DETERMINATION OF SUSCEPTIBILITY LEVELS OF MOSQUITOES TO NON-PERSISTENT INSECTICIDES BY MICROINJECTIONS[1]

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ABSTRACT. A new approach for determining susceptibility levels of adult mosquitoes to insecticides by subcuticle injections has been offered. Several field populations belonging to the genera *Culex, Aedes, Anopheles, and Uranotaenia* were tested against commonly used insecticides. Advantages and limitations of the new method are discussed.

INTRODUCTION AND OBJECTIVES. During the 1971 Venezuelan equine encephalitis epidemic in U.S. about 12 million acres had to be sprayed for mosquito control to contain the epidemic in a limited area. Emergency situations like this arise from time to time, and resorting to massive spraying becomes absolutely essential. In addition to this, mosquito control districts spray routinely to insure comfortable living and to minimize the threat of arthropod-borne diseases. It is not uncommon for a large district to cover one million acres in a year, and considering that there are 350 organized mosquito control districts active in the U.S. (Shelton 1971), it would not be unfair to assume that well over 100 million acres are sprayed for mosquito control alone in this country every year. It is, therefore, important that mosquito control workers not only use those insecticides which are non-persistent and proven to be safe, but these must be used in minimum amounts so that we do not add to the environment, intentionally or inadvertently, any more "bad" or "good" insecticides (a "good" insecticide may become "less good" by further knowledge) than are absolutely essential. However, fundamental information on minimum lethal dose (Min. L. D.) is not easily available, especially for the non-persistent insecticides that are used in mosquito control in this country. One reason for this lack of vital information is that a satisfactory laboratory test to determine the susceptibility of adult mosquitoes to these types of insecticides has not been heretofore available. The commonly used WHO test is not satisfactory for biodegradable insecticides. Therefore, the objectives of the present study were:

(1) Determination of the absolute minimum lethal dosage of commonly used insecticides that will kill the different species of mosquitoes.

(2) Development of a simple laboratory test to determine the susceptibility of field and laboratory-reared mosquitoes to non-persistent insecticides.

METHODS AND MATERIALS. In the laboratory, insecticides can be applied by three ways: topical application, infusion, or injection. It seems that the last of these (injection method) is more accurate. Table 1 compares the results obtained with the three methods using house flies as test species. Variation, as measured by the standard error (SE), was generally more in the topically-applied group than in the injected group. However, when the infused group was compared with the injected

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TABLE 1.—Variability of topical (top), injection (inj), and infusion (inf), results for female houseflies.

Comparison of Standard Errors*

Insecticide	top/inj	inf/inj
SD-11319	2	..
AD-11373	10	10
SD-11097	0.5	1
Monocrotophos	2	1

* From the data of Sun and Johnson (1971).

group variation was about the same, or more, in the infused group. Furthermore, most insecticides, if not all, act after they have been absorbed through the cuticle. Therefore, after careful considerations it was decided to inject the test insecticides under the cuticle (Fig. 1). The insecticide dilutions were made in acetone which in turn was further diluted in a balanced salt solution for the purpose of injecting mosquitoes. Each mosquito was inoculated with 1 μl (0.001 ml) of appropriate dilution and the mortality was recorded after 24 hours of injection, as has been described elsewhere (Gilotra *et al.*, 1972).

Several species covering the genera *Aedes, Culex, Anopheles,* and *Uranotaenia,* and two insecticides, namely malathion and dibrom-14 were used. These insecticides have good safety records, and their oral toxicity (LD_{50}) in laboratory-bred white rats is 2800 and 430 mg per kilogram weight of animal, respectively (Neumeyer *et al.,* 1969).

RESULTS. In order that results be comparable, for each test (except *Uranotaenia* mosquitoes) the mosquitoes were divided into two groups; one group was exposed to the test insecticide I (i.e., malathion), and the other to the test insecticide II (dibrom). Table 2 shows the response of *Aedes* mosquitoes to malathion. In general, these mosquitoes are susceptible. Even at a low dosage of 3 ngms (nanograms) per mosquito, more than 50 percent of *A. sollicitans* were killed. The LD_{90} for *A. sollicitans* and *A. taeniorhynchus* was about 9 ngms. However, *A. aegypti* was a bit more tolerant. The LD_{90} was about 29 ngms. Table 3 shows the response of mosquitoes from the same pool to dibrom. All *Aedes* species were highly susceptible. The LD_{90} was 4 ngms. or less.

In the next series of tests the responses of *C. quinquefasciatus* (several different populations) and *C. salinarius* (three populations) were explored. These mosquitoes are major problems in the State of Louisiana. The so-called laboratory susceptible strain BR-L of *C. quinquefasciatus* was moderately tolerant to malathion (Table 4); the LD_{90} being about 29 ngms. The other three strains of *C. quinquefasciatus* (SB-F, PL-F and JF-F) and three strains of *C. salinarius* (NO-F, SB-F, SB-F-I) which were field-collected, were at least two to four times more tolerant than the BR-L strain of *C. quinquefasciatus.* When the same populations were exposed to dibrom (Table 5) they were all highly susceptible. The LD_{90} varied from 4 to 8 ngms.

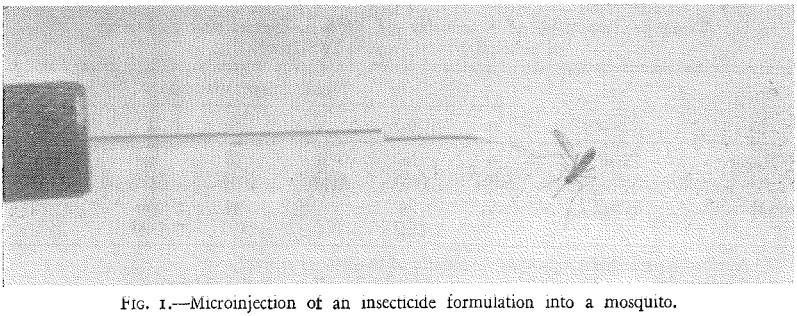

FIG. 1.—Microinjection of an insecticide formulation into a mosquito.

TABLE 2.—Percent kill of 2- to 5-day old *Aedes* mosquitoes after application of technical grade malathion by SC (subcuticle) route.

Species	Strain	Nanograms per mosquito						
		0	1	3	6	9	12	29
sollicitans	PL–F†	0 (10)*	6 (16)	60 (15)	88 (25)	96 (24)	100 (22)	NT
aegypti	NO–F	..	0 (6)	0 (5)	0 (10)	43 (7)	69 (16)	100 (10)
taeniorhynchus	PL–F	..	NT	NT	NT	82 (17)	100 (10)	NT

* Number in parentheses represents the number of mosquitoes tested.
† F indicates that test mosquitoes were reared from field collected larvae and pupae.
NT Not tested at that dosage.

Two populations of *An. crucians* were also tested, and they were moderately susceptible to malathion (LD_{90} was 29 to 58 ngms.) and highly susceptible to dibrom (LD_{90} was 4 to 8 ngms.). A few mosquitoes of *U. lowii* were also tested. Since these are very delicate and small, one wondered if they could be successfully injected. There was no mortality in the control, but the results were incomplete because *U. lowii* mosquitoes were not tested below the dosage of 29 ngms of malathion per mosquito and at this dosage there was 100 percent mortality. A summary of the results obtained from the 11 field-collected populations and 1 laboratory population with test insecticides is presented in Table 6. *A. sollicitans* and possibly *U. lowii* were susceptible to both the insecticides, while other populations were considerably more susceptible to dibrom as indicated by malathion/dibrom ratio in the last column of Table 6.

DISCUSSION. In injecting the insecticides under the cuticle, the physiological responses of mosquitoes to the test insecticide were measured. In other words, the authors tried to determine the minimum physiological lethal dose which should approximate the topically-applied dosage, should there be rapid absorption through the cuticle with no or minimum loss of active ingredients. There seems to be some reason to believe that this is what happens with the use of biodegradable insecticides, especially when they are applied as ULV concentrates. Field observations indicate that mosquitoes die rapidly, or in a short time after a suitable spray application. The implication is that the insecticide passes rapidly through the cuticle to initiate toxic effects which could not have happened if the insecticide had remained outside. Recently, rapid penetration of the cuticle by an insecticide has been very well documented by Benezet and Forgash

TABLE 3.—Percent kill of 2- to 5-day old *Aedes* mosquitoes after application of technical grade dibrom-14 by SC route.

Species	Strain	Nanograms per mosquito					
		0	0.2	1	2	4	8
sollicitans	PL–F†	0 (7)*	0 (16)	3 (30)	38 (29)	90 (29)	100 (18)
aegypti	NO–F	...	0 (14)	NT	35 (17)	100 (14)	NT

* Number in parentheses represents the number of mosquitoes injected.
† F indicates that test mosquitoes were reared from field collected larvae and pupae.
NT Not tested at that dosage.

TABLE 4.—Percent kill of 2- to 5-day old *Culex* mosquitoes after application of of technical grade of malathion by SC route.

Species	Strain	Nanograms per mosquito						
		0	6	12	29	58	88	117
quinquefasciatus	BR–L*	6 (17)	0 (11)	48 (29)	100 (26)	100 (27)	NT	100 (15)
	NO–L	4 (27)	0 (12)	36 (25)	50 (32)	88 (35)	NT	96 (25)
	SB–F†	5 (20)	0 (40)	3 (34)	12 (34)	21 (34)	NT	95 (20)
	PL–F	0 (30)	NT	15 (40)	55 (40)	88 (40)	NT	98 (20)
	JF–F	0 (30)	NT	30 (20)	75 (40)	83 (52)	95 (19)	100 (30)
salinarius	NO–F	0 (12)	NT	0 (20)	62 (21)	76 (17)	NT	100 (16)
	SB–F	0 (9)	NT	5 (21)	38 (29)	63 (49)	88 (40)	96 (24)
	SB–F–I	..	NT	NT	NT	96 (24)	100 (25)	NT

* L indicates that test mosquitoes were from laboratory colony.
† F indicates that test mosquitoes were reared from field collected larvae and pupae.
NT Not tested at that dosage.

(1972). They studied penetration of radioisotope-labelled malathion through the cuticle of the common house fly by use of radioautography. Their conclusions were: "The malathion moved through the cuticle and into the hemolymph within 15 seconds . . . the malathion being distributed by the hemolymph." Elsewhere

TABLE 5.—Percent kill of 2- to 5-day old *Culex* mosquitoes after application of technical grade dibrom-14 by SC route.

Species	Strain	Nanograms per mosquito					
		0	0.2	1	2	4	8
quinquefasciatus	BR–L*	17 (6)	6 (16)	NT	67 (15)	NT	100 (16)
	SB–F†	..	8 (12)	NT	0 (12)	NT	100 (12)
	PL–F	0 (19)	0 (20)	NT	36 (39)	100 (40)	100 (40)
	JF–F	..	25 (20)	8 (40)	55 (40)	100 (40)	100 (29)
salinarius	NO–F	..	NT	NT	40 (10)	81 (16)	100 (16)
	SB–F	0 (20)	10 (20)	NT	45 (58)	85 (40)	98 (48)
	SB–F–I	..	..	NT	NT	91 (21)	100 (25)

* L indicates that test mosquitoes were from laboratory colony.
† F indicates that test mosquitoes were reared from field collected larvae and pupae.
NT Not tested at that dosage.

TABLE 6.—Susceptibility of mosquitoes to malathion and dibrom-14—Summary at LD_{90-100} level.

Species	Strain	Malathion ngms/mosq.	Dibrom ngms/mosq.	Malathion/Dibrom
A. sollicitans	PL-F†	9	4	2
A. aegypti	NO-F	29	4	7
C. quinquefasciatus	BR-L*	29	8	4
	SB-F	117	8	15
	PL-F	117	4	29
	JF-F	88	4	22
C. salinarius	NO-F	117	8	15
	SB-F	117	8	15
	SB-F-I	58	4	14
An. crucians	SB-F	58	8	7
	SB-F-I	29	4	7
U. lowii	SB-F	<29	4	<7

* L indicates that test mosquitoes were from laboratory colony.
† F indicates that test mosquitoes were reared from field collected larvae and pupae.

in the text it was implied that it was actually considerably less than 15 seconds.

Since this is the first time the mosquitoes have been injected with insecticides, it is not possible to compare the results strictly with the results obtained by others. However, assuming close to 100 percent penetration of malathion through the cuticle, our results are almost identical with the ones obtained by Weidhaas and his colleagues (1970), by exposing mosquitoes in a wind tunnel and later determining the amount of insecticide per mosquito by chemical analysis. The LD_{100} for *A. taeniorhynchus* was 10 ngms as compared to between 9 and 12 ngms by our method. However, it may be pointed out that both the studies using aforementioned methods were done independently at different times and with different populations. Therefore, the possibility of a close agreement as a mere coincidence cannot be ruled out. The implication that the mosquito cuticle is an insignificant barrier still remains to be tested. In fact, the degree to which the cuticle acts as a barrier depends upon the type of insect and nature of insecticide formulation (Wintergham, 1969). Lovell (1963) tested german cockroach *Blatella germanica* and milkweed bug *Oncopeltus fasciatus* individually to malathion. LD_{50} ratios between the topically applied and injected groups were 18 and 2 respectively; these ratios were 2 and 1 respectively for isomalathion.

In our tests no topical application was made. Both malathion and dibrom were injected under the cuticle. The obvious question arises whether these differences between the two insecticides obtained by microinjections only, would remain more or less the same if insecticides were applied topically. This cannot be tested because so far it has not been technically possible to apply topically such small amounts of insecticides to individual mosquitoes as has been done with large insects. However, results obtained by the use of wind tunnel and caged mosquitoes at West Florida Research Laboratory, Panama City, Florida and at USDA Research Laboratory, Gainesville, Florida, indicate that our ratios between the malathion and dibrom for *Culex* and *Aedes* mosquitoes are not unrealistic (Personal communication). This is still circumstantial evidence since the tests were not done on the same population or even on the same species.

As unnatural as the new method may seem, we still believe it can be efficiently used to compare the effectiveness of different biodegradable insecticides which, by definition, must act in a short time, or run

the risk of being degraded. The actual method of testing by microinjections is simple, and can be used in the laboratory without any elaborate equipment. It should also satisfactorily measure the toxicity of persistent insecticides that rapidly pass through the cuticle.

With this type of quickly obtained comparative information easily obtainable one can then attempt to use the most efficient, as well as the most economical, insecticide in such a way that the quality of our environment is not jeopardized.

SUMMARY AND CONCLUSIONS. Mosquito populations of the same or different species are selectively susceptible to insecticides. It is essential that, before the start of any mosquito control operation, dominant species to be controlled be clearly identified and only minimum amounts of that insecticide, which on the basis of most recent tests has proven to be effective, safe, and economical, be used.

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