ARTICLES

EFFICACY OF THE ORGANIC SURFACE FILM ISOSTEARYL ALCOHOL CONTAINING TWO OXYETHYLENE GROUPS FOR CONTROL OF *CULEX* AND *PSOROPHORA* MOSQUITOES: LABORATORY AND FIELD STUDIES

R. LEVY,[1] J. J. CHIZZONITE,[1] W. D. GARRETT[2] AND T. W. MILLER, JR[1]

ABSTRACT. The efficacy of the organic surface film isostearyl alcohol containing two oxyethylene groups (ISA-20E) for controlling *Culex quinquefasciatus*, *Cx. nigripalpus*, *Psorophora columbiae* and *Ps. ciliata* was evaluated under laboratory and/or field conditions. Results of bioassays against *Cx. quinquefasciatus* indicated that ISA-20E would kill larvae and pupae, entrap and drown adults, and sink and inhibit the hatching of egg rafts. Field tests conducted under a wide range of environmental conditions at surface dosage of 0.20–0.45 ml/m² (0.21–0.48 gal/acre) indicated that effective control of larvae and pupae (90% or greater) of the *Culex* and *Psorophora* spp. would occur within 72 hr post-treatment. Factors influencing the larvicidal and pupicidal action of ISA-20E under bioassay and field conditions are discussed.

INTRODUCTION

The efficacy of the monomolecular organic surface film isostearyl alcohol containing two oxyethylene groups (ISA-20E)[3] in controlling natural populations of larvae and pupae of *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say breeding in effluent in settling, polishing, and evapo-percolation ponds of an industrial sewage treatment system of southwestern Florida was reported by Levy et al. (1980). Their data have indicated that ISA-20E can be sprayed in these polluted water habitats at surface dosages as low as 0.33 ml/m² (0.35 gal/acre) and control greater than 95% of the immature stages of the *Culex* spp. under a broad range of environmental conditions. Similar levels of control at comparable dosages of ISA-20E were achieved in ground and aerial spray tests against the immature stages of *Ae. taeniorhynchus* Wiedemann and *Ae. infirmatus* Dyar and Knab in salt-marsh habitats of southwestern Florida (Levy et al. 1981). The proposed kill mechanism of ISA-20E on immature mosquitoes, the influence of environmental factors on the rate of larvicidal and pupicidal action, the range of effectiveness on mosquito species and stages of development, the application methods, and the environmental safety of ISA-20E are discussed by Levy et al. (1980, 1981).

Culex nigripalpus and *Cx. quinquefasciatus* are potential vectors of St. Louis encephalitis in the southeastern United States (*Cx. nigripalpus* in southwestern Florida) and since the data generated from sewage and coastal salt-marsh trials were encouraging, additional tests were conducted to determine the spectrum of effectiveness of this surface-active chemical when used against permanent water

---

[1] Lee County Mosquito Control District, P.O. Box 06005, Ft. Myers, FL 33906.

[2] Environmental Sciences Division, Naval Research Laboratory, Washington, D C 20375.

[3] ISA-20E is a cosmetic ingredient manufactured under the name ®Arosurf 66-E2 by Sherex Chemical Company, Inc., P.O. Box 646, Dublin, OH 43017.

and floodwater mosquitoes in a variety of polluted and/or fresh water habitats.

MATERIALS AND METHODS

Bioassay. A series of bioassays were conducted to determine the effectiveness of ISA-20E in killing larvae and pupae, inhibiting eclosion of egg rafts, and entrapping and drowning caged adults of laboratory-reared *Cx. quinquefasciatus*. In tests against eggs and immature stages, desired amounts of ISA-20E were pipetted with a microsyringe in 400 ml glass beakers containing ca. 250 ml of well water purified by reverse osmosis (RO) filtration and 10–20 larvae and/or pupae (2–3 replications/test) or one egg raft (3 replications/test). Egg rafts were tested ca. 18 hr post-introduction of oviposition cups to gravid mosquitoes. Percentage mortality of larvae and pupae was recorded at 24 hr intervals. The number of 1st instar larvae recovered at 24 hr post-treatment was the criterion used to evaluate percentage eclosion. ISA-20E bioassay procedures used for immature mosquitoes were described by Levy et al. (1981); data were analyzed by analysis of variance.

The effect of ISA-20E on adults was determined in cages (45.6 and 61.0 cm^3) containing mixed populations of a few thousand males and females; females were blood-fed and egged once prior to testing. The mosquitoes were given sugar cubes and sugar water feeders as source of carbohydrates. One or two glass crystallizing dishes (125 mm O.D.) filled with ca. 250 ml of RO water treated with ISA-20E were introduced into a central area or corner of a screened cage, respectively. An RO water control containing no monomolecular film was placed in each cage on the opposite or adjacent corner of a crystallizing dish containing ISA-20E. Percentage entrapment of adults according to sex was recorded 24 hr post-treatment. All bioassays were conducted in a room maintained at ca. 26–27° C (ambient) and 80% RH.

Field Trials. Field tests to determine the efficacy of ISA-20E in the control of natural populations of immature stages of *Cx. nigripalpus* and *Cx. quinquefasciatus* were performed in settling, polishing, and/or evapo-percolation ponds of sewage treatment systems servicing an industrial complex, a trailer park, and an elementary school. In addition, trials were conducted against natural populations of *Cx. nigripalpus* breeding in fresh to brackish water in a road-side ditch, drainage swale, marsh impoundment, discarded tires, and a storm water detention/retention basin. The water surface area of the polluted and fresh water habitats ranged from 103.2 to 452.1 m^2 and <0.5 to 307.6m^2, respectively.

Semi-permanent habitats formed as a result of intermittent rain were treated with varying dosages of ISA-20E to control larvae and pupae of *Ps. columbiae*. Test sites included grassy fields, impounded areas, roadside ditches and drainage swales; the surface areas ranged from 15.2 to 2365.9 m^2.

ISA-20E was applied to the water surface with a plastic non-pressurized hand sprayer (Levy et al. 1980) which was adjusted to dispense ca. 1.2 ml of ISA-20E each time the spray trigger was depressed. Sampling of immature *Culex* and *Psorophora* spp. before and at 24 hr intervals after treatment, monitoring ISA-20E persistence with an indicator oil and determining the efficacy of ISA-20E against natural populations of larvae and pupae were conducted according to procedures reported earlier (Levy et al. 1980, 1981). Data were analyzed by analysis of variance. Certain climatological and water quality data were obtained at various intervals in each field trial along with data concerning the effect of ISA-20E on non-target organisms.

RESULTS AND DISCUSSION: BIOASSAY

Larvae and Pupae. In bioassays the mean percentage mortality of 1st–4th instar larvae of *Cx. quinquefasciatus* ranged from 0–37, 0–50, 13–80, and 42–85 at 24, 48, 72, and 96 hr post-treatment, respectively (Table 1), indicating that ISA-20E was not effective in killing larvae of this species under these conditions. However, data from field trials with ISA-20E against mixed populations of *Cx. nigripalpus* and *Cx. quinquefasciatus* in sewage treatment systems showed that cumulative mortality of 90% or greater was obtained in most field trials within 48 hr after treatment (Levy et al. 1980). A 90% mortality level was not achieved in bioassays until 120 hr post-treatment or longer (Table 1). Although larvae were highly tolerant of ISA-20E under bioassay conditions, pupae exhibited a significantly greater sensitivity (Table 1). Mean mortality of 30–96% and 97–100% was achieved with *Cx. quinquefasciatus* pupae at 24 and 48 hr post-treatment, respectively. In addition, comparable levels of mortality were observed when pupae were exposed to a reduced dosage of ISA-20E (Table 1). This increased sensitivity of the pupal stage was reported by Levy et al. (1980, 1981) in laboratory and field trials.

The significant difference in mortality obtained in laboratory and field tests against *Cx. quinquefasciatus* was attributed to the nature of the bioassay system, i.e. the test chamber and the quality of the

Table 1. Bioassay of immature stages of *Cx. quinquefasciatus* with ISA-20E at a surface dosage of 0.25 ml/m^2.

Tests according to stage (instar)	Cumulative percentage mortality of larvae, pupae, and/or emerging adults at indicated post-treatment time period (range)[1]			
	24 hr	48 hr	72 hr	96 hr
larval (1st)	0	0	—	—[2]
larval (2nd)	10.0(10.0)	25.0(20.0–30.0)	40.0(30.0–50.0)	55.0[3](50.0–60.0)
larval (2nd)	35.0(30.0–40.0)	50.0(50.0)	55.0(50.0–60.0)	55.0(50.0–60.0)
larval (2nd)	0	0	13.3(10.0–20.0)	30.0(20.0–40.0)[4]
larval (3rd)	13.3(0–30.0)	16.7(0–30.0)	16.7(0–30.0)	—[5]
larval (3rd)	16.7(10.0–25.0)	28.3(20.0–45.0)	30.0(20.0–45.0)	41.7(30.0–55.0)
larval (3rd–4th)	0	13.3(10.0–20.0)	36.7(20.0–50.0)	46.7(20.0–80.0)
larval (4th)	0	16.7(0–30.0)	46.7(30.0–70.0)	—[6]
larval (4th)	36.7(20.0–40.0)	43.3(20.0–60.0)	73.3(40.0–90.0)	—
larval (4th)	10.0(0–20.0)	35.7(28.5–42.9)	39.2(39.2)	64.3(64.3)[7]
larval (4th)	5.0(0–10.0)	—	—	85.0(80.0–90.0)[8]
larval (4th)	36.7(30.0–50.0)	36.7(30.0–50.0)	80.0(60.0–90.0)	—
larval (4th)	16.7(13.3–20.0)	23.3(20.0–26.7)	36.7(26.7–46.7	46.7(40.0–53.3)[9]
larval (4th) & pupal	57.6(49.1–59.3)	59.3(54.2–69.5)	—	96.6(89.7–100)
pupal[10]	25.0(20.0–30.0)	98.3(94.9–100)	—	—
pupal	53.6(46.4–57.1)	96.5(89.3–100)	—	—
pupal	30.0(20.0–40.0)	100	—	—
pupal	96.3(88.9–100)	100	—	—

[1] Mortality corrected by Abbott's formula.

[2] 43.3% (20.0–70.0) and 56.7% (30.0–80.0) mortality at 120 and 216 hr post-treatment, respectively.

[3] Larvae appear to be 2nd instar; 4th instar larvae and pupae in controls.

[4] 70.0% (60.0–80.0) and 96.7% (90.0–100) mortality at 144 and 288 hr post-treatment, respectively.

[5] 50.0% (10.0–90.0) mortality at 144 hr post-treatment.

[6] 93.3% (90.0–100) mortality at 168 hr post-treatment.

[7] 74.1% (57.1–67.8) mortality at 120 hr post-treatment.

[8] 95.0% (90.0–100) mortality at 120 hr post-treatment.

[9] 63.3% (60.0–66.7) mortality at 144 hr post-treatment.

[10] Surface dosage of 0.1 ml/m^2.

water used in the tests. Bioassays were effected by evaporation of water and subsequent loss of surface film on the sides of the glass beakers even though beakers were loosely covered with a sheet of polyethylene to retard evaporation. In several tests, the bead of excess ISA-20E that was produced at the test dosage (0.25 ml/m^2) was observed to cling to the side of the beakers as the water level dropped. The increased adherence of the bead of ISA-20E was also observed when plastic containers were used and when beakers were not thoroughly clean. The surface to volume ratio in the test chamber was not comparable to the field sites and therefore was presumed to enhance the aforementioned observation. In addition, bioassay observations indicated that larvae and pupae exposed to ISA-20E tended to congregate around the meniscus at the water-beaker interface more than mosquitoes in control containers. This condition possibly facilitated penetration of their siphons or trumpets through the interface and film to obtain atmospheric oxygen and prolonged survival.

Although these physical factors were presumed to adversely affect the action of ISA-20E in the bioassay system, a major factor accounting for the difference in the rate of larvicidal and pupicidal action of ISA-20E in laboratory and field tests appeared to be the differences in dissolved oxygen (DO) concentrations. Dissolved oxygen levels of 0.1–0.3 ppm were observed in the sewage systems in the present study. Slower ISA-20E-induced kill of immature *Culex* spp. was observed in sewage treatment basins when the DO level was 1.2 ppm (Levy et al. 1981). The DO in the RO water used in bioassays ranged from 3.8 to 4.8 ppm. Since *Cx. quinquefasciatus* can live in polluted water of extremely low oxygen concentration, prolonged survival at unusually high oxygen levels during exposure to the surface film can be expected to occur from cuticular respiration and air storage in the tracheal system. Also, various methods of filling the beakers with water was shown to differentially oxygenate the water just prior to addition of the surface film.

Laboratory tests against larvae of *Cx. quinquefasciatus* exposed to a surface dosage of 0.25 ml ISA-20E/m^2 in a closed bioassay system containing water deoxygenated by boiling and/or the addition of sodium sulfite resulted in a significant increase in the rate of larvicidal and pupicidal action (Levy et al. unpublished).

EGG RAFTS. No adverse effect on eclosion was observed when egg rafts of *Cx. quinquefasciatus* were topically treated with 0.0012 ml ISA-20E or when the water containing egg rafts was treated with a surface dosage of 0.25 ml ISA-20E/m^2 (Table 2). However, a significant inhibition in eclosion resulted when egg rafts were immediately submerged after being topically treated with ISA-20E. These egg rafts were observed to immediately sink to the bottom of the beaker and then

Table 2. Effect of ISA-20E on egg rafts of *Cx. quinquefasciatus*.

Bioassay procedure[1]	Mean no. eggs/raft (range)	Mean % eclosion (range)
Film applied topically to egg rafts	85.2 (43–148)	78.9 (55.8–85.2)
Film applied topically to egg rafts; egg rafts submerged	74.4 (45–86)	4.0 (0–17.8)
Film applied to water surface	42.8 (22–53)	90.7 (81.1–100)
Control—egg rafts submerged	54.5 (34–75)	83.5 (73.5–88.0)
Control—egg rafts not submerged	62.7 (52–75)	92.0 (86.5–100)

[1] ISA-20E applied at 0.0012 ml/beaker (0.0012 ml/beaker = 0.25 ml/m^2 surface dosage).

sometimes rise and temporarily maintain a position just under the film-coated water surface before again sinking to the bottom. These data suggest that an impact on reducing immature *Culex* populations may be enhanced in certain situations in natural habitats by using the appropriate application technique for ISA-20E. It is recommended that a high pressure spray system be utilized in areas along the vegetative perimeter where *Culex* spp. typically oviposit. Wetting, sinking, and subsequent inactivation of egg rafts may result from this procedure while simultaneously reducing existing larval and pupal populations. Application of ISA-20E to the water surface of a sewage treatment pond with a non-pressurized hand sprayer had little or no observable impact on egg rafts present around the vegetation perimeter (Levy et al. 1980).

Adults. Cage studies indicated that ISA-20E-treated water could entrap and drown males and females of *Cx. quinquefasciatus* within 24 hr after treatment (Table 3). A significantly greater percentage of males and females were entrapped in the ISA-20E-treated dishes when compared to the untreated controls and a significantly greater percentage of females were entrapped than males. A majority of the mosquitoes in the controls were still moving on the water surface and only a few had submerged and drowned. However, all mosquitoes entrapped in the container treated with ISA-20E were dead and ca. 90% of the adults had submerged. Although a total of 33 egg rafts were laid in the controls, only 7 were found in film-treated dishes. Several drowned females were observed to contain partially extruded egg bunches. It is presumed that the reduced surface tension caused by ISA-20E inhibited the ovipositing females from maintaining a normal egg-laying orientation at the surface of the water. Numerous dead adults (mainly females) were observed submerged and floating on the water surface in sewage treatment systems sprayed with ISA-20E (Levy at al. 1980).

CULEX FIELD TRIALS

Sewage Treatment Systems. Results of spray tests with ISA-20E against mixed populations of immature stages of *Cx. nigripalpus* and *Cx. quinquefasciatus* breeding in aeration and decomposition ponds of sewage treatment systems are presented in Table 4. Wind velocities recorded during the pre- and post-treatment sampling periods ranged from <3.2 to gusts up to 16.1 kmph but the direction of the wind remained constant during each test. Water temperature, DO, conductivity, pH and rainfall measurements at these sampling intervals ranged from 15–30° C, 0.2–0.3 ppm, 850–1,000 μmhos/cm, 7.4–7.5, and 0–0.5 cm, respectively.

Control of immatures at the termination of all trials ranged from 91.9–98.4%.

Table 3. The effectiveness of ISA-20E in entrapping caged adults of *Cx. quinquefasciatus.*[1]

Cage no.	Container	Number of adult mosquitoes entrapped			Percentage entrapped[2]		
		Males	Females	Total	Males	Females	Total
1	ISA-20E	531	386	917	69.8	96.0	78.8
	Control	230	16	246	30.2	4.0	21.2
2	ISA-20E	1225	678	1903	78.9	94.7	83.9
	Control	327	38	365	21.1	5.3	16.1

[1] Surface dosage of 0.25 ml/m².

[2] Based on total number entrapped in ISA-20E and control containers —

$$\text{(i.e. \% entrapped} = \frac{\text{ISA-20E}}{\text{ISA-20E + Control}} \text{ or } \frac{\text{Control}}{\text{ISA-20E + Control}}\text{)}$$

Table 4. Percent reduction of immature stages of *Cx. nigripalpus* and *Cx. quinquefasciatus* in sewage treatment systems after spray application of IAS-20E.

Test designation	Film dosage (ml/m²)	Pre-treatment mosquito samples: No. larvae by instar and pupae (P); Total (T)						Post-treatment												
									No. larvae by instar and pupae						% reduction of larvae by instar and pupae					
		1	*2*	*3*	*4*	*P*	*T*	Hr	*1*	*2*	*3*	*4*	*P*	*T*	*1*	*2*	*3*	*4*	*P*	*T*
1	0.25	51	513	613	841	203	2221	24	13	9	4	58	79	163	74.5	98.2	99.3	93.1	61.1	92.7
								48	3	4	4	20	167	198	94.1	99.2	99.3	97.6	17.7	91.1
2	0.30	0	227	159	530	1362	2278	24	0	61	27	92	5	185	—	73.1	83.0	82.6	99.6	91.9
								48	0	13	22	75	0	110	—	94.3	86.2	85.8	100	95.2
								96	0	49	3	12	0	64	—	78.4	98.7	97.7	—	97.2
								144	0	19	3	15	0	37	—	91.6	98.7	97.2	—	98.4
3	0.33	283	2646	1488	312	282	5011	24	531	1788	1690	638	232	4879	0*	32.4	0*	0*	17.7	2.6
								48	674	1172	715	884	208	3653	0*	55.7	51.9	0*	26.2	27.1
								72	46	495	1345	1360	178	3424	83.7	81.3	9.6	0*	36.9	31.7
								96	943	2025	1055	1115	101	5239	0*	23.5	29.1	0*	64.2	0*
								120[1]	—	—	—	—	—	—	—	—	—	—	—	—
								144	0	21	118	30	29	198	100	99.2	92.1	90.4	89.7	96.0
4	0.45	426	348	50	21	67	912	24	11	13	0	6	8	38	97.4	96.3	100	71.4	88.1	95.8

* Increase over pre-treatment samples.

[1] Retreated with ISA-20E at 0.33 ml/m², high concentrations of 1st–2nd instar larvae from new egg hatch.

The presence of ISA-20E was indicated at the final post-treatment sampling period in each test. Percentage control of immature *Culex* spp. in tests 1, 2, and 4 ranged from 91.9–95.8% within 24 hr post-treatment; however, control of immatures at 24 hr post-treatment in test 3 was only 2.6%. Tests 2 and 3 were conducted in the same sewage system under similar wind and oxygen conditions. However, the water surface during test 2 was clear and open while in test 3 it contained high concentrations of green algae. Poor mosquito control in test 3 was attributed to a combined effect from persistent unidirectional gusting winds and to an unusually high algae content over the entire surface of the test site inhibiting the normal spreading/respreading qualities of ISA-20E. High concentrations of egg rafts were found at various sampling intervals during test 3 and therefore affected the percentage reduction by increasing the 1st–2nd instar larval post-treatment populations. Use of the indicator oil in test 3 showed that the film was compacted in a small section in the downwind portion of the habitat; however, stability of the film was difficult to determine with the indicator because of the natural surface films produced by the high concentration of green algae. However, sampling for pupal skins indicated that no observable adult emergence had occurred during the 120 hr period. Dead females were also observed on the surface, possibly indicating film-entrapment during periods when wind effects were minimal.

Since control of immatures in test 3 was poor at 120 hr post-treatment, the habitat was retreated at the initial dosage. At this time the algae concentration was much reduced making application easier even though the wind continued to be a problem. Excellent control was achieved at 24 hr after the habitat had been retreated (144 hr postinitial treatment). It is recommended that a high pressure spray system can be used under these surface characteristics to assure better film penetration through algal or vegetative mats (Levy et al. 1981). Furthermore, preliminary observations have indicated that ISA-20E appears to have no detrimental impact on larvae of certain midge species (Chironomidae) but may be useful in controlling pupae and emerging adults of these nuisance species in sewage treatment systems (Levy et al. 1981).

FRESH/BRACKISH WATER HABITATS. Table 5 presents data on the control of immature stages of *Cx. nigripalpus* in fresh and brackish water habitats with ISA-20E. The wind speed in these tests ranged from <3.2 to gusts up to 24.1 kmph; wind direction was constant. The water temperature, conductivity, and pH of the test habitats ranged from 25–30° C, 600–7,000 μmhos/cm, and 7.2–7.6, respectively. In general, the mosquito habitats were too shallow to obtain accurate oxygen measurements with the oxygen probe utilized. Cumulative reduction of larvae and pupae in 5 field trials ranged from 72.4–99.9% and 67.7–100% within 24 and 48 hr post-treatment, respectively. Unidirectional winds gusting to 16.1 kmph and habitat drying recorded during test 2 were presumed to be responsible for the reduced mortality observed 48 hr after treatment. Disruption of ISA-20E integrity over the water surface by the wind resulting in poor kill of larvae and pupae has been reported by Levy et al. (1981) and is presumed to be the major factor responsible for ineffective or delayed control with this film. Results of fresh/brackish water field trials against *Cx. nigripalpus* were consistent with those obtained at sewage treatment systems against *Cx. nigripalpus* and *Cx. quinquefasciatus*.

PSOROPHORA FIELD TRIALS

Results of all field trials against *Psorophora* spp. (Table 6) with ISA-20E at surface dosages of 0.20–0.45 ml/m² (0.21–0.48 gal/acre) indicated that mortality of immature stages at the termination of an experiment after 24, 48, or 72 hr ranged from 93.1–100%. One hundred percent mortality of 3rd–4th in-

Table 5. Percent reduction of immature stages of *Cx. nigripalpus* in freshwater habitats after spray application of IAS-20E.

		Pre-treatment mosquito samples						Post-treatment												
		No. larvae by instar and pupae (P); Total (T)							No. larvae by instar and pupae						% reduction of larvae by instar and pupae					
Test designation	Film dosage (ml/m²)	*1*	2	*3*	*4*	*P*	*T*	Hr	*1*	2	*3*	*4*	*P*	*T*	*1*	2	*3*	*4*	*P*	*T*
1	0.40	0	0	0	8	12	20	24	0	0	0	1	0	1	—	—	—	87.5	100	99.9
								48	0	0	0	0	0	0	—	—	—	100	100	100
2	0.45	0	259	166	54	7	486	24	0	11	80	21	22	134	—	95.8	51.8	61.1	0*	72.4
								48[3]	0	0	35	72	50	157	—	100	78.9	0*	0*	67.7
								72[4]	—	—	—	—	—	—	—	—	—	—	—	—
3	0.45	92	94	164	24	2	376	24	0	3	11	12	2	28	100	96.8	93.3	50.0	0	92.6
								48	0	1	3	1	1	6	100	98.9	98.2	95.8	50.0	98.4
4	—[1]	25	35	50	75	20	205	48	0	0	0	0	0	0	100	100	100	100	100	100
5	1.0[2]	20	15	40	35	7	117	24	4	3	9	4	2	22	80.0	80.0	77.5	88.6	71.4	81.2
								48	0	0	0	0	0	0	100	100	100	100	100	100

* Increase over pre-treatment samples.

[1] One spray of ISA-20E per tire (ca. 1.2 ml).

[2] *Cx. quinquefasciatus* breeding among refuse; dense surface debris.

[3] Greater than 50% of habitat dry; immatures concentrated.

[4] Habitat completely dry.

Table 6. Percent reduction of immature stages of *Psorophora columbiae* in natural habitats after spray application of IAS-20E.

		Pre-treatment mosquito samples						Post-treatment												
		No. larvae by instar and pupae (P); Total (T)							No. larvae by instar and pupae						% reduction of larvae by instar and pupae					
Test designation	Film dosage (ml/m²)	*1*	2	*3*	*4*	*P*	*T*	Hr	*1*	2	*3*	*4*	*P*	*T*	*1*	2	*3*	*4*	*P*	*T*
1	0.45	0	3	28	0	0	31	24	0	0	7	0	0	7	—	100	75.0	—	—	77.4
								48[1]	—	—	—	—	—	—	—	—	—	—	—	—
								72	0	0	0	0	0	0	—	100	100	—	—	100
2	0.45	0	0	5	40	0	45	24	0	4	0	4	0	8	—	0*	100	90.0	—	82.3
								48	0	0	0	0	0	0	—	100	100	100	—	100
3	0.45	0	0	0	178	0	178	24	0	0	0	81	0	81	—	—	—	54.5	—	54.5
								48	0	0	0	24	0	24	—	—	—	86.5	—	86.5
								72	0	0	0	10	0	10	—	—	—	94.4	—[4]	94.4
4	0.45	0	0	0	56	92	148	48	0	0	0	0	5	5	—	—	—	100	94.6	96.6
5	0.45	0	0	0	0	108	108	24	0	0	0	0	0	0	—	—	—	—	100	100
6	0.45	0	0	0	0	37	37	24	0	0	0	0	0	0	—	—	—	—	100	100
7	0.40	0	0	0	0	23	23	24	0	0	0	0	0	0	—	—	—	—	100	100
8	0.20	0	0	0	0	82	82	24	0	0	0	0	0	0	—	—	—	—	100	100
9	0.20	0	5	0	25	42	72	24	0	5	0	5	0	10	—	0	—	80.0	100	86.1
								48	0	0	3	2	0	5	—	100	0*	92.0	100	93.1
10[2,3]	0.25	0	1	88	2	9	81	24	0	0	5	1	0	6	—	100	95.3	50.0	—	93.4

* Increase over pre-treatment samples.

[1] Rain increased size of test site 10–15%, new brood hatched increasing number of second instar (no samples collected).

[2] Mixed populations of *Ps. ciliata*.

[3] Habitat 90% dry 24 hr post-treatment.

[4] Dead pupae found.

star larvae of *Ps. ciliata* (Fabricius) occurred within 24 hr after treatment. High mortality of larvae of *Ps. columbiae* (>90%) was delayed beyond 24 hr in 4 out of 10 field trials. Nevertheless, the data indicated that ISA-20E was stable in all tests at the termination of an experiment and could persist in certain *Psorophora* habitats at high film pressure and kill larvae and pupae for at least 48 hr at a dosage as low as 0.20 ml/m^2 (0.21 gal/acre).

ISA-20E was sprayed by helicopter (Levy et al. 1981) at a rate of ca. 0.4 gal/acre to control mixed populations of 4th instar larvae and pupae of *Ps. columbiae* and *Ps. ciliata* and 1st–3rd instar larvae of *Cx. nigripalpus* in an ca. 1.4 acre grassy field. High concentrations of egg rafts were also observed prior to treatment. The water surface was not contiguous but was confined to several large low lying areas throughout the field. Water temperature and wind speed ranged from 28.5 to 32.0° C and <3.2 to 4.8 kmph, respectively. One hundred percent control of immature *Psorophora* spp. resulted within 48 hr post-treatment; however, high populations of 1st–3rd instar larvae of *Cx. nigripalpus* were still present in scattered areas at the termination of the *Psorophora* test. Further observation of this site at 120 hr post-application indicated that ISA-20E was persistent at high film pressure and that >95% of the *Culex* were dead. Prolonged survival of the *Culex* sp. was mainly attributed to the high oxygen level (5–6 ppm) in the field and to the continuous hatching of egg rafts throughout the observation period. Species and instar (stage) differences were also presumed to contribute to the difference in ISA-20E sensitivity between the *Culex* and *Psorophora* spp. In general, results indicated the effectiveness of a low volume ISA-20E aerial application system for use in uncanopied mosquito habitats.

Rain and constant wind fetch were observed to disturb the integrity of ISA-20E over the surface of *Psorophora* habitats, however, these conditions were usually temporary and 90–100% larval and pupal mortality was recorded at the termination of all tests conducted with 0–2.5 cm rain, in uni- and multi-directional winds and in wind speeds fluctuating from <3.2–12.9 kmph. In addition, the effective lateral spreading pressure of ISA-20E was indicated in test 1. Furthermore, water temperatures of 27.5–35.0° C, dissolved oxygen levels of 6.3–11.0 ppm, and pH values of 7.4–8.1 were recorded during these tests.

Results of 10 field trials to determine the efficacy of ISA-20E against larvae and pupae of *Ps. columbiae* have indicated that this monomolecular organic surface film can be used effectively to control this species as well as *Ps. ciliata* under natural breeding conditions without adverse environmental effects.

CONCLUSIONS

Results of bioassays to determine the efficacy of ISA-20E against *Cx. quinquefasciatus* larvae and pupae (Table 1) indicated that the levels of mortality obtained within 48 hr post-treatment were significantly lower than the percentage mortality observed in sewage treatment systems at this time period. However, observations for several days beyond the 48 hr post-treatment period in several bioassays indicated that mortality significantly increased with exposure time under the bioassay conditions. Bioassays showed that pupae were significantly more sensitive to ISA-20E than larvae; however, mortality was also delayed when compared to results of field trials. This phenomenon was mainly attributed to the nature of the test chamber and the quality of the water (i.e. the dissolved oxygen content) used in the experiments. Results of additional bioassays against *Cx. quinquefasciatus* indicated that ISA-20E could inhibit eclosion of egg rafts if the proper application technique was used (Table 2) and could entrap and drown males and females contacting the film-treated water surface to rest, drink or oviposit (Table 3). In general, results of bioassays indicated that ISA-20E could have a potential impact in certain field situations in significantly reducing the number of adults of *Cx. nigripalpus* and *Cx. quinquefasciatus* produced from natural habitats by adversely affecting eclosion of egg rafts, adult emergence, oviposition of females and by killing larvae and pupae.

Results of 9 field trials indicated that ISA-20E could be used to effectively control natural populations of larvae and pupae of *Cx. quinquefasciatus* and *Cx. nigripalpus* in most polluted (Table 4) and fresh/brackish water (Table 5) habitats varying in water quality and affected by natural climatological variations. Similar results were observed in 10 tests against natural populations of immature *Psorophora* spp. (Table 6). Mortality of larvae and pupae in excess of 90% at the dosages tested usually occurred within 72 hr after treatment; however, adverse wind conditions and surface characteristics were observed to disrupt the integrity of ISA-20E over the surface of the water and, therefore, inhibit effective kill of larvae and pupae. Recommended field surface dosages for controlling the immature stages of these species are 0.30–0.50 gal ISA-20E/acre. The dosage may vary with habitat surface characteristics, application techniques, environmental conditions, and stage of development. Observations on the general environmental safety of ISA-20E to fish and wildlife were consistent with reports by White and Garrett (1977) and Levy et al. (1981); however, some field observations indicated that the reduced surface tension induced by ISA-20E may adversely affect the surface orientation of some species of Gerridae, Hydrometridae, and Chironomidae under certain environmental conditions. In general, data concerning the efficacy of ISA-20E in controlling natural populations of *Culex, Aedes,* and *Anopheles* spp., stage-related sensitivity to ISA-20E, persistence of ISA-20E under field conditions, effective surface dosages, and the effects of certain environmental parameters on the larvicidal and pupicidal action of ISA-20E have been presented by Levy et al. (1980, 1981).

References Cited

Levy, R., J. J. Chizzonite, W. D. Garrett and T. W. Miller, Jr. 1981. Ground and aerial application of a monomolecular organic surface film to control salt-marsh mosquitoes in natural habitats of southwestern Florida. Mosq. News 41:291–301.

Levy, R., W. D. Garrett, J. J. Chizzonite and T. W. Miller, Jr. 1980. Control of *Culex* spp. mosquitoes in sewage treatment systems of southwestern Florida with monomolecular organic surface films. Mosq. News 40:27–35.

White, S. A. and W. D. Garrett. 1977. Mosquito control with monomolecular organic surface films: II–Larvicidal effect on selected *Anopheles* and *Aedes* species. Mosq. News 37:349–53.