Iran, showed an LC₅₀ of DDT ranging from 1.4% to 3.6%. Tests on samples of *A. sacharovi* revealed that DDT resistance had arisen in the Izeh area, where DDT residual spraying had been applied for 12 years and malathion for 3 years. (Table 1.)

The adult LC₅₀ of dieldrin in the villages of Mohammed-Bagloo in August 1970 and Diktash in July 1971 in the

Moghan-shahr area was found to be 0.45% and 0.43% respectively. The LC₅₀ of dieldrin was found to be 0.2% in Takabbandan in the Izeh area in June 1971.

The mortality on 3.2% and 5.0% malathion papers with 1 hr exposure was 100% at Bordboran.

A. culicifacies. After the application of DDT in 1959, the density of *A. culicifacies* decreased sharply. Susceptibility tests carried out in 1963 showed that the LC₅₀ was 0.5%.

In 1964 and 1965, Baluchestan was sprayed with dieldrin, and from 1967 to 1973, with DDT and malathion, 2 cycles per year with each insecticide. During this period of 10 years, the density of *A. culicifacies* was so negligible that it was not possible to perform susceptibility tests. By April and May of 1973, the density of *A. culicifacies* in Hit village increased to about 500 per unsprayed dwelling and 5 per sprayed dwelling. Susceptibility tests carried out at Hit, Said-abad and Kheir-abad showed that *A. culicifacies* is resistant to DDT (Zaini and Manouchehri 1973). The percentage of mortality after 1 hr exposure and 24 hr recovery was between 39 and 46%. (Table 2). When the time of exposure was increased to 4 hr the percent mortality after 24 hr recovery was 70%. Susceptibility tests carried out with dieldrin and malathion showed that *A. culicifacies* is susceptible to both insecticides and the LC₅₀'s were 0.05% and 0.55% respectively.

References

- Brown, A. W. A. and Pal, R. 1971. Insecticide resistance in arthropods. World Health Organization, Geneva, Monograph Series No. 38:95-143.
- Eshghy, N. and Ghiasseddin, M. 1966. Preliminary biological studies of *A. fluviatilis* in Iran. Sch. Pub. Hlth. & Inst. Pub. Hlth. Res. Teheran, Iran. Scient. Publ. 1527:29-32.
- Eshghy, N., Zaini, A., Manouchehri, A. V. and Javadian, E. 1970. Studies of the susceptibility level of anopheline mosquitoes to insecticides in different areas of Iran. Sch. Pub. Hlth. & Inst. Hlth. Res. Teheran, Iran. Scient. Publ. 1816: 2-4.

- Manouchehri, A. V., Eshghy, N., Seyedi Rashti, M. A., Zaini, A. and Mottaghi, M. 1972. Studies on the susceptibility level of vector anopheline mosquitoes in Iran. Sch. Pub. Hlth. & Inst. Pub. Hlth. Res. Teheran, Iran. Scient. Publ. 1874:2-24.
- Mofidi, Ch., Samimi, B., Eshghy, N. and Ghiaseddin, M. 1958. Further studies of anopheline susceptibility to insecticides in Iran: results of Busvine and Nash method. Inst. Parasit. & Malariol. Teheran, Iran, Publ. 585:1-7.
- Mofidi, Ch. and Samimi, B. 1960. Resistance of *A. stephensi* to dieldrin in Iran. Inst. Parasit. & Malariol. Teheran, Iran. Publ. 650:3-4.
- Shahgudian, E. R., Eshghy, N., Zaini, A. and Seyedi Rashti, M. A. 1965. Report on the preliminary entomological studies of a malathion pilot project conducted in the shahrestan of Bandar Abbas and Minab, south Iran. Sch. Pub. Hlth. & Inst. Pub. Hlth. Res. Teheran, Iran. Scient. Publ. 1370:3-4.
- W.H.O. 1970. Insecticide resistance and vector control. 17th report of the W.H.O. Expert Committee on Insecticides. Tech. Rep. Ser. 443:47-65.
- Zaini, A., Eshghy, N., Seyedi Rashti, M. A., Javadian, E. and Saebi, E. 1971. Susceptibility of anopheline mosquitoes to insecticides in different areas of Iran. Sch. Pub. Hlth. & Inst. Pub. Hlth. Res. Teheran, Iran. Scient. Publ. 1845:2-3.
- Zaini, A. and Manouchehri, A. V. 1973. Preliminary notes on the development of DDT resistance in *Anopheles culicifacies* in Baluchestan province, southern Iran. Iranian J. Pub. Hlth. 3:156-162.