GROUND AND AERIAL APPLICATION OF A MONOMOLECULAR ORGANIC SURFACE FILM TO CONTROL SALT-MARSH MOSQUITOES IN NATURAL HABITATS OF SOUTHWESTERN FLORIDA[1]

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ABSTRACT. The efficacy of monomolecular organic surface films of isostearyl alcohol containing 2 oxyethylene groups was evaluated at varying dosages against natural populations of *Aedes taeniorhynchus* Wiedemann in several salt-marsh habitats. Results of ground and aerial spray application at surface dosages of 0.30 – 0.45 ml/m² (0.32 – 0.48 gal/acre) indicated that this chemical can be used effectively to control larvae and pupae of this *Aedes* sp. under a wide range of field conditions. Methodology for field application, mode of action of this chemical on immature mosquitoes, and environmental factors which influence the surface-film method are discussed.

When applied to the surface of a mosquito habitat a thin film of isostearyl alcohol containing 2 oxyethylene groups (ISA-20E)[4] can disrupt the normal orientation of immature mosquitoes at the air-water interface by reducing the water surface tension to less than 29 dynes/cm. This physical process can kill larvae and pupae by increasing the wetting of tracheal structures and subsequently causing anoxia (suffocation). Also it can adversely affect the emergence of adult mosquitoes. Since ISA-20E-induced mortality of immature mosquitoes is presumed to be produced by physical factors and not by classical chemical toxicity induced by many conventional larvicides, resistance to this material is not expected to develop. This monomolecular organic surface film is a non-ionic, non-petroleum, biodegradable surface active chemical that should have a long shelf-life, can be used in the field over a wide temperature range, and will not spread over its own monomolecular film (Garrett and White 1977). Tests have indicated it has little or no adverse effects on mammals (Reynolds, personal communication) and several species of non-target aquatic organisms (White and Garrett 1977, Levy et al. 1980).

The efficacy of ISA-20E in controlling natural populations of larvae and pupae of *Culex nigripalpus* Theobald and *Cx. quinquefasciatus* Say in an industrial sewage treatment system was reported by Levy et al. (1980). Results of field trials in sewage settling, polishing, and evapo-percolation ponds indicated that 97% control of immature *Culex* spp. could be obtained at a spray dosage as low as 0.33 ml ISA-20E/m² water surface (0.35 gal/acre). Data from laboratory and/or simulated field studies have indicated that ISA-20E is highly effective in killing 4th instar larvae and pupae of *Anopheles quadrimaculatus* Say (White and Garrett 1977) and pupae of *Aedes aegypti* (L.) (Garrett 1976) at a surface dosage of 0.04 ml/m² (0.043 gal/acre). However, at the same surface dosage, they showed that ISA-20E was not effective in controlling larvae of *Ae. taeniorhynchus* Wiedemann (White and Garrett 1977) and *Ae. aegypti* (Garrett 1976).

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[1] Mention of a brand name or proprietary product does not constitute a guarantee or warranty by Lee County Mosquito Control District, and does not imply its approval to the exclusion of other products that may also be suitable.

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[4] ISA-20E is manufactured for use as a cosmetic ingredient by Sherex Chemical Co., Inc., P. O. Box 646, Dublin, Ohio 43017, under the trade name Arosurf 66-E2.

In addition to the control of *Culex* spp. in polluted and fresh water areas, the major portion of the Lee County Mosquito Control District program is aimed at the control of flood-water mosquitoes breeding in semi-permanent habitats created by intermittent tides and/or rain. Although *Psorophora columbiae* Dyar and Knab is a severe pest during the rainy season, our principal target species throughout the year is *Ae. taeniorhynchus* which breeds in the 66,000 acres of coastal salt-marsh of Lee County, Florida. Therefore, investigations were conducted to determine the efficacy of ISA-20E for controlling larvae and pupae of *Ae. taeniorhynchus* in natural habitats of Lee County, Florida.

METHODS AND MATERIALS

Salt-marshes mainly characterized by white, black and red mangrove, cenicella, and brazilian pepper, as well as potholes, ditches, ponds, and fields breeding immature stages of *Ae. taeniorhynchus* were sprayed with varying concentrations of ISA-20E. The water surface area of the test habitats ranged from 6.5–3582.7 m².

Surface applications were made with a small plastic hand-activated pump sprayer (Levy et al. 1980) at dosages of 0.30–0.45 ml/m² (0.32–0.48 gal/acre). Aerial applications were made from a Bell 47G helicopter equipped with a Simplex® spray system having one 52 gal tank filled with ca. 2 gal of ISA-20E and a boom (9.75m) fitted with 18 TeeJet® nozzles (No. 6135) with D2 orifices. This spray system was calibrated to dispense ca. 0.4 gal ISA-20E/acre or 0.37 ml ISA-20E/m² water surface at a pump pressure of 11–12 psi. Three helicopter tests were conducted at an air speed of ca. 74 kmph and at an altitude of ca. 9–15 m. To achieve the desired surface dosage (ca. 0.37 ml/m²), 2–4 spray swaths were flown (1 swath = ca. 15.2–18.3 m) over the test areas. Wind speed at the time of application was 12.9–16.1 kmph with gusts up to 25.7 kmph in test 1, 8.0–11.3 kmph in test 2, and $<$3.2–4.8 kmph in test 3.

About 1 hr prior to treatment with ISA-20E, 3–25 mosquito samples were obtained with a pint dipper from several locations in each test site. The number of mosquito samples collected was based on the size of the habitat as well as on the concentration and spatial distribution of immatures at the time of sampling. The numbers of *Ae. taeniorhynchus* pupae and larvae according to instar were recorded as well as certain physical and environmental measurements, meteorological characteristics, and water quality characteristics associated with each test site. Samples were obtained at 24, 48, 72, 96, and/or 144 hr post-treatment in a manner consistent with pre-treatment procedures. Persistence (stability) of ISA-20E was monitored at each post-treatment sampling period with an indicator oil in a manner previously described by Levy et al. (1980).

Percentage reduction of larvae and/or pupae at each sampling interval was the main criterion used to evaluate the efficacy of ISA-20E against *Ae. taeniorhynchus*. Data were evaluated according to stage of development and dosage by analyses of variance. The effect of ISA-20E on non-target vertebrate and invertebrate aquatic organisms was also recorded.

A series of bioassays was conducted against laboratory-reared larvae and pupae of *Ae. taeniorhynchus*. These tests were conducted in 400 ml glass beakers containing ca. 250 ml of 50% sea water (sea water diluted with distilled water) and 10 immature *Ae. taeniorhynchus*/beaker (2–3 replications/test). The salinity, conductivity, dissolved oxygen, and pH of the sea water ranged from 15.5–16.1 ppt, 26,800–27,000 μmhos/cm, 4.3–6.9 ppm, and 6.5–7.6, respectively. ISA-20E was applied to each beaker with a microsyringe at a surface dosage of ca. 0.25 ml/m². Larvae were fed ca. 10 drops of a ground, rabbit-chow, water-suspension prior to the addition of the monomolecular surface film. Beakers were loosely covered with a sheet of clear polyethylene to help prevent evaporation and subsequent loss of ISA-20E on the sides of the beakers. Percentage mortality of each immature stage was recorded at various post-treatment intervals. Tests were conducted in a room maintained at ca. 26.7°C (ambient) and ca. 80% RH.

RESULTS AND DISCUSSION

Results of tests to control natural populations of larvae and pupae of *Ae. taeniorhynchus* with ISA-20E from hand spray applications are presented in Tables 1 and 2. Data obtained at 24 hr post-treatment Table 1, tests 1 and 4–9 indicated that high mortality (98–100%) of immature stages could be achieved in certain salt-marsh/salt water habitats at a spray dosage of 0.45 ml ISA-20E/m² of water surface. In addition, ca. 15–20% of the larvae sampled in test 7 were identified as *Ae. infirmatus* Dyar and Knab, and this *Aedes* sp. was also highly susceptible to ISA-20E at the dosage applied. Helicopter application of ISA-20E (Table 2, test 1) at a surface dosage of ca. 0.37 ml/m² also resulted in 100% mortality of larvae and pupae 24 hr post-treatment. Although high mortality of larvae and pupae was observed at 24 hr post-treatment in 8 trials, results of 5 additional hand sprayer tests indicated that ISA-20E-induced mortality of most larvae of *Ae. taeniorhynchus (i.e.* 90% or greater) was delayed to 48 or 72 hr post-treatment (Table 1). Observations at 24 hr post-treatment in 5 additional field trials indicated that very low mortality of larvae (5–20%) had occurred even though persistence of ISA-20E at high film pressure was indicated in each habitat. However, a significant increase in the mortality of immature stages of *Ae. taeniorhynchus* was noted 48 hr post-treatment. High concentrations of living larvae were also present in some of these areas having high film pressure. Even though significant mortality was delayed in these 5 tests, 90% or greater reduction in the natural population of immatures was obtained by 48 or 72 hr post-treatment. Similar results indicating effective but delayed mortality were also observed at 48 and 72 hr post-treatment (Table 2, tests 2 and 3, respectively) in helicopter trials with ISA-20E. The presence of high concentrations of dead larvae and pupae (floating and/or submerged) was always observed in a test site when sampling indicated significant acute or delayed mortality.

Little or no detectable differences in film persistence and percentage mortality of immature *Ae. taeniorhynchus* were noted at the dosages utilized at the termination of field trials (Tables 1–2). However, a time-related sensitivity of *Ae. taeniorhynchus* according to stage of development was noted in these trials, *i.e.* pupae usually exhibited a very noticeable stress response characterized by abdominal flexing within minutes after application of ISA-20E at dosages ranging from 0.30–0.45 ml/m². Mortality of pupae was usually observed 5–7 hr post-application. Comparable levels of larval mortality were not observed until 24–72 hr after treatment even though 3rd and 4th instar larvae were observed to react to the presence of ISA-20E almost immediately after contacting the surface film. They dropped from the surface and formed an "0" shape in the process of trying to remove the film from the opening of their siphons with their mouthparts. Molting of larvae in the presence of ISA-20E was observed in tests 1B and 11–12 (Table 1) and tests 2 and 3 (Table 2). It appears that the molting of larvae to higher instars and larvae to pupae at various intervals during a test may have obscured the mortality data associated with individual instars in some tests. However, some observations indicated that there was a film-induced retardation in the molting sequences of 3rd to 4th instar and 4th instar to pupae when compared to the molting of larvae in untreated areas. In addition, in several tests mortality similar to that produced by certain insect growth regulators was observed.

Bioassays (Table 3) against *Ae. taeniorhynchus* in 400 ml beakers at surface dosages of ca. 0.25 ml ISA-20E/m² indicated that there did not appear to be a

Table 1. Percent reduction of *Aedes taeniorhynchus* larvae and pupae in natural habitats after hand spray application of ISA-20E at 0.45 ml/m^2.[1]

Test no. (habitat designation)[2]	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(A)	96	303	23	6	0	428	24	0	5	3	0	0	8	100	98.3	87.0	100	—	98.1
(B)	56	1642	4	0	0	1702	24	0	8	8	0	0	16	100	99.5	0*	—	—	99.1
2(A)	0	257	198	111	0	566	48	0	124	101	62	0	287	—	51.8	49.0	44.1	—	49.3
							72	0	2	0	2	0	4	—	99.2	100	98.2	—	99.3
3(C)	0	361	0	0	0	361	48	0	0	0	0	0	0	—	100	—	—	—	100
4(C)	0	212	1395	1691	512	3810	24	0	0	0	0	0	0	—	100	100	100	100	100
5(D)	1	21	39	57	0	118	24	0	0	0	2	0	2	100	100	100	96.5	—	98.3
6(E)	0	0	11	0	0	11	24	0	0	0	0	0	0	—	—	100	—	—	100
7(F)[3]	0	5	1	8	0	14	24	0	0	0	0	0	0	—	100	100	100	—	100
8(G)	50	0	0	0	0	50	24	0	0	0	0	0	0	100	—	—	—	—	100
9(H)	34	240	19	0	0	293	24	0	6	0	0	0	6	100	97.5	100	—	—	98.0
							48	0	0	0	0	0	0	100	100	100	—	—	100
10(I)	0	445	125	3389	91	4050	72	0	0	0	0	12	12	—	100	100	100	86.8	99.7
11(I)	0	88	3276	0	0	3364	48	0	0	65	283	0	348	—	100	98.0	0*	—	89.7
							72	0	0	7	207	0	214	—	—	99.8	0*	—	93.6
							96	0	0	0	169	4	173	—	—	100	0*	0*	94.9
12(I)	0	219	43	2596	0	2858	72	0	0	46	5	10	61	—	100	0*	99.8	0*	97.9
							144	0	0	56	1	5	62	—	100	0*	99.9	0*'	97.8

[1] Tests 10–12 were treated at 0.41, 0.33 and 0.30 ml/m^2, respectively.
[2] Repeat letters indicate re-test of same site.
[3] Mixed population of *Ae. infirmatus.*
* Increase over pre-treatment sample.

Table 2. Percent reduction of *Aedes taeniorhynchus* larvae and pupae in natural habitats after helicopter spray application of ISA-20E at 0.37 ml/m².

Test no. (habitat designation)[1]	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(J)	0	0	0	97	10	107	24	0	0	0	0	0	0	—	—	—	100	100	100
2(J)	0	12	200	95	0	307	24	0	0	0	140	0	140	—	100	100	0*	—	54.4
							48	0	0	0	15	4	19	—	—	—	84.2	0*	93.8
							72	0	0	0	0	0	0	—	—	—	100	100	100
3(I)	0	2376	1585	0	0	3961	24	0	478	2423	621	0	3522	—	79.9	0*	0*	—	11.1
							48	0	0	203	1756	278	2237	—	100	87.2	0*	0*	43.5
							72	0	0	72	111	0	183	—	—	95.5	0*	100	95.4
							96	0	0	0	0	0	0	—	—	100	100	—	100

[1] Repeat letters indicate re-test of same site.
* Increase over pre-treatment sample.

clearly defined relationship between percentage mortality of larvae according to instar at the various post-treatment intervals. However, actively molting, mixed larval populations seemed to show less acute sensitivity to ISA-20E than a single larval instar. Mortality was seen to increase with exposure time therefore indicating that ISA-20E at this dosage rate could persist in the beakers at least 4 days and kill *Ae. taeniorhynchus* larvae. In general, data generated from field trials concurred with bioassay data and showed no significant correlation between percentage mortality of larvae according to instar at the dosages tested. One hundred percent mortality of *Ae. taeniorhynchus* pupae resulted by 24 hr post-treatment in laboratory tests with ISA-20E at a surface dosage as low as 0.10 ml/m² (Table 3). Field and laboratory observations of mixed populations of pupae and larvae exposed to ISA-20E were consistent.

Observations indicated that ISA-20E is very stable in the field and can be effective in killing mosquito larvae and pupae in some salt-marsh habitats for at least 6 days when applied at a surface dosage as low as 0.30 ml/m² (0.32 gal/acre). In field test 2A (Table 1), ISA-20E at a surface dosage of 0.45 ml/m² persisted for the first 48 hr post-treatment but was not present 72 hr post-treatment. This was attributed to wind gusts and rainstorms which occurred at 24 hr after application. Nevertheless, 99% mortality was recorded at the 72 hr sampling period. With the exception of this test, persistence of ISA-20E was observed at all surface dosages at the termination of all experiments. Water temperature dropped to 14°C at 144 hr post-application in test 12 (Table 1) and was presumed to be responsible for the retarded decay rate of the material. In addition, ISA-20E did not usually persist for longer than 48 hr post-application in sewage treatment systems (Levy et al. 1980). The difference between field stability of ISA-20E applied to salt-marshes and sewage treatment systems was mainly attributed to the microbial and/or chemical degradation of

Table 3. Bioassay of immature stages of *Aedes taeniorhynchus* with ISA-20E at a surface dosage of 0.25 ml/m².

Test no.	Stage (instar)	Cumulative mean percentage mortality of larvae and/or pupae at indicated post-treatment time period (range)			
		24 hr	48 hr	72 hr	96 hr
1	larval (1st-2nd)	10.0(10.0)	96.7(90.0–100)	96.7(90.0–100)	100
2	larval (1st-2nd)	30.0(20.0–40.0)	50.0(40.0–60.0)	65.0(50.0–80.0)	—[4]
3	larval (2nd)	100	—	—	—
4	larval (2nd)	76.7(60.0–90.0)	100	—	—
5	larval (2nd)	55.0(30.0–95.0)	100	—	—
6	larval (3rd)	100	—	—	—
7	larval (3rd)	90.0(90.0)	95.0(90.0–100)	95.0(90.0–100)	100
8	larval (3rd)	86.7(80.0–90.0)	—	—	—[5]
9	larval (3rd-4th)	3.3(0–10.0)	23.3(0–50.0)	—	66.7[6](50.0–90.0)
10	larval (3rd-4th)	36.7(20.0–50.0)	56.7(50.0–60.0)	66.7(60.0–70.0)	73.3(60.0–80.0)
11	larval (4th)	79.3[1](58.6–100)	100	—	—
12	pupal	100	—	—	—
13	pupal	100[2]	—	—	—
14	pupal	100[3]	—	—	—

[1] Mortality (3.3%(0–10.0%)) corrected by Abbott's formula—No control mortality in other tests.

[2] 100% mortality at 24 hr at a surface dosage of 0.1 ml/m²

[3] 100% mortality at 4.5 hr post-treatment.

[4] 100% mortality at 120 hr post-treatment.

[5] 96.7% (90.0–100%) mortality at 120 hr post-treatment.

[6] 86.7% (80.0–90.0%) and 93.3(90.0–100%) mortality at 120 and 144 hr post-treatment, respectively.

ISA-20E in the sewage and industrial effluent, and the surface fluctuations caused by intermittent pumping and draining sequences used in the sewage treatment process.

Detectable quantities of ISA-20E were not present at each post-treatment sampling location within a test site. This was mainly attributed to wind speed and direction, and to the amount and location of emergent and/or floating vegetation and not to the dosages of ISA-20E that were utilized. Salt-marsh habitats breeding high concentrations of immature *Ae. taeniorhynchus* are usually densely vegetated, and subsequently the areas of water surface treated with ISA-20E are protected from wind-induced translocation and compaction of the film. Irreversible translocation and compaction of ISA-20E can be expected to occur in habitats subjected to relatively strong (16.1 kmph or greater) and unidirectional winds for several days. Observations on the movement of ISA-20E under these adverse treatment conditions have indicated that insufficient area control will usually result. Wind speeds in field tests against *Ae. taeniorhynchus* usually ranged from <3.2–16.1 kmph, however, gusts up to 25.7 kmph were also recorded in one trial.

Hand treatment of densely vegetative habitats was usually by intermittent spraying at various locations within the site and insured even coverage and better film penetration. At the dosages applied, a helicopter, wide fan hand spray, or misting application of ISA-20E over the water surface was observed to produce millions of tiny beads or droplets of excess material. In contrast, much fewer, larger and more localized lenses of excess surface film resulted when a pin-stream spray pattern was used with the hand sprayer. It is presumed that spray application utilizing a wide fan spray pattern would insure a greater particle distribution over the water surface and subsequently reduce the chance that significant concentrations of localized lenses of floating ISA-20E would be lost due to wind-induced translocation and/or drying of a habitat in various locations. This technique would also reduce loss of material from possible adherence to floating or emergent vegetation and other organic material.

One helicopter evaluation was conducted strictly to determine the efficacy of aerial penetration of ISA-20E at a dosage of 0.4 gal/acre in a highly vegetated salt-marsh habitat containing no immature mosquitoes. The wind speed at the time of aerial application was 3.2 kmph. The water surface of this habitat was not continuous but was confined to numerous potholes and low lying areas containing dense concentrations of pickerelweed. Samples showed that material was present in ca. 90% of the treatment area immediately after application but that degradation over the entire habitat had occurred within 24 hr post-treatment. Therefore, a sufficient quantity of the total dosage ($0.37\ ml/m^2$) did not penetrate the vegetation to provide the film pressure and stability necessary for effective mosquito control. It should be noted that helicopter application of ISA-20E at a dosage of ca. 0.40 gal/acre resulted in satisfactory penetration of film through a moderately vegetated mangrove habitat mainly characterized by dense areas of emergent cenicella (Table 2, test 3).

Recent data (Levy et al. unpublished) have indicated that ISA-20E can be suspended in water with agitation at recommended dosages for mosquito control and therefore be applied at a rate of 5–7 gal/acre to facilitate penetration through heavily vegetated areas. Results of preliminary field tests with a hand-held compressed air sprayer with a water-based ISA-20E formulation at ca. 5 gal/acre (0.35 gal ISA-20E/acre) against larvae and pupae of *Ae. taeniorhynchus* and *Ps. columbiae* have indicated that there were no significant differences in percentage mortality of immatures and field persistence between ISA-20E applied alone or as a water suspension (Levy et al. unpublished). Additional data (Levy et al. unpublished) indicated that spray treatment of mosquito habitats at pressures of 100–300 psi with a trailer mounted spray system greatly increased penetration of ISA-20E into densely vegetated areas and assured better coverage and subsequent control of immature mosquitoes.

INFLUENCE OF ENVIRONMENTAL FACTORS. Observations in salt-marshes 24 hr after treatment indicated that ISA-20E was usually present in areas that were far removed from the initial points of hand spray application. Thus information was obtained about the effective lateral spreading pressure of this monomolecular film when applied at little or no spray pressure. In moderate to high winds (12.9 kmph or greater) initial hand spray application of ISA-20E was at the upwind portion of a habitat. This procedure allowed the wind-pushed film to translocate larvae and pupae to the downwind portion of a habitat where increased film and population pressures could maximize mortality. Even though displacement of ISA-20E over the water surface was significant under certain wind conditions, redistribution of the film was observed when the wind speed decreased or the wind direction shifted. These observations were also reported by Levy et al. (1980) in their spray tests with ISA-20E against *Culex* spp. larvae and pupae in an industrial sewage treatment plant.

Rain (ca. 1 cm) was recorded at 24 hr post-treatment in test 2A (Table 1) with a slight increase in water surface area of the original habitat, and little or no mortality was observed. Nevertheless, the dosage of ISA-20E utilized ($0.45\ ml/m^2$) was sufficient to cover the new areas with film for 48 hr or longer and kill 99% of the larvae and pupae at 72 hr post-treatment, even though effective film pressure was not evident 72 hr after treatment. Again, a high film pressure and 100% mortality was observed during test 3 (Table 2) following a 1.3 cm rain 96 hr after treatment. Similarly, rain had little effect on the action of ISA-20E on *Culex* mosquitoes breeding in sewage treatment systems (Levy et al. 1980).

It is presumed that utilizing high dosages of ISA-20E (>0.45 ml/m^2) that would provide lenses of excess material can be an effective means of controlling a new brood of larvae in newly flooded areas adjacent to a treatment site. Therefore, an overdosing technique could be utilized in selected mosquito habitats that are known to increase in size as a result of tidal fluctuation or persistent rain. In this way, effective film pressure and uniform coverage could be expected over the entire habitat.

Reiter (1978) reported that levels of dissolved oxygen in a mosquito habitat were important in evaluating larvicidal approaches which depend on suffocation. He indicated that effective levels of larval control were most likely to be expected with a monomolecular surface film when the dissolved oxygen of the water was less than 30% saturated, the exact value depending on the mosquito species. It is presumed that prolonged survival of larvae that were prevented from normally penetrating the water interface to obtain atmospheric oxygen by a surface film such as ISA-20E was via cuticular respiration and air storage within the tracheal system. Dissolved oxygen levels of 2.8, 1.2, 0.9, 1.1, and 0.7 ppm were recorded at pre-treatment, 24, 48, 72, and 96 hr post-treatment, respectively during test 3 (Table 2). Perhaps a factor accounting for the acute sensitivity of pupae to ISA-20E was their presumed inability to carry on cuticular respiration. Dissolved oxygen levels of 0.6, 0.6, 0.7, and 3.2 ppm were recorded before treatment in tests 1A and B, 4, and 12, respectively. Total percentage mortality of larvae and pupae at 24 hr post-treatment in tests 1A and B and 4 was 98.1, 99.1 and 100, respectively (Table 1). Substantially less mortality of larvae (<10%) was observed at 24 hr post-treatment in test 12 (Table 1) thus indicating a significant negative correlation between the acute or delayed larvicidal action of ISA-20E on larvae of *Ae. taeniorhynchus* and the dissolved oxygen concentration of the mosquito habitat. Similar results were obtained in test 3 (Table 2).

High mortality (ca. 90%) of larvae and pupae of *Culex* spp. was usually obtained at 24 hr post-treatment in sewage treatment systems (Levy et al. 1980) having dissolved oxygen concentrations of 0.1–0.3 ppm. However, ISA-20E-induced mortality of larvae of *Cx. quinquefasciatus* of 90% or greater was not obtained until 96 hr post-treatment in one test in a sewage treatment polishing pond having a dissolved oxygen concentration of 1.2 ppm (Levy et al. unpublished). High acute mortality of larvae of *Cx. quinquefasciatus* exposed to ISA-20E was not obtained in laboratory bioassays conducted in highly oxygenated fresh water, further indicating the relationship between the amount of dissolved oxygen and the effectiveness of a monomolecular film.

Temperature and salinity are factors that have an effect on the solubility of oxygen in water (Spotte 1979). Temperature and the solubility of oxygen are inversely related. In addition, a rise of 10°C will cause the rate of oxygen uptake in a poikilothermic organism such as a mosquito larva to double or triple. The rate of development would also be proportionally increased. Reiter (1978) has presented data on the survival times of larvae at different temperatures. He showed that at a low concentration of dissolved oxygen larvae could survive longer at lower temperatures, presumably due to the reduced rate of respiration. Furthermore, he showed that at 32 C° the critical level of dissolved oxygen was about 50% higher than at 27°C. Pre- and post-treatment water temperatures for all *Ae. taeniorhynchus* habitats ranged from 14–38°C. Furthermore, temperature is inversely related to surface tension (Anonymous 1980).

Salinity is also inversely related to the concentration of dissolved oxygen in a mosquito habitat (Spotte 1979). An increase in salinity would cause a decrease in the amount of dissolved oxygen. For example, the salinity in test 3 (Table 2) fluctuated from 8.0–15.0 ppt over the 96 hr test period. Salinity in all test sites ranged from 3.5–22 ppt at pre-treatment.

Furthermore, the concentration of free carbon dioxide in the water is a function of pH (Spotte 1979). As the pH decreases there is an increase in the concentration of carbon dioxide. It should be noted that respiration and photosynthesis, a function of the amounts of plants and animals in a mosquito habitat, affect the pH of the water, *i.e.* respiration would cause the pH to decline while photosynthesis produces a pH increase. *Ae. taeniorhynchus* test sites were observed to range in pH from 6.7–8.3 at pre-treatment.

Within and between species differences in the tolerance of mosquito larvae to reduced levels of oxygen were presumed also to affect the acute mortality induced by ISA-20E. Field and laboratory bioassay observations of *Ae. taeniorhynchus* larvae exposed to ISA-20E indicated that there was a differential sensitivity or survival rate of larvae of the same instar over the 24 hr observation periods. Reiter (1978) has indicated that *Cx. quinquefasciatus,* a mosquito typically found in polluted habitats having low dissolved oxygen, had cuticular adaptations that allowed it to tolerate significantly lower concentrations of dissolved oxygen than 2 *Anopheles* spp. Laboratory tests at a dosage of 0.25 ml/m² have shown that *Cx. quinquefasciatus* assayed in highly oxygenated fresh water was significantly less sensitive to ISA-20E than was *Ae. taeniorhynchus* of the same instar that were tested in 50% sea water (Levy et al. unpublished). Fourth instar larvae of *Ae. taeniorhynchus* have been observed to contact vigorously the surface film in repeated attempts to penetrate through the film with their siphons at various angles to obtain atmospheric oxygen. These observations indicate that many of these angular penetrations are successful since some larvae in film-treated beakers seem to be hanging from the surface in a manner comparable to larvae in controls, thereby presumably obtaining atmospheric oxygen and prolonging survival. Furthermore, laboratory observations (Table 3) of mixed instars of *Ae. taeniorhynchus* larvae exposed to ISA-20E have suggested that prolonged survival may be partially attributed to molting at various intervals during an experiment. It is presumed that this process would remove internal and external portions of the respiratory siphon that were plugged or coated with the monomolecular film and allow larvae to attempt to repenetrate the surface film to obtain atmospheric oxygen. Therefore, the age of larvae within an instar could be important in evaluating larvicidal effects induced by a monomolecular organic surface film such as ISA-20E.

Qualitative data on non-target animals and plants (Table 4) exposed to various dosages of ISA-20E during field trials to control mosquitoes have indicated that this film will cause little or no adverse effects to the environment. Some mortality of pupae and/or emerging adults of certain midge species (Chironomidae) breeding in aeration and decomposition ponds at sewage treatment systems was noted; however, significant mortality of midges was also observed in some control (untreated) sewage ponds containing a similar layer of natural surface scums. Therefore, the true impact of ISA-20E on the reduction of the midge population is not known. Adult dragonflies were observed to oviposit in water treated with ISA-20E and a *Gambusia* sp. was observed eating large lenses of floating ISA-20E with no apparent adverse affects. Field and laboratory tests indicated that predation and asexual reproduction of the mosquito planarian *Dugesia dorotocephala* (Woodworth) and the infectivity and development of the mosquito nematode *Romanomermis culicivorax* Ross and Smith were not adversely affected by ISA-20E at surface dosages of 0.4–0.5 ml/m². Therefore, this monomolecular film may be employed in integrated mosquito control programs. Furthermore, no mortality, defoliation or discoloration of vegetation was observed in habitats treated with

Table 4. Non-target organisms observed in natural habitats before and after experimental application of ISA-20E to control immature mosquitoes.

Animals			Plants
Insects	Crustaceans	Amphibians Reptiles Fish Others	Algae Emersed Submersed Floating
Baetidae	Astacidae	Ranidae	Characeae (*Chara* spp.)
Libellulidae	Notostraca	Trionychidae (*Trionyx ferox*)	Typhaceae (*Typha* spp.)
Aeschnidae	Conchostraca	Emydidae	Poaceae (Graminae)
Gomphidae	Daphnidae	Alligatoridae	Lemnaceae
Coenagrionidae	Astacidae	(*Alligator mississippiensis*)	(*Lemna minor, Spirodela polyrhiza*)
Corixidae	Isopoda	Colubridae (*Natrix* spp.)	Verbenaceae (*Lippia lanceolata*)
Notonectidae		Poeciliidae	Avicenniaceae (*Avicennia germinans,*
Nepidae		(*Gambusia* sp.)	*Rhizophora mangle, Laguncularia*
Belostomatidae		Vorticellidae	*racemosa*)
Dytiscidae		Amoebidae	Aizoaceae (*Sesuvium portulacastrum*)
Gyrinidae		Planariidae	Anacardiaceae
Hydrophilidae		(*Dugesia dorotocephala*)	(*Schinus terebinthefolius*)
Psychodidae		Mermithidae	Schrophulariaceae
Chironomidae		(*Romanomermis culicivorax*)	(*Micranthemum umbrosum*)
Syrphidae		Tubificidae	Umbelliferae (*Hydrocotyle umbellata*)
		Hydrachnidae	Pontederiaceae (*Eichhornia crassipes*)

ISA-20E when compared to controls. Although no quantitative data were obtained concerning the effects of ISA-20E on the natural populations of animals and plants (Table 4), general observations indicated that there appeared to be no increased mortality of non-target organism when compared to untreated or control areas. Furthermore, no acute or chronic skin or eye irritation or respiratory effects were reported by spray applicators exposed to ISA-20E during experimental applications. ISA-20E has an acute oral LD_{50} for rats of 20,000 mg/kg, and has been classified as non-toxic orally and non-irritating to the eyes and skin (Reynolds, personal communication).

In summary, our data indicated that ISA-20E can be sprayed by conventional ground and aerial systems at surface dosages of 0.32–0.48 gal/acre and control larvae and pupae of *Ae. taeniorhynchus* in natural habitats varying in water qualities and climatological conditions without producing adverse health and environmental effects. However, at the dosage applied, aerial application of ISA-20E in densely vegetative salt-marsh habitats with the spray system utilized is expected to result in little or no penetration of material through the canopy. In addition, strong and persistent unidirectional winds can significantly reduce the mosquito control effectiveness of this monomolecular organic surface film. New and more effective solid and liquid formulations of ISA-20E have been developed and are presently being evaluated under a variety of field conditions.

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