MOSQUITO CONTROL WITH MONOMOLECULAR ORGANIC SURFACE FILMS: II - LARVICIDAL EFFECT ON SELECTED *ANOPHELES* AND *AEDES* SPECIES [1]

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ABSTRACT. The effectiveness of 4 monomolecular surface films was evaluated against 4th instar *Anopheles quadrimaculatus* and *Aedes taeniorhynchus* larvae. At an application rate of 0.04 milliliters per square meter (0.043 gallons/acre), 2 of the 4 compounds tested provided virtually 100 percent control of *Anopheles* larvae in both laboratory and field tests. Laboratory tests indicate that, at the above application rate, none of the films tested effectively controlled *Aedes* larvae.

INTRODUCTION

Recent concern over the undesirable biological impact of synthetic organic insecticides has led to the curtailed use of many such materials in the control of mosquito populations. Consequently, alternatives to these compounds which do not insult the environment or pose a health hazard to man are urgently needed.

Garrett and White (1977) reported upon a novel technique for mosquito control. The approach uses nonionic surface-active liquids which spread spontaneously over the surface of water and form films which are nearly monomolecular. They reported that theoretically the quantity of these materials required for the control of certain mosquito larvae and pupae was at least 70 times less than the amounts of petroleum-based oils presently used. In contrast to the petroleum oils, it appears likely that these thin, nontoxic films might be suitable for use on reservoirs and irrigation water bodies. Furthermore, they apparently do not interfere with gas exchange across the air-water interface, and thus should not result in oxygen starvation in the underlying water. The current studies were undertaken to determine the efficacy of these monomolecular films as larvicides against selected *Anopheles* and *Aedes* species.

METHODS AND MATERIALS

LABORATORY. Four monomolecular films were selected for evaluation. These compounds included: diethylene glycol monolaurate (DEGMOL); sorbitan monooleate, 75% + 2 – ethyl butane, 25% (SMO75/2EB); sorbitan monooleate, 37.5% + lauryl ether containing 4 oxyethylene groups, 50% + 2 – ethyl butanol, 12.5% (SMO75/2EB + POE(4)LE); and isosteryl alcohol containing 2 oxyethylene groups (ISA-2 OE). Each compound was tested at a surface concentration of 40 microliters/sq.m. against larvae. In each test, 100 4th-stage larvae of *Anopheles quadrimaculatus* Say and *Aedes taeniorhynchus* Wiedemann were placed into each of 5 small enamel pans

[1]The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. Mention of product in this paper does not constitute a recommendation or an endorsement of the product by the U.S. Navy.

containing 1000 ml of tap water. The required amount of toxicant was added to the water surface in each pan, and mortality was recorded at 24 hr intervals. Accompanying each series of 5 pans was a similar pan containing 100 untreated control larvae. Each complete test was replicated a minimum of 3 times.

FIELD. Following evaluation of these compounds in the laboratory, each was evaluated against larvae in 4 sq.m., polyethylene-lined artificial pools created in a woodland environment. In these initial semi-field tests, 5000 4th-instar *An. quadrimaculatus* larvae were placed into each of 5 artificial pools. Each of the test compounds was applied to a respective pool at a rate of 40 microliters/sq.m. The fifth pool served as a control. To determine the effectiveness of each compound, total counts of live larvae were made in treated pools every 24 hr. Tests with ISA-2OE were replicated twice. Each of the remaining compounds were evaluated in single, unreplicated tests.

Those compounds which proved most effective in initial semi-field tests were further evaluated against 4th-instar *An. quadrimaculatus* larvae in swimming pools ranging from 24 - 180 sq.m. in size. Each compound was again applied at a rate of 40 microliters/sq. m. Large pools containing 3000 untreated larvae each served as controls. The effectiveness of each compound was determined by making total counts of live larvae in treated pools at 24-hour intervals. Except where indicated, each compound was evaluated in a single test.

RESULTS AND DISCUSSION

The results obtained in these experiments demonstrated that certain monomolecular films effectively control *An. quadrimaculatus* larvae. These larvae reacted immediately to the application of each compound tested. Each compound instantaneously dispersed the randomly distributed larvae to the edges of the pan. All larvae immediately assumed an *Aedes*-like position at the air-water interface and experienced considerable difficulty in maintaining contact with the surface. Once contact was lost, larvae experienced similar difficulty in reorienting themselves at the air-water interface. Submerged larvae became hyperactive. Larval movement rapidly became frenzied. The degree of hyperactivity appeared to vary with the compound tested.

Laboratory results obtained with *An. quadrimaculatus* larvae are presented in Table 1. The data indicate that DEGMOL and SMO75/2EB + POE(4)LE, as monomolecular films, are only marginally effective as larvicides. However, SMO75/2EB and ISA-2 OE provided levels of control ranging from 96-100%. ISA-2 OE appeared to be the most effective compound tested. Within 20 min. of exposure to this compound all larvae were either dead, moribund, or submerged and thrashing about in a violent, snake-like fashion. Within 35 min. approximately 95% of all larvae tested were either dead or moribund.

Each of the 4 monomolecular films was evaluated against *An. quadrimaculatus* larvae in initial semi-field tests. Prior to applying the test compounds, the water surface in the small artificial pools was covered by a moderate sprinkling of pine needles. The *Anopheles* larvae were uniformly distributed over the surface. As each compound was applied to its respective pool the films again dispersed the larvae, pine needles, and surface scums to the opposite end of the pool. Initially, many larvae were protected by the combination of pine needles and surface scums. However, within an hour the films dispersed the surface scums exposing the larvae to the action of the monomolecular films. In these tests the larvae reacted to the films in a manner similar to that observed in the laboratory tests. Larval activity appeared less frenzied, however.

Each film tested appeared to be readily biodegradable. Films apparently affect only organisms intimately associated with the air-water interface. Several aquatic insect groups, including species of Gerridae, were active in the pools. Shortly after

Table 1. Effect of monomolecular films on *Anopheles quadrimaculatus* larvae in laboratory tests.

Compound tested	Cumulative mortality after indicated hours of post-treatment exposure		
	Hours	Larval mortality	% Cumulative mortality[a]
DEGMOL[b]	24	1267	50.4
	48	1326	52.6
	72	1355	53.4
SMO 75/2EB[c] + POE(4)LE	24	939	62.3
	48	959	63.2
	72	962	63.0
SMO 75/2EB[b]	24	2407	96.3
	48	2411	96.4
	72	2414	96.6
ISA–20E[c]	24	1490	99.3
	48	1499	99.3
	72	1500	100.0

[a] Percent mortality corrected by Abbott's formula.
[b] Data reflect results of 5 replicates; 500 larvae/test.
[c] Data reflect results of 3 replicates; 500 larvae/test.

applying the compounds the gerrids submerged and struggled to reach the margins of the pools. However, species of Dytiscidae were totally unaffected. Approximately 24 hr. after treatment, gerrids were again active on all pools.

The results obtained with the 4 experimental films in these small pool tests are presented in Table 2. Both SMO75/2EB and ISA-2 OE provided 100% mortality within 24 hr. It is interesting to note that the 2 compounds which were only moderately effective in laboratory tests were more effective in the field. Interestingly

Table 2. Effect of monomolecular films on *Anopheles quadrimaculatus* larvae in small experimental pools.[a]

Compound used	Hours of exposure	Cumulative larval mortality	% Cumulative mortality
DEGMOL	24	3554	71.1
	48	3786	75.7
	72	4065	81.1
SMO 75/2EB + POE(4)LE	24	3850	77.0
	48	4156	83.1
	72	4365	87.2
SMO 75/2EB	24	5000	100.0
ISA–20E	24	10000[b]	100.0

[a] Except where indicated, data reflect results of single, unreplicated tests; 5000 larvae tested against each compound. Percent mortality corrected by Abbott's formula.
[b] Results reflect results of 2 replicates.

Table 3. Effect of selected monomolecular films on *Anopheles quadrimaculatus* larvae in large experimental pools.[a]

Compound tested	Pool size	Number larvae used	Mortality after 24 hours exposure	Percent mortality
SMO 75/2EB	85 sq. m.	9000	8994	99.9
ISA-20E	24 sq. m.	3000	3000	100.0
ISA-20E	180 sq. m.	15000	15000	100.0

[a] Data reflect results of single, unreplicated tests. No mortality occurred in control pools containing 3000 larvae.

too, the mortality achieved with DEGMOL and SMO75/2EB + POE(4)LE increased 10% over a 72-hr. period despite the fact that the films had apparently been biodegraded.

The mechanisms by which the films cause mortality in mosquito larvae are not exactly known. Mortality probably can be attributed to physical effects of the surface-active chemical rather than to toxic influences. Lowered surface tension probably precludes proper larval orientation at the air-water interface. However, Garrett and White (1977) proposed a second mechanism. Greatly lowered surface tension could result in wetting of the internal hydrophobic structure of larval tracheae. The result might lead to liquid blockage and interference with respiration. This mechanism might explain the increase in larval mortality observed in these tests.

Table 3 presents the results obtained in large artificial pool tests with the most ef-

Table 4. Effect of monomolecular films on *Aedes taeniorhynchus* larvae in laboratory tests.

Compound tested	Cumulative mortality after indicated hours of post-treatment exposure: Hours	Larval mortality	% Cumulative mortality[a]
DEGMOL	24	50	2.5
	48	89	4.5
	72	126	6.0
	96	154	7.1
SMO 75/2EB + POE(4)LE	24	58	1.9
	48	124	4.8
	72	154	6.1
	96	172	6.7
SMO 75/2EB	24	128	6.4
	48	226	11.0
	72	254	12.4
	96	270	13.2
ISA-20E	24	105	5.0
	48	237	11.2
	72	520	25.4
	96	724	35.7

[a] Percent mortality corrected by Abbott's formula. Data reflect results of 4 replicates; 500 larvae/test.

fective films. Both compounds tested provided essentially 100% control of *Anopheles* larvae. Each pool contained a natural assortment of non-target species including: frogs, toads, gerrids, nymphal stages of Odonata and Ephemeroptera, as well as several species of aquatic Coleoptera. Again, only those species intimately associated with the air-water interface were affected at the dosage levels tested. It is postulated that probably no gerrid mortality would have even occurred if the sides of the pools had not prevented them from leaving the water.

The promising results obtained against *Anopheles* larvae encouraged testing of these films as larvicides against *Ae. taeniorhynchus*. The results of laboratory tests with this species are presented in Table 4. At the dosage level tested, the effect of monomolecular films appears genus-specific. None of the compounds tested effectively controlled *Ae. taeniorhynchus* larvae. However, those compounds which were most effective against *An. quadrimaculatus* larvae were also the most effective against the *Aedes* larvae tested. Perhaps the compounds tested would be effective against *Ae. taeniorhynchus* larvae if the dosage rate were increased.

In certain circumstances, monomolecular films appear to provide an acceptable alternative to the insecticides normally used to control mosquito larvae. The surface film approach to mosquito control is based upon the modification of physical processes necessary to the development of the mosquito in the aquatic environment. The poor results obtained against *Ae. taeniorhynchus* larvae should not discourage further research into this novel approach to mosquito control. Further investigations might or might not verify the genus specificity observed during these tests. If monomolecular films do prove to be specific against *Anopheles* larvae, the technique still promises to provide a harmless and uncomplicated means of controlling one of the world's major disease vectors—the malaria mosquito.

Reference Cited

Garrett, W. D. and S. A. White. 1977. Monomolecular organic surface films: I - Selection of optimum film-forming agents. Mosquito News 37:344–350.