ARTICLES

RESISTANCE OF ANOPHELINES TO CHLORINATED INSECTICIDES IN GREECE

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After the generalized campaign with DDT launched in Greece in 1946 by the Malaria Division of the Athens School of Hygiene, various domestic insects (housefly, *Culex molestus, Pulex irritans*), which had originally disappeared from DDT-sprayed quarters, showed, consecutively, during the immediately following years resistance to this insecticide (1, 2, 3). However, no hint had ever been made in any communication until 1951 of any kind of resistance to DDT by anophelines. In the autumn of that year, from personal communications by sanitary inspectors working in the field it was for the first time brought to our attention that in certain areas of Greece, where the amended malaria control program we had prepared was carried out, especially in Peloponnese, an unusual anopheline density was observed in DDT-sprayed quarters at varying intervals.

After a thorough check was made of these communications, we considered it advisable, owing to the importance of the subject, to present those observations, with all possible reservation, in a preliminary communication to the Malaria Section of the World Health Organization, which was circulated as WHO/Mal/74 Doc. on Dec. 18, 1951, under the title "Do Anophelines Acquire Resistance to DDT?"

In the following year (1952), we proceeded, with our associate Dr. Georgopoulos, to a more specific investigation of the above problem in an area of Peloponnese (Scala-Laconia). From the above investigation, it was ascertained experimentally that in two villages of that area (Asterion and Souli), *A. sacharovi* had developed a clear physiological resistance to DDT and gammexane (5), and that in another village of the same area (Leimonas), the resistance of *A. sacharovi* had also extended to chlordane (6).

In this way, the development of resistance to chlorinated insecticides by one anopheline species in Greece was for the first time brought to light. The above observations made clear the necessity of studying the phenomenon in other areas of the country and of looking into its possible consequences on malaria incidence in Greece. To this end, an extended investigation program was worked out, which included: (1) the carrying out of epidemiological surveys in various areas of Greece, and (2) the determination of the degree of resistance eventually developed by the local vectors to chlorinated insecticides, through appropriate experiments and tests.

The results of the investigations are summarized below.

The findings of the epidemiological survey made in 1953 and 1954 were as follows:

(1) Besides Scala area, in other areas of Greece also (Drama, Cavalla etc.) *A. sacharovi* is very little, if at all, affected by DDT house spray.

(2) It was also observed that the effect of sprayings, repeated twice or three times in the same malaria season with alternately used chlorinated insecticides (DDT, chlordane, gammexane etc.) on *A. Sacharovi* density in various areas of Greece (Salonica, Serres, Chalkididi, Elia, etc.) was a very moderate one, if any.

(3) The other two local vectors (*Anopheles superpictus* and *A. maculi-*

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pennis) were also very little affected by chlorinated insecticides.

(4) The development of resistance to chlorinated insecticides by anopheline vectors in some areas was followed by an obvious increase in malaria cases, while, in others, it resulted in an increase of malaria incidence assuming sometimes the character of local epidemic outbreaks.

(5) The aforementioned epidemic outbreaks were exclusively due to *Plasmodium vivax*. On the other hand, *P. falciparum* cases were sporadic and there was no indication whatever of noticeable reconstruction of the cycle of the latter plasmodium.

A more detailed investigation made concurrently (7) in 1953 and 1954 on the effectiveness of chlorinated insecticides in stables sprayed experimentally in an area of Greece (Scala-Laconia), gave the following results:

(1) *A. sacharovi* density * in a DDT-sprayed stable † was high from the first 5-day period after spraying, and rose quickly to the control levels during the subsequent two 5-day periods. The mortality of *A. sacharovi* caught in the above stable after a 24-hour stay in the cages was low from the first 5-day period after spraying (35.7%), and decreased to 23.7 per cent during the second 5-day period and to 23.9 per cent during the third 5-day period.

(2) *A. sacharovi* density, although it had a considerable decline in a chlordane-sprayed stable †† during the first 5-day period after spraying, showed an abrupt rising during the second 5-day period after spraying with this insecticide, and during the subsequent 5-day periods the fluctuations of mosquito density were practically the same as those observed in the control (Fig. 1). On the other hand, the mortality of *A. sacharovi* caught in the chlordane-sprayed stable after their 24-hour stay in the cages, was already from the first 5-day period after spraying abou the same as that observed in the contro (Fig. 2).

(3) Dieldrin, though clearly more ef fective than DDT and chlordane, als showed reduced action. From the firs 5-day period, *A. sacharovi* catches in dieldrin-sprayed stable ††† were positiv and during the fourth 5-day period afte spraying, there was an evident increase i the mosquito density (Fig. 1). The mor tality of mosquitoes caught in the abov stable after their 24-hour stay in th cages remained at relatively high level during the first two 5-day periods afte spraying; thereafter there was an abrup decline (Fig. 2).

(4) The diazinon-dieldrin combinatio seems to be superior to dieldrin, indi vidually applied. The mortality of *A sacharovi* after spraying with the abov combination,‡ after their 24-hour stay i the cages, was 100 per cent during th first 5-day period and 86.9 per cent durin the second 5-day period after spraying Thereafter, in this case also, there was a abrupt decline in mortality (Figs. 1 an 2).

On the other hand, from an investiga tion (8) made in three areas of Greece b topical application to adult *A. sacharov* and *A. maculipennis* with microdoses o DDT, in accordance with the Ludwi technic (9) and by the application o Buswine and Nash method (10) on total of 21,723 *A. sacharovi* and 144 *maculipennis* tested, there were the fol lowing findings:

(1) The LD_{90} DDT on *A. sacharov* in the village of Elos of Scala area i 1953 was 0.36 microgram per mosquito In 1954, in the same village the LD_9 DDT on *A. sacharovi* amounted to 0.4 microgram per mosquito (Fig. 3).

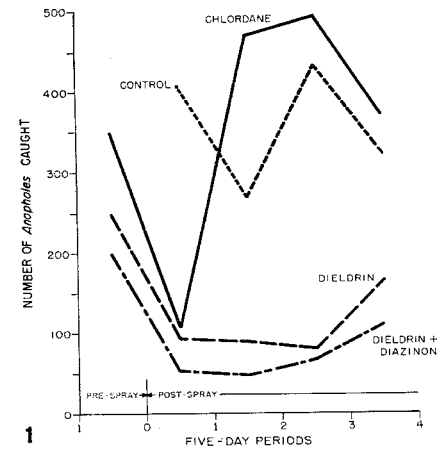
* The estimation of the vector density was in all cases made on the basis of daily catches of a 15 minutes duration always carried out by the same person.

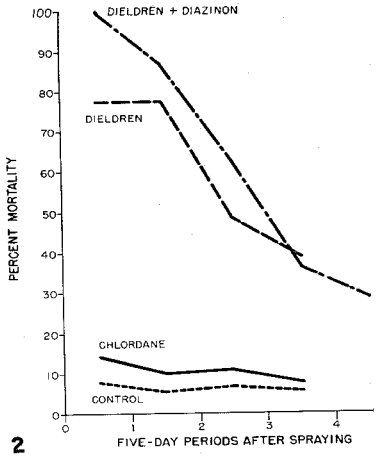
† Dose of DDT used: 2.0 gr. active ingredient per square meter.

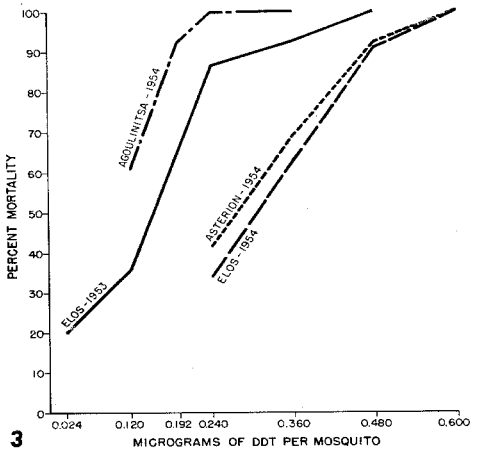
†† Dose of chlordane used: 1500 mg. act. ingred. per sq. meter.

††† Dose of dieldrin used: 400 mg. of act ingred. per sq. meter.

‡ Dose used: 320 mg. diazinon and 250 mg dieldrin per sq. m.







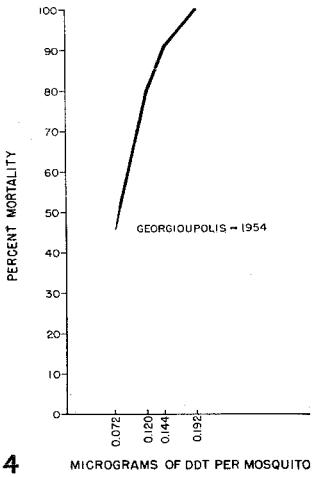


FIG. 1. Density of *Anopheles sacharovi* in stables sprayed with different insecticides and in an unsprayed stable (control). Asterion, Aug.-Sept. 1954.

FIG. 2. Twenty-four hour mortality of *Anopheles sacharovi* caught in stables sprayed with different insecticides and in an unsprayed stable (control). Asterion, Aug.-Sept. 1954.

FIG. 3. Twenty-four hour mortality of *Anopheles sacharovi* (adults) after topical application of different microdoses of DDT (expressed in micrograms per mosquito).

FIG. 4. Twenty-four hour mortality of *Anopheles maculipennis* (adults) after topical application of different microdoses of DDT (expressed in micrograms per mosquito).

(2) The LD_{90} DDT on *A. sacharovi* in the village of Asterion, located 2 kilom. from the former village, in 1954 was also 0.48 microgram per mosquito (Fig. 3).

(3) The LD_{90} DDT on *A. sacharovi* in Agoulinitsa (Elia) in 1954 was 0.19 microgram per mosquito (Fig. 3). It should be noted that in this area of Peloponnese, from recently made epidemiological investigations, it was found that *A. sacharovi* showed clear resistance, not only to DDT, but also to chlordane and gammexane.

(4) The LD_{90} DDT on *A. maculipennis* in Georgioupolis (Crete) in 1954 was 0.14 microgram per mosquito (Fig. 4). In this area, although the house spray carried out by the malaria control service has been suspended since 1951, the population is said to continue privately, from time to time, the use of chlorinated insecticides, with a view to getting rid of the nuisance of the domestic insects.

(5) The average mortality, after 24 hours, of *A. sacharovi* exposed to a 2 per cent DDT solution for 60 minutes, in accordance with the Buswine and Nash method, amounted in Agoulinitsa, in 1954, to 71.2 per cent. In the same year, in the village of Asterion the average mortality of *A. sacharovi* exposed to a 2 per cent DDT solution for 60 minutes, after 24 hours, was 22 per cent. It rose to 96.9 per cent after exposure to a 4 per cent DDT solution (Fig. 5).

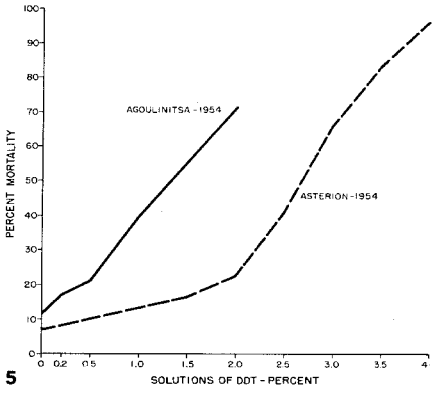


FIG. 5. Twenty-four hour mortality of *Anopheles sacharovi* (adults) after exposure of one hour to different solutions of DDT in paper-lined vials.

CONCLUSIONS

From the investigations made in Greece during the period 1952–1954, the following conclusions can be drawn:

(1) Malaria vectors, particularly *A. sacharovi,* developed clear physiological resistance to DDT and other chlorinated insecticides. This fact had a varying effect on malaria incidence in Greece, at various intervals.

(2) Measurement of the degree of resistance developed by *A. sacharovi* to DDT is not possible, due to the lack of data regarding the degree of susceptibility showed by this species during the initial stages of the application of above insecticide.

(3) The degree of resistance to DDT developed by *A. sacharovi* varies from area to area and tends to increase from year to year.

(4) The minimum LD of DDT found in Greece on *A. sacharovi* (0.19 microgram per mosquito) is larger than that found for *A. maculipennis* (0.14 microgram per mosquito), and is very probably larger than even the LD of DDT on the susceptible strains of *A. sacharovi.*

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JEEP MOUNTED MIST BLOWER

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The Marin County Mosquito Abatement District has built and has been using a one-man operated mist blower. This rig is a self-contained unit and is operated by the driver of the jeep. Most of the important features can be seen in the 2 views shown in Figure 1.

The motor, blower, and pump are mounted on a ¼-inch steel plate, 12" x 36". This plate is fastened by four bolts and is placed over the left rear fender of the jeep. Originally, the jeep had two extra leaves in each rear spring. After installation of the mist blower, it was found that two additional leaves had to be added to the left rear spring.

A 4½ HP Lawson gasoline engine (4-cycle, 1 cylinder) is placed directly behind the left side of the cab of the jeep. The power is supplied to the pump and blower by pulleys and belts.

A ½-inch Flex-Roller pump is mounted beside the blower with a bypass. It pumps insecticide from a 50-gallon tank located in the center of the jeep directly behind the cab. The liquid is pumped through a flexible rubber tube to the discharge end of the blower. A removable #5 whirljet nozzle is firmly attached near the center of this discharge pipe and sprays the material into the air stream. The insecticide is pumped at the rate of about one gallon per minute. A valve in this line controls the spray; it is operated by a lever from the driver's seat.

The blower is a war surplus unit and puts out approximately 600 cubic feet per minute at 4200 rpm. It is attached immediately behind the motor and blows toward the rear of the jeep. The discharge opening is 4" x 6", reduced to a 5 inch circular pipe. This is fitted to a rotating 90°, 5-inch elbow. With the aid of bicycle sprockets and a bicycle chain, this discharge pipe can be rotated by a crank located to the immediate left of the driver; thus, the mist can be directed straight up, straight down, horizontally away from the jeep, or in any intermediate direction.

A "T" joint with a valve is placed just beyond the discharge end of the pump, connected by a length of hose with the old gun-type nozzle for catch-basin or other spraying.

Another "T" joint and valve is located by the intake end of the pump. This is attached to another length of hose, which can be put into a ditch or any other convenient body of water and used to suck up water for refilling the insecticide tank. The intake end of the refilling hose is inclosed with a fine screen to eliminate debris from the tank.

Two of these rigs were built and mounted on jeeps last fall and have been in almost continuous use. No trouble has been encountered; on the contrary, the results have been far better than during the past years of marsh spraying. The opera-