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ASSESSMENT OF THE RESIDUAL ACTIVITY OF DIELDRIN USING BIOASSAY IN THE FIELD IN IRAN

(Abstracts from a report)

by

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Introduction

During the period June to November 1958 a WHO team, ATME 2,[1] in co-operation with the Institute of Malariology and Parasitology of Teheran, carried out field trials of bioassays of dieldrin residues in the Kazeroon area of south-western Iran.

This area had previously been sprayed with DDT, but in 1957 A. stephensi developed resistance to this insecticide. Thus a change was made to dieldrin 50% w.d.p., which was used for the first time in the spring of 1958.

The tests started 107 days after the spraying of dieldrin and continued until 193 days after spraying. The dosages referred to in the report are the amounts of active ingredient which theoretically should be placed on each square metre of sprayed surface.

1. Description of area

Kazeroon lies about 150 km west of Shiraz in south-western Iran. The climate is arid. The temperatures in Kazeroon range from 40°C to 45°C during the summer but drop to 24°C in the autumn. The mean relative humidity was generally below 50% except in villages along flowing rivers, where it was up to 75%.

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[1] The WHO team consisted of Dr Mohan Rao, Mr J. W. Armstrong, sanitarian, and Messrs K. Thymakis, J. Petrides and P. Caprari, laboratory technicians. The work was co-ordinated by Dr Ch. Mofidi, Director of the Institute of Malariology, and by Dr G. Houel, Senior WHO Malaria Adviser in Iran.

The villages in which bioassays were carried out were in a valley surrounded by high mountains. The roads were very rough, bumpy and dusty, which made transporting mosquitos difficult. The average number of houses per village was 45, stables 50 and thatched huts 12.

The houses and stables were usually of mud bricks and covered with roofs of reed grass alone or reed grass woven as mats placed on top of palm or wooden beams. The stable walls were rough, but houses were usually plastered with smooth mud or limewashed. The thatched huts were built of reed grass secured with sticks.

Fifty-one houses, 41 stables, 11 grass matting surfaces, seven wooden and four palm beams, and six lime-plastered surfaces were bioassayed. They were distributed in 11 villages.

2. Materials and methods

(a) Test insects. Adult females of A. stephensi were used in all bioassay tests. During July and August these were collected wild in two villages 70 km from Kazeroon, but in September and October mosquitos from the Insectarium established at Kazeroon were used.

(b) Equipment. For bioassay tests both WHO conical chambers and WHO plastic Petri dish type chambers were used.

Aspirators made of glass tubing 0.9 mm in diameter were found to give less injury to the insects when transferring them from cages to the test chambers than those of larger diameters. Due to high temperatures, bad roads, and the long distances over which it was necessary to transport mosquitos, high mortalities of mosquitos occurred at first. In order to minimize these a special transportation box was designed. This had a wooden frame of internal dimensions 35 cm x 35 cm x 35 cm. The wooden frame was closed on all sides except one with pressed fibre boards. On the open side a sliding door of the same material was fitted. In another fibre board of 35 sq. cm, a number of holes of about 1.5 cm diameter were bored, and this board was placed in the box at about a height of 8 cm from the bottom. It was so placed that it could be slid out. Below this perforated board, a tin pan of 30 cm x 30 cm x 5 cm was kept and a towel saturated with water was kept in the tin pan; on all sides except on the bottom and the sliding door, four holes of about

0.75 cm were bored. During the period of collection of mosquitos, transportation and bioassay, a wet cotton towel was wrapped round the cage containing mosquitos. This cage, in which the mosquitos were kept, was 25 cm x 25 cm x 25 cm and made of steel wires of about 0.5 cm and was enclosed in a sack of nylon mosquito netting. When the temperature rose above 30°C, the sliding door was raised to about 8-10 cm and firmly secured with sticks. Three of these boxes fitted securely in the back part of a station wagon. No mortalities in controls were observed even when the mosquitos were transported up to 150 km. For the recovery of exposed mosquitos plain, unwaxed paper cups (10 DV Mono Containers Ltd) were used.

(c) Method of bioassay. The spraymen wrote the date of spraying and the dosage on the walls of every sprayed house, thus facilitating the selection of sprayed houses. Houses were visited and if possible those with smooth walls selected; if these were not available, walls without holes were chosen, and which were not too rough. The plastic Petri dish type chamber can be used only on smooth walls, while the conical type can be used on uneven and rough surfaces. It was necessary however to choose places where the conical chambers could be secured without leaving gaps between the cones and the wall. The conical chambers were used also on reed grass or grass matting roofs.[1]

After suitable surfaces for bioassay had been selected, the conical chambers or plastic Petri dishes were placed on the wall surfaces at different heights and secured firmly with thumb tacks. Any gaps left between the chamber and the wall were plugged with cotton wool. Sometimes, on more uneven walls, two or even three

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[1] [Editor's note] It would be interesting to know how many spots were selected for testing on the same day in one room (or on one type of surface) and how many replicate tests were performed on each spot.

Both points are important for the evaluation of bioassay tests. Since in any general spraying programme there are large unintentional variations in the dosage applied to different spots, the selection of too few spots for testing may lead to large experimental errors in the attempt to assay the residual toxicity of the nominal dosage. Likewise, the use of a small number of mosquitos on a spot may give a distorted result in view of the normal variability of insecticide tolerance within the population.

layers of plastic sponge strips were attached to the bottom of the conical chambers. Adhesive tape (Scotch tape) was found useless for securing them to the rough walls.

For control tests (in which the mosquitos do not come in contact with sprayed surfaces) two layers of wrapping paper were placed on the wall, held with thumb tacks, and the conical chamber was placed on top of the paper. Usually the chambers used in these "control" tests were kept separately and were used exclusively for that purpose. The time of exposure in all tests and controls was 30 minutes and about ten mosquitos at a time were transferred into each chamber.

During the period of exposure, the paper cups were prepared by covering them with mosquito netting of adequate size, cutting a hole of about 3 cm in the middle of the netting and putting a rubber band to hold the netting.

After exposure the mosquitos were removed with the collecting tube sent with the WHO kit. It was quite easy, with a little experience, to collect all the mosquitos from the conical chamber. The technique used was to suck the collected mosquitos very gently, quickly remove the bent end and transfer the mosquitos into the recovery cage. A wet cotton pad was immediately put on the opening in the mosquito netting and the pad firmly held with two rubber bands running vertically across the paper cup. This technique enabled us to transport the recovery cages over long distances to Kazeroon where they were usually kept for 24 hours after exposure.

The method recommended by WHO was used for plastic Petri dish chambers. The method, in short, was to slide a red cardboard sheet under the dish and remove the dish from the wall, then inverting a paper cup, of which the bottom was cut off and replaced with mosquito netting, over the cardboard slide and slowly removing the slide without letting the mosquitos escape. The paper cup was then secured with two rubber bands.

The residual toxicities of three dosages of dieldrin (250 mg, 500 mg and 1 g per m^2) and of two dosages of BHC (500 mg and 1 g per m^2) applied to various surfaces were bioassayed by the team.

It was not always possible to select dark places for the tests, but it was observed that diffuse sunlight did not affect the behaviour of <u>A. stephensi</u>. Direct sunlight, however, irritated the mosquitos in the cones, making them fly incessantly. It was observed that the light which irritated the mosquitos in the cones did not disturb them in transportation cages.

The bioassay chambers were always wiped with a cotton swab soaked in 95% alcohol. When the plastic sponge got dirty it was removed, the chamber washed with soap and water and then dipped in pure gasoline, followed by another washing in soap and water.

Recovery cages were kept on tables, isolated from everything else, and the legs were in containers of water. This was to prevent the mosquitos from being eaten by ants.

3. Results and discussion

The results are divided into three groups, according to the dosage of dieldrin: villages sprayed with 0.5 g/m^2 (the usual amount aimed at in Iran); villages sprayed with 1 g/m^2; and villages sprayed with 0.25 g/m^2. Only a few experimental villages were sprayed with these last two dosages.

(a) Results obtained in villages treated with dieldrin at 0.5 g/m^2

These results are shown in table 1. They are arranged in two groups - mud surfaces and others (as described above). In all cases where the control mortality exceeded 5% the percentage mortalities are corrected by Abbott's formula. It should be remembered that the mean number of mosquitos introduced into each bioassay chamber was ten; thus the mean number of tests is approximately one-tenth of the total number of mosquitos given for each series of tests.

It can be seen that the percentage mortalities in these tests varied considerably over the period of the experiment (107 to 193 days after spraying). The activity of the residues did not decline slowly with time as might be expected.

However, the mortalities in general show a pattern of decline, and at about 164 days after spraying are below 50% and usually below the 25% level.[1]

In order to show this apparent pattern of decline in biological activity of dieldrin, the per cent. mortalities obtained in the tests done at 100 days after spraying and presented in the table are grouped at ten-day and twenty-day intervals. From these results it can be clearly observed that this pattern of the declining biological activity of dieldrin was gradual from 100 to 150 days, then quite steep to 170 days, and reduced still further at 193 days after spraying, when the tests were discontinued. Grouped at ten-day intervals the trend of mortalities was uneven; when grouped at twenty-day intervals it was smoother and revealed the basic pattern of the declining biological activity of dieldrin. The results were not statisically analysed. It was felt that better results would be obtained if the same houses could be revisited. This was done four times in one house, three times in another and twice in two other houses. The same spots were not retested at the successive visits to these houses.[2]

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[1] [Editor's note] It is possible that, rather than a gradual decline in bioassay mortalities, one might expect the rate to remain consistently at 100% over a longer or shorter period and then to fall away rather rapidly once the residual toxicities fell within the critical range for the test insect. That this did not happen is only partly explained by the long interval which elapsed between spraying and the start of the tests. The results obtained might lead the reader to suspect that the A. stephensi of the Kazeroon area possessed, in some individuals, a gene for resistance to dieldrin.

We are informed that Dr Rao performed susceptibility tests by the WHO method, concurrently with his bioassay tests. In various tests on wild-caught A. stephensi run from July to September 1958 the LC_{50} was found to range from about 0.09 to 0.16% dieldrin. At the highest test-concentration recorded, 0.4%, there were two survivors out of 205 mosquitos tested.

[2] [Editor's note] In any bioassay tests performed over an extended period in the field there are many variable factors, each producing a risk of experimental error. Among such factors in the Kazeroon area are the roughness of the wall surfaces, the degree of openness (or closure) of the rooms, the wear of surfaces at different heights, and the competence of different spraymen. All these factors have probably influenced the results at each spot tested. The procedure of greatest value, therefore, would have been the serializing of tests at a number of marked spots, preferably at many such spots in a few selected rooms. Above everything such an approach might have revealed the order of variation from spot to spot in the dosage applied, and would in any case have yielded results of good comparability. Tests performed at different spots should not be regarded as replicates.

The results of these "follow-up" tests are given in table 2. From these results it might be inferred that: (i) there was a definite pattern in the decrease of mortalities of the bioassays inasmuch as the mortalities are mostly below 20% at six months after spraying; (ii) the four villages, situated in different areas, in which we carried out the repeated bioassay tests showed a similar and consistent pattern of the decline of biological activity below the 20% level; and (iii) there was a considerable variability of results at different spots.

The results of tests done with conical chambers and Petri dishes in the same room (table 5) do not show much difference between the two methods.

(b) Results obtained in villages treated with dieldrin at 1 g/m^2 and at 0.25 g/m^2

Fewer tests were done in villages sprayed with 1 g/m^2 since only three villages were sprayed, for experimental purposes, with this dosage.

The results are presented in table 3. The average mortalities are grouped at ten-day intervals as before. From the results, here again one can see the same pattern of the sharp decline of the biological activity of dieldrin between 150 and 175 days after spraying, falling below 20% mortality on mud surfaces.

Tests were done in only one village in which the spraying was made at 0.25 g/m^2. The results presented in table 4 show only a slight activity of dieldrin on the 137th day after spraying and, paradoxically, a higher mortality ten days later. This is probably due to an experimental error.

4. Summary

(a) A series of bioassay tests was carried out in field conditions in the Kazeroon area, Iran, using an improvised "transportation box" which enabled the transport of mosquitos without any mortalities in the controls.

(b) In the four villages where bioassays were done in the same room, we observed a varying pattern of decline in biological activity of dieldrin up to 165 days after spraying and a sharp decline thereafter, giving mortalities below 20% at 180 days after spraying. From observations in other villages where the tests were not done at regular intervals, the same pattern was also evident. This pattern, evident on both mud surfaces and non-mud surfaces, was obvious not only in villages sprayed with 0.5 g/m^2, where most of the bioassay tests were done, but also in villages where a concentration of 1 g/m^2 was used.

(c) It is probable that experimental huts would serve the purpose of evaluating the duration of activity of an insecticide much better than ordinary houses, although this might not reflect the normal course of events in sprayed houses.

Graphs showing the temperature and relative humidity variations might be necessary in interpreting the results of bioassays.

An attempt should be made to correlate bioassay results (mortalities) with the mortalities occurring among free mosquitos which visit a sprayed room. This might well be done with the aid of sprayed and unsprayed experimental huts fitted with window traps, where, besides the bioassays, numbers of mosquitos could be released and their behaviour and mortalities observed.

Any bioassay tests done should be carried out in conjunction with other entomological surveys like anopheline densities, behaviour, etc. so as to give an overall and comprehensive picture of the status of the vector in a given area.

5. Acknowledgements

The team wishes to express its thanks to Dr Ch. Mofidi, Director of the Malaria Institute, Teheran, for his interest and help in giving facilities to carry out these investigations; to Dr G. Houel, Senior WHO Adviser in Iran, for his active help in many ways; to the members of the Institute of Malariology, especially those in Kazeroon, and to the United Nations Technical Assistance Board, Iran, and WHO Regional Office, EMRO, for much help.

TABLE 1. ANALYSIS OF BIOASSAY TESTS AT 10-DAY INTERVALS OF (A) MUD AND (B) NON-MUD SURFACES TREATED WITH 0.5 g/m^2 OF DIELDRIN IN VILLAGES NEAR KAZEROON, IRAN, IN 1958 USING Anopheles stephensi

	Number of days after spraying	Number of villages in which tests were done	Control			Bioassay of mud surfaces			
			Number dead	Total number used	Per cent. mortality	Number dead	Total number used	Per cent. mortality	Corrected per cent. mortality
A	101-110	1	8	31	22.0	53	56	95.0	93.6
	111-120	2	1	176	0.1	201	272	73.9	73.9
	121-130	1	0	107	0.0	152	152	100.0	100.0
	131-140	2	1	185	0.5	322	492	64.8	64.8
	141-150	3	4	119	3.3	264	444	59.4	59.4
	151-160	4	23	196	11.7	524	692	75.1	71.7
	161-170	7	4	301	1.3	373	1276	29.2	29.2
	171-180	1	0	65	0.0	61	389	15.6	15.6
	181-190	1	0	29	0.0	29	236	12.3	12.3
	191-200	1	6	48	12.5	16	98	16.3	4.3
B	111-120	1	0	80	0.0	104	142	73.2	73.2
	141-150	3	4	119	3.3	45	77	58.4	58.4
	151-160	4	23	196	11.7	42	78	53.8	47.7
	161-170	7	4	301	1.3	166	423	39.2	39.2
	171-180	1	0	65	0.0	27	53	50.9	50.9

TABLE 2. ANALYSIS OF BIOASSAY TESTS OF MUD SURFACES IN REVISITED HOUSES IN VILLAGES NEAR KAZEROON, IRAN, SPRAYED WITH 0.5 g/m^2 OF DIELDRIN IN 1958, USING *Anopheles stephensi*

Number of days after spraying	House	Control			Bioassay of mud surfaces			
		Number dead	Total number used	Per cent. mortality	Number dead	Total number used	Per cent. mortality	Corrected per cent. mortality
107	I	8	31	22.0	53	56	95.0	93.6
111	I	0	78	0.0	87	99	87.8	87.8
123	I	0	107	0.0	142	142	100.0	100.0
164	I	0	24	0.0	8	176	4.5	4.5
116	II	1	98	1.0	114	173	65.8	65.8
133	II	1	103	0.9	216	244	88.5	88.5
164	II	0	24	0.0	41	194	21.1	21.1
137	III	0	82	0.0	106	253	41.5	41.5
141	III	2	34	5.8	73	106	68.8	66.9
165	III	0	21	0.0	13	138	9.4	9.4
152	IV	8	56	14.2	222	261	85.0	82.4
180	IV	0	41	0.0	15	205	7.3	7.3
162	V	3	35	8.5	101	111	90.9	88.9
184	V	0	43	0.0	29	236	12.3	12.3

TABLE 3. ANALYSIS OF RESULTS OF BIOASSAY TESTS OF SURFACES SPRAYED WITH 1 g/m^2 OF DIELDRIN IN VILLAGES NEAR KAZEROON, IRAN, IN 1958, USING *Anopheles stephensi*

A - mud surfaces; B - non-mud surfaces

	Number of days after spraying	Number of villages in which tests were done	Control			Bioassay			
			Number dead	Total number used	Per cent. mortality	Number dead	Total number used	Per cent. mortality	Corrected per cent. mortality
A	140-149	1	1	201	0.5	103	224	45.9	45.9
	170-179	3	0	177	0.0	240	1280	19.0	19.0
B	140-149	1	1	201	0.5	209	211	99.0	99.0
	170-179	3	0	177	0.0	90	183	49.1	49.1

TABLE 4. ANALYSIS OF RESULTS OF BIOASSAY TESTS OF MUD WALLS SPRAYED WITH 0.25 g/m^2 OF DIELDRIN IN A VILLAGE NEAR KAZEROON, IRAN, IN 1958, USING *Anopheles stephensi*

Number of days after spraying	Number of villages in which tests were done	Control			Bioassay of mud walls			
		Number dead	Total number used	Per cent. mortality	Number dead	Total number used	Per cent. mortality	Corrected per cent. mortality
130-139 (137)	1	2	131	1.5	58	189	30.7	29.3
140-149 (147)	1	0	30	0.0	62	121	51.2	51.2

TABLE 5. ANALYSIS OF BIOASSAY TESTS USING CONICAL AND PETRI DISH TYPE CHAMBERS AND *Anopheles stephensi* IN VILLAGES NEAR KAZEROON, IRAN, SPRAYED WITH DIFFERENT DOSAGES OF DIELDRIN AND BHC

No. of days after spraying	Insecticide and dosage g/m2	Nature of surface bioassayed	CONICAL CHAMBER							PETRI DISH CHAMBER						
			Control			Bioassay				Control			Bioassay			
			No. dead	Total no. used	% mortality	No. dead	Total no. used	% mortality	% corrected mortality	No. dead	Total no. used	% mortality	No. dead	Total no. used	% mortality	% corrected mortality
111	DLD 0.5	Mud	0	38	0.0	50	58	87.0	87.0	0	40	0.0	37	41	90.0	90.0
		Lime plaster	0	43	0.0	46	66	69.7	69.7	0	39	0.0	58	76	69.7	69.7
116	DLD 0.5	Mud	1	53	1.9	65	93	68.5	68.5	0	45	0.0	49	80	61.2	61.2
123	DLD 0.5	Mud	0	51	0.0	78	78	100.0	100.0	0	56	0.0	74	74	100.0	100.0
133	DLD 0.5	Mud	1	54	0.5	151	171	88.3	88.3	0	49	0.0	65	73	89.0	89.0
137	DLD 0.25	Mud house	2	66	3.3	14	58	23.0	23.0	0	65	0.0	27	56	50.9	50.9
		Mud stable	2	66	3.3	5	42	7.6	7.6	0	65	0.0	12	37	32.0	32.0
143	DLD 0.25	Mud	0	20	0.0	22	40	55.0	55.0	0	10	0.0	13	35	37.0	37.0
143	DLD 1.0	Mud	0	57	0.0	14	56	25.0	25.0	0	63	0.0	32	46	69.5	69.5
		Lime plaster	0	57	0.0	70	71	99.0	99.0	0	63	0.0	41	41	100.0	100.0
147	DLD 1.0	Mud	1	36	2.7	35	88	44.0	44.0	0	45	0.0	22	42	52.0	52.0
		Lime plaster	1	36	2.7	58	60	97.0	97.0	0	45	0.0	39	39	100.0	100.0
65	BHC 0.5	Mud	0	53	0.0	37	98	37.7	37.7	0	30	0.0	16	72	22.2	22.2
			0	49	0.0	41	115	35.0	35.0	0	24	0.0	4	33	12.0	12.0