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THE TOXICITY OF DDT TO *ANOPHELES CLAVIGER* (MEIGEN) IN SARDINIA AND ON THE ITALIAN MAINLAND[1]

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In the past several years an increasing number of papers have appeared reporting the development of acquired resistance by house flies and culicine mosquitoes to DDT and certain other insecticides, following the repeated exposure of these insects to these toxicants. It has become necessary to reconsider the role that these materials may play in the control, and particularly in the possible eradication, of various disease carrying insects.

Fay, Baker and Grainger (1949) have demonstrated in the laboratory a slight acquired resistance by adult *Anopheles quadrimaculatus* Say, following the exposure of one generation to residually treated surfaces, but they did not find any subsequent increase in resistance when they tested the offspring of the mosquitoes surviving exposure for the next three generations. When selective exposure was then omitted, the following generation reverted to the initial level of sensitivity to the toxicant. At this time, there are apparently no reports in the literature of the appearance of acquired resistance to DDT by the larvae of any anopheline species, although such resistance has been reported for several culicine species. A further search of the literature reveals that the basic minimum lethal dose (MLD) of various insecticides has been established almost entirely by experiments on a very few species of commonly colonized mosquitoes, such as *Anopheles quadrimaculatus* Say, *Culex quinquefasciatus* Say and *Aedes aegypti* (L.), although Bushland (1947) has reported results obtained with *Anopheles punctulatus* Dönitz, *Armigeres milnensis* Lee, and *Culex annulirostris* Skuse in New Guinea; and Yates (1950) has tested *Aedes nigromaculis* (Ludlow) and *Culex stigmatosoma* Dyar as well as mixed culture of *Aedes vexans* (Meig.) and *A. sticticus* (Meig.) in Oregon. The results obtained by Bushland (1947) indicate that the 48-hour MLD of DDT for *Anopheles punctulatus* in New Guinea was less than one part DDT to 40 million parts water, whereas the MLD of DDT for *A. quadrimaculatus* in the United States has been variously reported as being between 1 to 100 million and 1 to 200 million (see table 1).

Cognizance of the role that acquired resistance may play in the effectiveness of chlorinated insecticides in the field of medical entomology has made it increasingly important to determine the initial toxicity of these various chemicals to mosquito species of medical importance, as a means of establishing a base-line with which subsequently determined toxicities may be compared. The recent work of King (1950) would indicate that resistance on the part of adults may also be reflected in larvae;

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at least he found this to be the case in *Aedes taeniorhynchus* (Wiedemann) and *Aedes sollicitans* (Walker). If the basic toxicity of various chemicals to mosquito larvae in different parts of the world is to be established for comparative purposes, it is desirable that a standard testing method be agreed upon. Particular attention should be given to standardizing the method of dispersing in water the toxicant under test, the volume of fluid used, the number of larvae per test, the temperature at which the test should be run, the duration of the exposure period, the number of replications which should be considered adequate, the question whether it is more desirable to express the results of the tests as MLD's (as has been most generally done) or as LD^{50}'s (which is the more usual pharmacological procedure) and whether the results are consistent enough to render the test valid for comparative purposes. Some variation in results obtained with different lots of larvae from a single laboratory colony has been reported.

In Sardinia, where an intensive program has been in progress over the past four years seeking the total eradication of the malaria vector species *Anopheles l. labranchiae* Falleroni, by all possible means, including both larviciding of water surfaces and residual spraying for adults, the question of a possible acquired resistance to DDT arose during the 1950 breeding season. Larvae of this species could not be obtained in adequate numbers for toxicity tests to establish the MLD, but adequate material was available for work on *Anopheles claviger* (Meigen). As the breeding places of this species as well as those of *A. l. labranchiae* has been extensively and repeatedly treated with DDT larvicides and as there was some doubt as to the effectiveness of the larvicidal treatments, it seemed desirable to determine the MLD of this species. The MLD of DDT to the larvae of this species had not previously been established. Therefore it was not possible to compare the results of the toxicity tests performed by me with similar results obtained before the intensive use of DDT in Sardinia. It was possible to establish, however, that DDT larvicides had not been used in the springs at Ninfa, in Latina Province on the Italian mainland, and to duplicate the toxicity tests performed on field-caught larvae of *A. claviger* from Ozieri, Sardinia, with larvae of the same species at Ninfa on the mainland.

METHODS

There is considerable variation in the methods various workers have used in making toxicity tests, and in how the results are reported. I have followed the methods most frequently used, within the limitations of the equipment available, so the results could be compared with those of other workers. DDT was dissolved in acetone in such proportions that the acetone-DDT solutions could be mixed with 250 cc. of water, so that not more than one cc. of the acetone solution was used to obtain the desired strongest concentration of DDT in water. Other workers have indicated that as much as one per cent of acetone in water was not toxic to mosquito larvae. The tests reported in this paper were conducted in 400 cc. beakers; 250 cc. of water was poured into each. About 25 cc. of water was then poured from each of these into adjacent 50 cc. beakers and 20 early fourth-instar larvae were introduced into the small beakers. The appropriate amount of DDT-acetone solution to obtain the desired concentration of DDT was then added to the remaining water in the 400 cc. beakers, and the mix-

ture stirred with a glass rod. The larvae and water contained in the small beakers were then poured into the large beakers. This procedure was used to avoid the shock of introducing the larvae directly into the DDT-water suspension. In all test runs two controls were used; one with the larvae in water alone, and one with the larvae in water to which had been added the maximum amount of acetone used to obtain the highest concentration of DDT-acetone in that test run. In Sardinia the tests were run in tap water from the city supply at Cagliari, while at Ninfa clear spring water in

TABLE 1

Average 48-hour mortality of 4th instar anopheline larvae in DDT-acetone water suspensions

(Results are given in per cent.)

Species	*Anopheles claviger*		*Anopheles quadrimaculatus*									*Anopheles punctulatus*
Location	Ozieri, Sardinia, Italy	Ninfa, Latina, Italy	Florida, U. S. A.									New Guinea
No. of replications	5	4	–	3	3	3	3	3	4	5	3	1 or 2
Parts DDT per million parts water.												
1 to 2.5	100											
1 to 10	98	95										
1 to 25	85	73										
1 to 40												90
1 to 50	69	55										90
1 to 75												80
1 to 100	45	35				98		100	100	100		45
1 to 200	10	20	98	97	84	71	100	100	100	94	98	30
1 to 300				93								
1 to 400			85	82	53	24	100	68	61	55	76	10
1 to 600											41	
1 to 800					16		19					
Authority for test			1	2	3	3	3	4	4	5	6	7

Authorities for the data given under *A. quadrimaculatus* and *A. punctulatus* are as follows: 1 = Deonier, Jones and Incho (1946); 2 = Deonier and Jones (1946); 3 = Incho and Deonier (1950); 4 = Deonier, Raun, Peek, Davis and Nottingham (1949); 5 = Deonier, Maple, Jones, Hinchey and Edie (1945); 6 = Incho and Deonier (1947); 7 = Bushland (1947). Percentages are given to the nearest whole number.

which the larvae were living was used. The larval mortality was read at 48 hours. Any larvae which had pupated during the 48-hour test period were omitted from the calculations of the results, as pupae are less sensitive to DDT suspensions than larvae. All tests were run at room temperature which maintained the liquids used in the tests at an average temperature of 22° to 23°C. As the chemical used was a sample of technical grade DDT, recrystalized DDT not being available, it was desirable to check this material for its toxicity. A portion of the sample of DDT used in these tests was checked by Dr. W. McDuffie in comparison tests with recrystallized DDT against

A. quadrimaculatus at the Orlando, Florida, laboratory of the U. S. Department of Agriculture, where it produced the same mortality as the standard material in use there.

RESULTS

The results of the tests are given in table 1 along with comparable data from the literature. Two interesting results are to be noted:

1. The 48-hour MLD of DDT for *A. claviger* is about 1 part DDT to 10 million parts water. The work of other investigators, whose results are summarized in Table I, indicates that the 48-hour MLD of DDT for *A. quadrimaculatus* is about one part DDT in 200 million parts water. It would appear, therefore, that *A. claviger* is only about one-twentieth as sensitive to DDT as is *A. quadrimaculatus*.

2. Larvae of *Anopheles claviger* from Ozieri in Sardinia, where DDT larvicides have been intensively and repeatedly used, and larvae of this species from Ninfa on the Italian mainland, where they have not been used, are about equally sensitive to DDT. There is no evidence of an acquired resistance in the Sardinian larvae of this species.

As a check on these results, toxicity tests were run on larvae of *Culex molestus* Forskal (= *Culex pipiens* var. *autogenicus* Roubaud) obtained near Cagliari, in the same beakers with *A. claviger*. While *A. quadrimaculatus* has been reported to be more sensitive to DDT than *Culex quinquefasciatus* (= *Culex fatigans* Wiedemann) by all authors working with these two species (Incho and Deonier, 1947; Deonier et al., 1949), the writer found the reverse to be true in the comparison of *A. claviger* with *C. molestus;* the MLD for *C. molestus* approximated that reported for *C. quinquefasciatus*.

SUMMARY

The 48-hour MLD of DDT for *Anopheles claviger* in Sardinia and on the Italian mainland was found to be one part DDT in 10 million parts of water, about one twentieth that reported for *A. quadrimaculatus* in the United States. Despite this relative lack of sensitivity of *A. claviger* to DDT, no evidence was found of an acquired resistance in Sardinia, where DDT has been intensively and repeatedly used, since the same MLD was found for larvae from an area on the Italian mainland, where DDT larvicide had not been used.

It would be desirable to establish the MLD (or the LD^{50}) of DDT and the other recently developed synthetic insecticides against the larvae of the important malaria vectors of the world, to fix a base-line from which any subsequently developed resistance might be measured. Methods of conducting and reporting toxicity tests against mosquito larvae should be standardized, for ready comparison of results from different parts of the world.

SOMMARIO

La MLD di DDT di 48 ore contra l'*Anopheles claviger* in Sardegna e nell'Italia continentale si è vista composta di 1 parte DDT in 10 milioni di parti d'acqua, per cui la sensibilità del *claviger* è circa una ventesima parte di quella riportata per *A. quadrimaculatus* negli Stati Uniti. Malgrado questa relativa mancanza di sensibilità dell'*A. claviger* al DDT non si è trovata nessuna prova di una resistenza acquistata in Sardegna, dove il DDT è stato usato intensivamente e repetutamente, dacche la

stessa MLD fu trovata per le larve di una zona nell'Italia continentale, dove il larvicida DDT non era stato usato.

Sarebbe desiderabile di stabilire la MLD ("dose minima mortale"), o la LD^{50} ("dose mortale, 50 percento") di DDT, e degli altri insetticidi sintetici recentemente prodotti contra gli importanti vettori di malaria, allo scopo di fissare una base dalle quale misurare qualsiasi resistenza che si sviluppasse nel futuro. I metodi per condurre e riportare le prove di tossicità contra le larve della zanzara dovrebbero essere standardizzati per poter prontamente comparare i risultati ottenuti nelle diverse parti del mondo.

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RESUMEN

La dosis letal mínima cuarenta y ocho horas (48-hour MLD) de DDT para *Anopheles claviger* en Sardinia y en el Continente Italiano resultó ser una parte de DDT por 10 millones de partes de agua, aproximadamente una vigésima parte de lo reportado en Estados Unidos para *Anopheles quadrimaculatus*. A pesar de esta relativa falta de sensibilidad de *A. claviger* al DDT no se notó ninguna evidencia de

resistencia adquirida en Sardinia, donde DDT se había usado extensamente y repetidamente pues se observó la misma dosis letal mínima contra larvas procedentes de una área del Continente Italiano donde el larvicida DDT no se había usado previamente.

Resultaría conveniente establecer la dosis letal mínima (o la dosis letal 50) de DDT y de otros insecticidas sintéticos descubiertos recientemente contra las larvas de los importantes vectores de malaria en el mundo para fijar una línea de base que serviría para medir resistencias desarrolladas subsiguientemente. Los métodos usados en la ejecución y reporte de pruebas de toxicidad contra las larvas de mosquitos deben ser normalizadas para lograr comparaciones ligeras de los resultados obtenidos en diferentes partes del mundo.