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# Field Experiment with the Use of Gamma-BHC

## for the Control of DLN and DDT Resistant A. Stephensi Mysorensis in the Khesht Area, Kazeroun, South of Iran

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## Introduction

After the development of resistance in *A. stephensi mysorensis* (the main malaria vector of Southern Iran) to DLN (and consequently to other cyclodiene compounds) in 1960-61, spraying with this group of insecticides was automatically discontinued in the whole of Southern Iran, including the area where, after withdrawal of DDT in 1957 because of the development of resistance in *A. stephensi*, BHC (Lindane) was the only insecticide used for the control of malaria vectors. Following the suggestion put forward by the Thirteenth WHO Expert Committee on Insecticides, 1963, about the possible use of BHC against DLN resistant vectors, and in order to determine the value of this insecticide in the interruption of malaria transmission in an area where its main malaria vector(*A. stephensi*) is resistant to DLN, an extended

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field trial was carried out during 1963 in Khesht area, Kazeroun, southern Iran.

## Area of Operation

### Geography

The experimental area (Khesht) is a rather isolated plain 565 m. above sea level, with an area of about 145 sq. km. It is a part of the central foothills of the southern slope of the Zagros Mountains, located southeast of Kazeroun about 150 kms. from the Persian Gulf. A permanent river, the Shahpur, crosses the area from east to west and serves as the main source of water for the area, producing at the same time the main anopheline breeding places.

### Climate

The project area is part of central southern Iran with a sub-tropical climate. It has a long hot summer and a short mild winter. The maximum and minimum temperatures range from 15° to 46° and from 6° to 30° C. respectively (Graph 1). The annual rainfall is not considerable in this area (average of 150-200 mm. recorded over a number of years). The main rainy season covers the period from December to March.

### Agriculture

The main crop in this area is dates. Crops such as wheat and barley are cultivated either by using river water or by dry farming. In some villages, cultivation of rice and tobacco is practised.

### Demography

The experimental area consisted of 32 villages with a population of about 11,173 people (2132 families), giving an average density of 76.8 people per sq. km. of surface, 5.25 persons per family and 349 persons (66.6 families) per village.

The total number of buildings in these 32 villages is estimated as 11,897, with an average sprayable surface of about 78 sq. m. per building. An average of 27 % of the total number of buildings is of a temporary or semi-temporary nature (huts, summer huts, Koomeh, etc.); the remaining 73 % are permanent buildings (rooms, stables, stores, etc.). The percentage of absorbent surfaces (mud, plaster, lime-washed, etc.) is estimated as 50-55 % against 45-50 % for non-absorbent surfaces (wood, rush-mat, thatch, palm-date leaves, etc.). The average sprayable surface per capita, per family and per village is estimated as 83 sq. m., 440 sq. m. and 29,000 sq. m. respectively. Details of these data are given in Table 1.

## Population Movement

The movement of population is not considerable in this area. A small group of tribes crosses the northern portion of the area on their way to and from their winter quarters, and stays in the area for only two or three days. Another type of local and temporary movement is the immigration of laborers from neighboring areas during the harvesting of crops and date-picking and packing. Also a small group of shepherds enters the area during summer and stays there for 2-3 months to collect fodder for their buffaloes.

## Malarometric Background

The experimental area is located in the tropical zone of southern Iran. The central part of this zone is usually characterized by a long single-peaked transmission season of about 8 months (April-December). In some years, the transmission season takes the shape of a double-peaked curve, with the higher peak in April-July and the lower in September-December, separated by 4 to 6 weeks of low vector activity. The main malaria vector of the area is *A. stephensi mysorensis* which covers, more or less, the above-mentioned single or double-peaked transmission season. *A. fluviatilis*, and to some extent *A. superpictus*, are present in the area, usually with a very low density

*Table 1 — Details of the specific data of the experimental villages (sprayed with BHC).*

| Serial No. | Name of village | Population | No. of families | No. of structures | estimated sprayable surf. (1000 m2) |
|---|---|---|---|---|---|
| 1 | Booraki-Olya | 600 | 120 | 477 | 60 |
| 2 | Booraki Fereydun | 55 | 10 | 59 | 5 |
| 3 | Shahbaz-Khani | 376 | 66 | 259 | 26 |
| 4 | Tchaghsayi | 324 | 62 | 246 | 22 |
| 5 | Booraki-Sofla (F) | 122 | 22 | 94 | 6 |
| 6 | Tchiti | 122 | 21 | 67 | 5 |
| 7 | Boneh-Ali | 78 | 14 | 123 | 12 |
| 8 | Mahal-el-din | 282 | 50 | 351 | 28 |
| 9 | Booraki-Vosta | 615 | 123 | 656 | 66 |
| 10 | Bezin | 333 | 69 | 245 | 12 |
| 11 | Booraki Sofla | 448 | 83 | 406 | 27 |
| 12 | Khajeh-Jamali | 262 | 52 | 206 | 16 |
| 13 | Tchar-Borj | 221 | 40 | 273 | 22 |
| 14 | Shah-Davood | 180 | 31 | 207 | 16 |
| 15 | Mafi-Abad | 165 | 23 | 124 | 15 |
| 16 | Boneh-Seyd Reza | 385 | 81 | 492 | 56 |
| 17 | Hassan Abad | 101 | 18 | 81 | 8 |
| 18 | Koreh-Lishoo | 39 | 8 | 29 | 2 |
| 19 | Borji-Seyed | 343 | 64 | 356 | 30 |
| 20 | Kamankashi | 310 | 59 | 302 | 24 |
| 21 | Tanghi | 406 | 68 | 505 | 36 |
| 22 | Konar-Takhteh | 1165 | 224 | 1478 | 120 |
| 23 | Tchoroom | 148 | 30 | 154 | 11 |
| 24 | Roodak | 134 | 24 | 60 | 10 |
| 25 | Moghissabad | 15 | 2 | 30 | 2 |
| 26 | Tal-espeed | 95 | 18 | 90 | 6 |
| 27 | I-mani | 359 | 62 | 442 | 35 |
| 28 | Jafar-jeen | 233 | 79 | 503 | 30 |
| 29 | Gooriga | 490 | 86 | 551 | 35 |
| 30 | Khesht | 2082 | 381 | 2595 | 150 |
| 31 | Rostam Abad | 270 | 47 | 228 | 20 |
| 32 | Nemat Abad | 201 | 36 | 209 | 17 |
| Total | 32 villages | 11173 | 2132 | 11897 | 930 |

and mainly during the first (May-June) or second (October-November) part of the year. They do not play an important role in transmitting and maintaining malaria in this area.

Among the other anopheline species (not yet incriminated as vectors) present in the area are *A. d'thali*, *A. turkudi*, *A. sergenti*, *A. apoci*, and *A. pulcherrimus.*

The intensity of malaria during its static stage (before 1950), was known to be hyperendemic or highly mesoendemic in the experimental area, with both *P. falciparum* and *P. vivax* as prevalent parasite species (in some years the former and in some years the latter was the predominant species of parasite). Epidemiological studies of malaria in the experimental area have shown that malaria transmitted by *A. stephensi mysorensis* is of an unstable character (stability index estimated as 0.33). Studies carried out in the experimental area during September 1962 and July 1963 (prior to spraying) have given the following estimated figures for *A. stephensi mysorensis*:

| | | |
|---|---|---|
| a — | Anoph. density (No. of Anoph. per individual) | 58 to 281.6 |
| b — | Gametocyte rate | 4.5 % to 10.8 % |
| c — | Percentages of potentially dangerous females | 2 % to 10.2 % |
| d — | Human Blood Ratio | 5.5 % to 17 % |
| e — | Man biting habit | a = 0.027-0.08 |
| f — | No. of bites received by an individual in one night | ma = 1.97-8 |
| g — | The proportion of infective mosquitos (all parasite species) | h = 0.0037-0.0232 |
| h — | Entomological infection potential | EIP = 7.04-45.96 |
| i — | Reproduction Rate | z = 1.7-6.25 |
| j — | Number of transmissions | 0.39-2.07 per 24 hours |

## Operational History

The expe.imental area had been sprayed irregularly with 5 cycles of DDT (2 grs. per sq. m., one cycle per annum) during the period of malaria control (1951-57). After the development of resistance to DDT in *A. stephensi mysorensis* in 1957 and the replacing of DDT by DLN (1958), the experimental area (Kheshi) and neighboring areas (Zeidoun and Mahour-milati) were sprayed with BHC (500 mg. per sq. m., two cycles per annum). As a result the control of *A. stephensi* and other malaria vectors in the area and to a great extent, the interruption of malaria transmission and the reduction of the global parasite rate, were successful for the period of 1959-1960. During 1961. after the development of resistance to DLN in *A. stephensi mysorensis*, another cycle of BHC was used. As effective control of *A. stephensi* was not achieved (except for a short period), spraying with this insecticide was completely halted. During the autumn of 1961 and the spring of 1962, the whole of one village and a few huts in another village of the project area, were sprayed with Fenthion (1-2 grs. base per sq. m.) on an experimental basis.

The experimental area has been under regular monthly parasite survey (in selected villages or in the entire area) since 1958. The results of these surveys are presented in Table 2, which shows the gradual reduction of A.P.I. from a higher figure in 1957 (a year of epidemic) first to 5.1 % (1958) and then to 0.8 % (1959), 0.5 % (1960) and 0.6 % (1961) due to the application of BHC. As the table shows, after suspension of BHC spraying the API increased, first to 4.2 % (1962) and later to 13.2 % (up to August 1963).

*Table 2 — The results of parasite surveys carried out in Kheshi area over the period 1958-1963.*

| Year | Spraying status | No. of slides collected | A.P.I. | P. R. | Mx. monthly Peak month |
|---|---|---|---|---|---|
| 1958 | BHC (500/sq.m.) | 4881 | 5.1 | 13.5 | July |
| 1959 | BHC " | 4900 | 0.8 | 2.1 | July |
| 1960 | BHC " | 4198 | 0.5 | 1.9 | October |
| 1961 | BHC " | 4337 | 0.6 | 1.4 | July - Aug. |
| 1962 | Unsprayed | 3523 | 4.2 | 9.7 | Oct. - Nov. |
| 1963 (first part) | " | 9370 | 13.2 | 27 | August |
| 1963 (second part) | Exp. BHC (1000 mg/sq.m) | 13994 | 5.3 | 16.2 | Sept. |

## Material and Methods

### Spraying

32 villages in the area with an approximate surface of 930,000 sq. m. were sprayed with Lindane (Hortex — 50 % w.d.p., 1000 mg. base per sq. m.) The spraying took place from 6-25 July 1963 (20 working days), after the building and setting up of new summer huts and temporary shelters. 99 % of the total buildings and all known natural outdoor shelters were included in the spraying.

## Evaluation

### Parasitological Evaluation

The entire population of the experimental area (11,173 persons) underwent a regular monthly parasite survey with the following considerations:

a — Collection of blood samples from all children below 2 years.

b — Collection of blood from all fever and suspected cases and from previous positive cases.

c — Mass blood collection in 3 pilot villages.

d — Collection of blood from 20 % of the remaining population, selected by random sampling.

e — Investigation of all positive cases found after spraying. These cases were administered a single dose of chloroquine (600 mg. base, adult dose).

### Entomological Evaluation

For this purpose, the three villages with the highest mosquito potentiality were selected and subjected to regular entomological survey on a ten-day interval basis. In every village at least six fixed catching stations of different types (room, stable, summer hut) were selected, and four artificial shelter pits were dug and regularly checked. The following techniques were used in the survey:

a — Estimating vector density (per shelter, per individual of indoor and outdoor mosquito population).

b — Determination of larval density.

c — Night man biting rate (outdoor-indoor).

d — Window-trap and floor-sheet collection and estimate of overnight mortality and mosquito survival rate.

e — Use of test-hut by releasing laboratory bred *A. stephensi mysorensis* into the hut and estimating overnight mortality.

f — Survey of artificial shelter pits and other outdoor resting places.

g — Determination of the vectors' level of susceptibility to the insecticides.

h — Determination of the value of some important epidemiological factors (EIP, ma, etc.).

i — Other studies such as age determination, blood preference, etc. (as far as was necessary and possible.).

## Biological Evaluation

Bio-assay tests were carried out in one pilot village on a seven-day or biweekly interval basis. Various surfaces (mud, plaster, tent, wood and rush-mat, etc.) were included, and different time exposures were used.

*Table 3 — Susceptibility level of A. stephensi mysorensis to chlorinated insectisides in the Kheshi area, using WHO test Kit. (time exposure 60 minutes-number of Anoph. tested in brackets).*

**DDT**

| Year & Month | Temp. O. C. | R. H. | % mortalities in following concentrations | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1 | 2 | 4 | Control |
| Nov. 1960 | 24 | 65 | 7(41) | 14(112) | 17(108) | 32(106) | 2(106) |
| July 1961 | 35 | 50 | — | — | 6(119) | 24(109) | 0(110) |
| July 1962 | 37 | 45 | — | 8(152) | 11(150) | 29(150) | 2(101) |
| July 1963 | 35 | 30 | — | 0(42) | 11(106) | 23(72) | 0(69) |
| Oct. 1963 | 29 | 35 | — | — | 12(60) | 24(57) | 0(52) |
| July 1964 | 32 | 40 | 3(100) | 10(100) | 14(100) | 26(100) | 1(100) |
| " " | 32 | 40 | 9(100) | 23(100) | 70(100) | 98(100) | 0(100) |

**DLN**

| Year & Month | Temp. O. C. | R. H. | % mortalities in following concentrations | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.2 | 0.4 | 0.8 | 1.6 | 4 | Control |
| Nov. 1960 | 24 | 65 | 42(125) | 55(120) | 22(117) | 73(114) | 87(117) | 0(127) |
| July 1961 | 35 | 50 | — | — | 2(109) | 4(111) | 14(114) | 0(109) |
| July 1962 | 37 | 45 | — | — | — | 7(150) | 25(150) | 2(100) |
| July 1963 | 35 | 30 | — | — | 0(44) | 6(36) | 26(45) | 0(47) |
| Oct. 1963 | 29 | 65 | — | — | 0(42) | 5(71) | 11(59) | 0(77) |
| July 1964 | 32 | 40 | 0(99) | 0(100) | 0(99) | 0(99) | — | 0(100) |
| " "* | 32 | 40 | 11(100) | 11(100) | 19(100) | 27(100) | — | 0(100) |

* Exposure time = 240 minute.

# Results

## *Entomological Evaluation*

### Susceptibility of vectors

The susceptibility level of *A. stephensi mysorensis* to DDT and DLN (using WHO standard Test Kit) was determined both prior to and after spraying with BHC, and the results are given in Table 3. As the table shows, the susceptibility level of *A. stephensi* to DDT remained more or less the same from 1960 to 1964, showing a mortality rate ranging from 24 % to 32 % in a one-hour exposure to 4 % DDT concentration. These results, in combination with those obtained in a 4-hour exposure period (98 % mortality to

*Table 4 — A. stephensi density (adult & larvae) in the pilot experimental villages (before & after spraying) for the period April 1962-March 1964. (Average monthly figures, based on records at intervals of ten days)*

| Period (Month) | 1963 - 1964 adults per shelter | 1963 - 1964 adults per indiv. | 1963 - 1964 larvae per 10 dips | 1962 - 63 adults per shelter | 1962 - 63 larvae per 10 dips |
|---|---|---|---|---|---|
| April | 1 | 0.1 | 4 | 1.3 | 4 |
| May | 9 | 0.8 | 27.5 | 3.6 | 12 |
| June | 116 | 54.3 | 67 | 2.3 | 76 |
| July (before spray.) | 366 | 146 | 143 | 288 | 130 |
| July (after spray.) | 2 | 0.3 | 45 | — | — |
| August | 2.8 | 0.5 | 36 | 478 | 135 |
| September | 21 | 14.5 | 76 | 201 | 115 |
| October | 47 | 12.5 | 123 | 141 | 125 |
| November | 23 | 6.3 | 99 | 10.5 | 21 |
| December | 8 | 1.7 | 16 | 3 | 8 |
| January | 2.5 | 0.8 | 1 | 1.6 | 1 |
| February | 0 | 0 | 0 | 0.6 | 0 |
| March | 0.3 | 0 | 0 | 0.25 | 0 |

4 % DDT concentration), show that *A. stephensi mysorensis* is resistant (with intermediate intensity) to DDT. In the case of *A. stephensi* and DLN, after the development of resistance in late 1960, the susceptibility remained more or less at the same level up to July 1963 (showing 4-7 % mortality and 14-26 % mortality in a one-hour exposure to 1.6 % and 4 % DLN concentrations). After spraying with BHC in July 1963, the tests performed during October of the same year (2 months later) showed a slight reduction of the susceptibility level (5 % and 11 % mortalities obtained in a one-hour exposure to 1.6 % and 4 % DLN concentrations). Tests performed in the following year (July 1964) showed a greater reduction of the susceptibility level occurring under the BHC selection pressure (showing no mortality in a one-hour exposure to the same concentration). The susceptibility level of *A. stephensi mysorensis* to BHC was not determined prior to spraying, but the tests performed during October 1963 (3 months after spraying with BHC using the Busvine & Nash method) showed a mortality rate of 5.5-8.3 % in a one-hour exposure to 0.005 %-0.02 % BHC concentrations; mortality rates observed in a one-hour exposure to 0.08 % and 0.16 % BHC concentrations were 40 % and 54 % respectively.

### Determination of Anopheline Density

Determination of the indoor density of *A. stephensi*, as well as of the larval density and outdoor collection of anophelines, were carried out according to the schedule outlined on the previous pages; the results are summarized in Tables 4 and 5. These tables show that, following spraying in mid-July, the vector mosquitoes were under control for almost 6-8 weeks, as expressed by adult and larval density of *A. stephensi* and outdoor mosquito population, taking into consideration the percent mortality after 24 hours holding.

### W.T. and Floor Sheet Collections

These techniques were carried out in the pilot villages following the schedule previously described. The results, expressed as values of K (number

*Table 5 — Results of outdoor collection of anophelines (from artificial shelter pits) in the pilot villages, for the period April 1963-March 1964.*
*(number of anophelines collected and their stages-Monthly record)*

| Month | A. stephensi | | | | A. fluviatilis | | | | A. superpictus | | | | % A. stephensi survived after 24 hrs. holding |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Un-fed | blood-fed | gravid | Total | Un-fed | blood-fed | gravid | Total | Un-fed | blood-fed | gravid | |
| April 1963 | 5 | 0 | 1 | 4 | 112 | 4 | 95 | 13 | 6 | 0 | 3 | 3 | 88% |
| May " | 26 | 1 | 21 | 4 | 377 | 31 | 291 | 55 | 28 | 3 | 11 | 14 | 98% |
| June " | 1374 | 479 | 641 | 254 | 358 | 10 | 328 | 20 | 28 | 0 | 22 | 6 | 100% |
| July " | 2814 | 1431 | 1042 | 341 | 16 | 0 | 16 | 0 | 7 | 2 | 4 | 4 | 100% |
| Aug. " | 996 | 706 | 271 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11% |
| Sept. " | 769 | 427 | 325 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42% |
| Oct. " | 115 | 43 | 69 | 3 | 0 | 0 | 0 | 0 | 36 | 5 | 31 | 0 | 98% |
| Nov. " | 160 | 68 | 84 | 8 | 3 | 0 | 3 | 0 | 17 | 4 | 13 | 0 | 95% |
| Dec. " | 9 | 2 | 5 | 2 | 1 | 0 | 1 | 0 | 2 | 0 | 2 | 0 | |
| Jan. 1964 | 3 | 1 | 0 | 2 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | |
| Feb. " | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| March " | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

*Table 6 — Value of K and detailed W.T. and floor sheet collections of A. stephensi mysorensis in the pilot villages for the period April — Dec. 1963. (monthly figures, based on records at ten days intervals)*

| Period (Month) | No. of Anoph. in outleet W. T. | | | | | | | | No. of Anoph. in inlet W.T. | | | | | | | | No. of Anoph. on floor sheet | | | | Value of K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dead | | | | Alive | | | | Dead | | | | Alive | | | | | | | | |
| | Un-fed | blood fed | gravid | Total | Un-fed | blood fed | gravid | Total | Un-fed | blood fed | gravid | Total | Un-fed | blood fed | gravid | Total | Un-fed | bllod fed | gravid | Total | |
| April - May | — | — | 1 | 1 | — | 10 | 5 | 15 | — | — | — | — | — | — | — | — | — | — | — | — | 0.06 |
| June | 2 | 1 | 4 | 7 | 13 | 89 | 2 | 104 | — | — | — | — | 5 | 12 | 1 | 18 | — | — | — | — | 0.06 |
| July (before spray.) | 9 | 4 | 60 | 73 | 36 | 198 | 6 | 240 | 3 | — | 2 | 5 | 20 | 39 | — | 59 | — | — | — | — | 0.23 |
| July (after spray.) | 29 | 19 | 1 | 49 | 7 | 21 | — | 28 | 33 | 20 | — | 53 | 3 | 11 | — | 14 | 173 | 252 | 21 | 446 | 0.96 |
| August | 41 | 23 | 14 | 78 | 3 | 27 | 1 | 31 | 29 | 5 | — | 34 | 37 | 58 | — | 95 | 45 | 47 | 1 | 93 | 0.85 |
| September | — | 12 | 4 | 16 | 33 | 109 | 4 | 146 | 8 | 5 | — | 13 | 16 | 30 | — | 46 | 51 | 51 | — | 102 | 0.45 |
| October | 2 | 14 | 3 | 19 | 9 | 44 | 2 | 55 | 4 | 2 | — | 6 | 6 | 14 | — | 20 | 2 | 6 | — | 8 | 0.33 |
| November | 1 | — | — | 1 | 1 | — | — | 1 | — | — | — | — | 3 | 5 | — | 8 | — | — | — | — | — |
| December | — | — | — | — | — | 1 | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — |

of dead anoph. in W.T., plus number of dead on floor, divided by number of dead on floor plus number of dead and alive in W.T.) are expressed in Table 6. According to these results, the value of K is fully significant (in favor of the insecticide pressure) for a period of almost 6-8 weeks.

## Hut-Test

This method showed an overnight mortality rate for *A. stephensi mysorensis* (released into the experimental hut) of over 75 % for a period of 12 weeks after spraying (Table 7).

## Age Composition of Mosquitoes

Age determination was carried out in the pilot village of Bezin, using the Polovadova-Detinova method. About 2000 *A. stephensi*, were dissected during the period April-December 1963, and a maximum of 5 gonotrophic cycles was observed in the month of November. The results of these studies, expressed as a percentage of potentially dangerous females and the ratio of

*Table 7 — Details of Hut-Test, carried out in pilot villages, after spraying wuth BHC. (records at intervals of 10-14 days)*

| | Number of A. stephensi mysorensis | | | | | | % mortality | |
|---|---|---|---|---|---|---|---|---|
| Days after spray. | Dead in W. T. | Alive in W. T | Dead on floor | Alive in hut | Total collected | Total dead | Test | Control |
| 17 | 45 | 0 | 85 | 0 | 130 | 130 | 100% | 2% |
| 27 | 29 | 0 | 66 | 0 | 95 | 95 | 100% | 7% |
| 34 | 37 | 0 | 113 | 0 | 150 | 150 | 100% | 0 |
| 45 | 42 | 4 | 89 | 0 | 135 | 131 | 97% | 5% |
| 56 | 31 | 4 | 87 | 2 | 124 | 118 | 95% | 5% |
| 67 | 29 | 8 | 99 | 19 | 155 | 128 | 82% | 5% |
| 78 | 28 | 14 | 100 | 28 | 170 | 128 | 75% | 1% |
| 89 | 25 | 11 | 124 | 32 | 192 | 149 | 76% | 4% |
| 100 | 19 | 33 | 90 | 32 | 174 | 109 | 62% | 3% |
| 116 | 14 | 28 | 52 | 82 | 176 | 66 | 37% | 1% |
| 126 | 4 | 28 | 46 | 169 | 247 | 50 | 20% | 0.5% |
| 136 | 1 | 10 | 52 | 203 | 266 | 53 | 20% | 0.5% |
| 147 | 1 | 2 | 44 | 161 | 208 | 45 | 21% | 0 |
| 157 | 1 | 5 | 54 | 211 | 271 | 55 | 20% | 0 |

*Parous/Nulliparous*, are presented in Table 8. As the table shows, the insecticide was reasonably effective in keeping the age of the female vectors below the dangerous age for a period of 5 weeks (completely) or 8 weeks (low percentage). Furthermore the ratio of P:N remained below (50:50) for a period of 8 weeks after spraying.

*Table 8 — Results of age determination of A. stephensi mysorensis in the pilot village (before & after spraying) — April-Dec. 1963.*
*(expressed as percentage of P.D. and ratio of Parous/Nulliparous)*

| Period (Month) | No. of Anoph. dissected | % P. D. | Ratio $\frac{P}{N}$ |
|---|---|---|---|
| April 1963 | 27 | — | 0.59 |
| May " | 75 | — | 0.89 |
| June " | 722 | 6.3 | 0.73 |
| July (before spray.) | 211 | 7.6 | 0.85 |
| July (after spray.) | 46 | 0 | 0 |
| 1 - 10 Aug. 1963 | 122 | 0 | 0.15 |
| 11 - 20 " " | 73 | 0 | 0.17 |
| 21 - 30 " " | 62 | 1.6 | 0.34 |
| September " | 196 | 3.5 | 0.60 |
| October " | 117 | 2.9 | 0.83 |
| November " | 126 | 1.6 | 0.94 |
| December " | 96 | 0 | 0.78 |

## Mathematical Epidemiology

The estimated value of some epidemiological factors was worked out and calculated in the pilot village of Bezin; the results of this work are presented in the Table 9. According to the figures given in this table, no transmission could have occurred during the 6 weeks following spraying. The chance of transmission in the following period was not very high, since it coincided with the end of mosquito activity and of the natural transmission season (December).

### *Biological Evaluation*

Bio-assay tests were performed at 7-10 day intervals on various surfaces

*Table 9 — Estimated value of some epidemiological factors in the village (Bezin), before and after spraying April-Dec. 1963.*

**for A. stephensi mysorensis**

| Period (Month) | Ratio Anoph./Man | ma | EIP | G. R. | estimated number of transmission/day |
|---|---|---|---|---|---|
| April | 0.10 | 0.004 | 0 | 9.4 | 0 |
| May | 0.94 | 0.043 | 0 | 9.6 | 0 |
| June | 4.31 | 0.25 | 1.3 | 12.3 | 0.16 |
| July (before spray.) | 145.7 | 8.35 | 38.8 | 4.5 | 1.7 |
| July (after spray) | 0.3 | 0.017 | 0 | 12 | 0 |
| August | 0.5 | 0.029 | 0.01 | 15 | 0 |
| September | 14.5 | 0.83 | 1.7 | 10.8 | 0.18 |
| October | 12.5 | 0.72 | 2.1 | 4.7 | 0.1 |
| November | 6.3 | 0.38 | 1.1 | 2.4 | 0.03 |
| December | 1.7 | 0.07 | 0 | 1.7 | 0 |

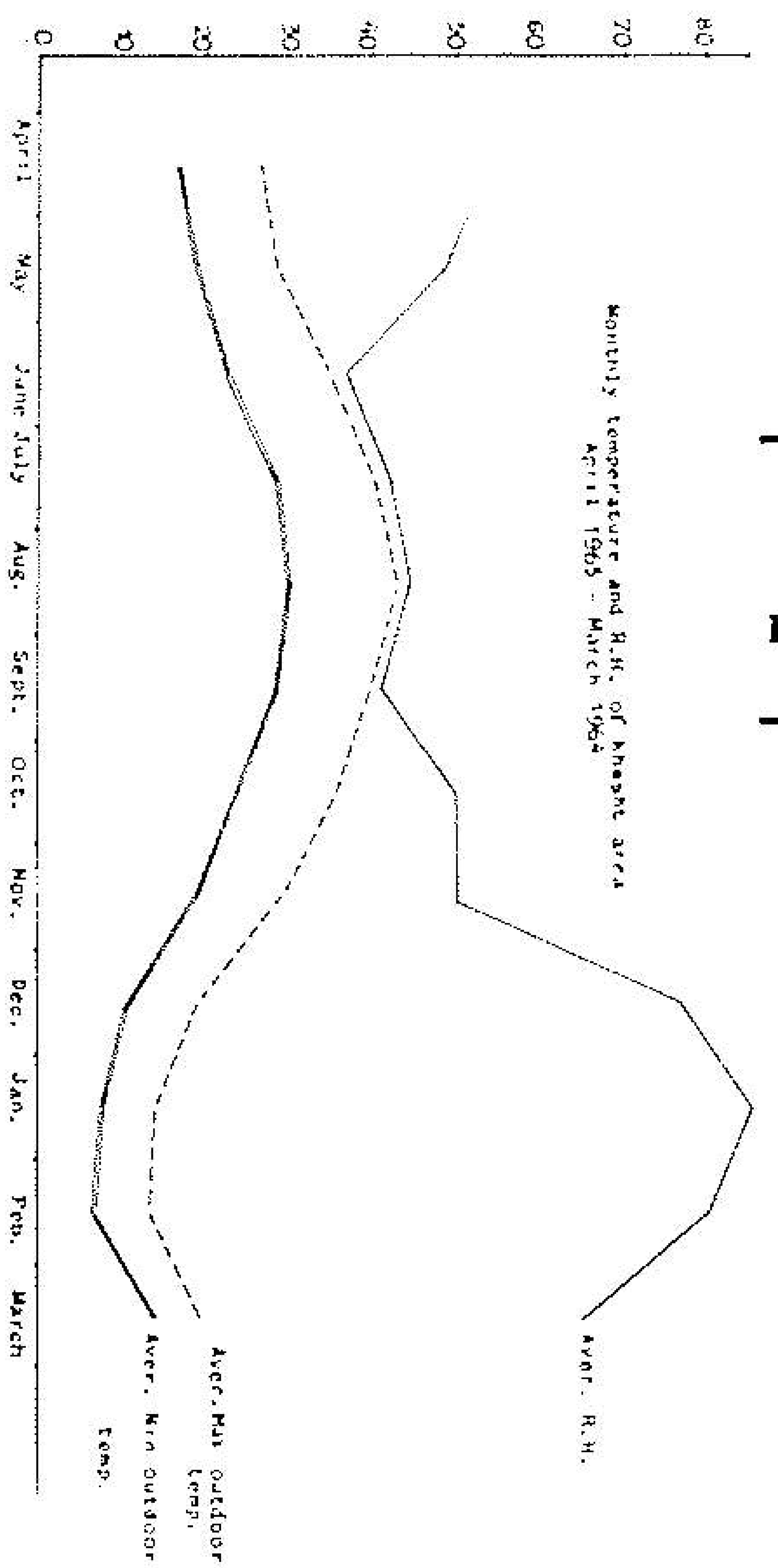
of the pilot village (Bezin), using laboratory bred *A. stephensi mysorensis* resistant to DLN and DDT. The results of these tests are summarized in Table 10. According to these results, the average mortality rate of over 40 % (for all surfaces) in 30-60 minutes exposure time, was only observed 5-6 weeks after spraying. It should be mentioned that the Bio-assay tests carried out on various surfaces sprayed with BHC (1000 mg. base per sq. m.) in the same area during 1959, showed a mortality rate of over 40 % in 30 minutes exposure time (average for all surfaces using laboratory bred *A. stephensi mysorensis* resistant to DDT but susceptible to DLN) for a period of 16-20 weeks.

### *Parasitological Evaluation*

The monthly parasite survey was carried out in the experimental villages, following the program outlined in the previous pages. The results of these surveys, expressed as a monthly parasite rate (all species) for infants (0-2 years) and people of all ages and also as a specific parasite rate (for *P. falciparum*) are presented in Table 11. They are compared with those observed during the previous year (March 1962-March 1963). According to these

*Table 10 — Results of Bio-assay tests carried out on various surfaces sprayed with Gamma BHC (1000 mg/sq. m.) — July-Dec. 1963. (giving percent mortalities of laboratory bred A. stephensi mysorensis)*

| Days after spraying | Temp. O. C. | R. H. | 30 minutes expos. Mud. surf. | 30 minutes expos. non-absorbent surf. | 60 minutes expos. Mud surf. | 60 minutes expos. non-absorbent surf. | 120 minutes expos. Mud surf. | 120 minutes expos. non-absorbent surf. | 240 minutes expos. Mud surf. | 240 minutes expos. non-absorbent surf. | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 32 | 34 | 78 | 100 | — | — | — | — | — | — | 5.8 |
| 11 | 33 | 40 | 50 | 92 | — | — | — | — | — | — | 0 |
| 18 | 35 | 46 | 36 | 71.5 | — | — | — | — | — | — | 0 |
| 22 | 33 | 48 | — | — | 83.5 | — | — | — | — | — | 0 |
| 25 | 34 | 48 | 27.4 | 72.3 | — | — | 89.8 | — | — | — | 0 |
| 32 | 33 | 44 | 23.8 | 51.4 | 52.8 | — | — | — | — | — | 4.2 |
| 39 | 32 | 45 | 21.2 | 44.4 | — | 80.8 | 73.7 | — | — | — | 5.5 |
| 46 | 31 | 45 | 19.2 | 37.2 | — | 66.2 | 68 | — | — | — | 4.2 |
| 55 | 33 | 45 | 13.8 | 24.5 | — | 37 | 45.5 | — | — | — | 3.9 |
| 60 | 30 | 45 | 9.5 | 17.4 | — | — | — | 58.3 | 72.8 | — | 0 |
| 67 | 30 | 44 | 7.8 | 12.3 | — | — | — | — | 69.5 | 88.8 | 4 |
| 74 | 27 | 50 | 5.6 | 10.2 | — | — | — | — | 68.8 | 82.5 | 0 |
| 81 | 26 | 55 | — | — | — | — | — | — | 65 | 78 | 4 |
| 88 | 25 | 55 | — | — | — | — | — | — | 62.2 | 73.9 | 3.3 |
| 107 | 26 | 48 | — | — | — | — | — | — | 61.9 | 70.8 | 3.3 |
| 117 | 24 | 58 | — | — | — | — | — | — | 59.4 | 67.3 | 3 |
| 127 | 22 | 65 | — | — | — | — | — | — | 57.8 | 63.4 | 0 |
| 138 | 21 | 65 | — | — | — | — | — | — | 55.2 | 61.4 | 3.5 |
| 148 | 14 | 70 | — | — | — | — | — | — | 54.8 | 59.2 | 3.3 |



results and in concurrence with data collected on vector potentiality (ma, *EIP*), the transmission of malaria is apparently interrupted or greatly reduced after the completion of spraying in late July, until some time in September or even October. The reduction of the number of malaria cases among adults and particularly infants, and in particular the greater reduction of the number of *P. falciparum* cases among both groups during the period of August-October, support the abovementioned assumption.

## Discussion and Conclusion

It is not surprising that BHC, applied at the recommended rates, should not work effectively against a DLN resistant malaria vector such as *A. stephensi mysorensis* in southern Iran. The only scientific support for the possible use of BHC in the control of DLN resistant mosquitoes is in the results of the susceptibility tests, which show that a DLN resistant strain is up to 800 times less susceptible than a normal strain, while in the case of BHC, difference between resistant and susceptible strains is only 30-50 times.

The present experiment, carried out in the Khesht area with the use of Gamma-BHC (Lindane) at a rate of 1000 mg. base per sq. m., as a residual spray against DLN resistant *A. stephensi mysorensis* showed that the test insecticide, under the prevailing conditions of the experimental area, can reasonably control the natural population of *A. stephensi mysorensis* for a period of about 6 to 8 weeks. This was shown by the application of various entomological techniques such as: determination of *A. stephensi* density, W.T. and floor collection, determination of age composition, estimating some epidemiological factors related to the vector potentiality, and application of biological assessment through Bio-assay tests. During this period, i.e., 6-8 weeks, the transmission of malaria is apparently interrupted or at least reduced to a much lower degree, as is proved by parasitological evaluation which shows a great reduction in the parasite rates for infants and people of all ages, and particularly in the specific parasite rate for *P. falciparum*. Beyond this period, reasonable effectiveness should not be expected from this in-

*Table 11* — *Results of monthly parasite survey of infants and all ages in the experimental area, before and after spraying 1962-1964.*
*(expressed as monthly parasite rate and specific parasite rate of infants and people of all ages)*

| | 1962 - 63 | | | | 1963 - 64 | | | |
|---|---|---|---|---|---|---|---|---|
| | Infant 0-2 years | | All ages | | Infant 0-2 years | | All ages | |
| Period | P. R. | Specific P. R. P. falciparum | P. R. | Specific P. R. P. falciparum | P. R. | Specific P. R. P. falciparum | P. R. | Specific P. R. P. falciparum |
| 21 March - 20 April | 0 | 0 | 0.2 | 0 | 3.2 | 1.3 | 5 | 1.5 |
| 21 April - 20 May | 0 | 0 | 0.9 | 0 | 2.6 | 0.8 | 6.1 | 1.3 |
| 21 May - 20 June | 0.7 | 0.7 | 1.3 | 0.2 | 3.1 | 0.4 | 10.5 | 1 |
| 21 June - 20 July | 4 | 1 | 4.5 | 1.4 | 2.7 | 0.2 | 7.6 | 0.9 |
| 21 July - 20 August | 9 | 3.6 | 13 | 5.2 | 20.5 | 3.5 | 27 | 2.8 |
| 21 Aug. - 20 Sept. | 10.8 | 7.6 | 13 | 9.8 | 4 | 0.1 | 16.2 | 0.3 |
| 21 Sept. - 20 Oct. | 10 | 8.3 | 11.2 | 8.2 | 1.2 | 0 | 4.6 | 0.2 |
| 21 Oct. - 20 Nov. | 12.4 | 4.2 | 17.5 | 11.6 | 0.8 | 0.15 | 4.6 | 1.2 |
| 21 Nov. - 20 Dec. | 2 | 1 | 8.4 | 6.2 | 0.6 | 0.15 | 1.4 | 0.3 |
| 21 Dec. - 20 Jan. | 2.2 | 1.1 | 7.6 | 5.2 | 0.15 | 0 | 0.7 | 0 |
| 21 Jan. - 20 Feb. | 28.5* | 14.3* | 32.7* | 19.2* | 0.15 | 0 | 0.7 | 0 |
| 21 Feb. - 20 March | 20* | 6.6* | 13.2* | 3.1* | 0.4 | 0 | 1.7 | 0 |

* Biased figures because of insufficient number of samples

secticide at the rate used, especially during the main mosquito activity season. The application of BHC at a higher rate is not economic and probably not very advantageous or promising.

However, on the basis of the results obtained from this experiment, it might be concluded that BHC at the rate used in this experiment is reasonably effective against DLN resistant *A. stephensi mysorensis* for about 2 months and can be used in emergency spraying in DLN resistant *A. stephensi* areas.

## Summary

In order to study the possible use of BHC against DLN resistant *A. stephensi mysorensis* and with a view to determining the value of this insecticide in the control of malaria transmitted by this vector, a Field Experiment (area scale) was carried out in the Khesht Area, Kazeroun, southern Iran, during 1963.

32 villages with a population of 11,173 persons were sprayed with gamma-BHC (Lindane) at the rate of 1000 mg. base per sq. m., during July 1963.

The effectiveness of the insecticide was assessed by the application of various entomological techniques and a monthly parasite survey of the entire population of the experimental area was performed; some epidemiological factors related to the potentiality of transmission were worked out to see whether or not BHC was able to interrupt malaria transmission.

These studies have shown that BHC, at the rate used in this experiment, can reasonably control the natural population of *A. stephensi mysorensis* for a period of about 2 months, during which the transmission of malaria was also interrupted or at least reduced to much lower rates.

It is concluded that, under the prevailing epidemiological and environmental conditions of the experimental area (and similar areas) BHC can be used as an emergency spray against DLN resistant malaria vectors.

## Résumé

En 1963, une expérience a été tentée à Khecht près de Kazéroun, au sud de l'Iran, pour démontrer la possibilité d'utilisation de Gamma-BHC, contre les *A. stephensi mysorensis* résistants au DLN, dans l'intention de contrôler le paludism transmis par ce vecteur.

32 villages (11173 habitants) ont été pulvérisés au Gamma-BHC (Lindane) à raison de 1gr./m.$^2$, durant Juillet 1963.

L'activité du produit a été jugée par divers téchniques enthomologiques en particulier par la suivre du parasite chez la population entière et par les facteurs épidémiologiques mesurant le potentiel de la transmission de la maladie.

De ces expériences il apparaît que le BHC peut arrêter la transmission de la maladie ou la diminuer considérablement durant une période de 2 mois, par le contrôle de la population des *A. stephensi mysorensis*. En conclusion, il apparaît qu'en cas de resistance des vecteurs du paludisme contre le DLN, le BHC peut être utilisé comme insecticide de secours (dans les conditions identiques à celles de l'expérience sus cité).

## References

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