FIELD EVALUATIONS OF ISA-20E FOR MOSQUITO CONTROL AND EFFECTS ON AQUATIC NONTARGET ARTHROPODS IN EXPERIMENTAL PLOTS

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ABSTRACT. The proposed mosquito control agent ISA-20E was applied to the water surface of field test plots near Bakersfield, California to determine whether or not mortality occurs in populations of indigenous mosquitoes and aquatic nontarget arthropods. Five sampling and monitoring methods were used to determine effects of application rates at 0.25, 0.5 and 1.0 ml/m² surface area.

ISA-20E was effective in reducing 3rd and 4th instars of *Aedes nigromaculis* and *Ae. melanimon* populations at 0.5 and 1.0 ml/m² surface rates, averaging 88% and 96% mortality reduction respectively. It was also effective in reducing pupae of the above species at all 3 rates. For the test on mixed *Culex tarsalis* and *Culex quinquefasciatus* populations, mortalities ranged from 49% to 100% for the 3-day test period at the 3 rates used.

Nontarget arthropods which showed acute lethal effects were corixids (*Corisella* spp.), notonectids (*Notonecta unifasciata*), clam shrimp (*Eulimnadia* sp.), and *Tropisternus lateralis* beetle adults. Nontargets that did not exhibit mortality were mayfly (*Callibaetis* spp.) naiads, chironomid larvae and copepods.

INTRODUCTION

Mosquito control utilizing ISA-20E[1], a liquid isostearyl alcohol containing 2 oxyethylene groups and which creates a monomolecular film over water surfaces, was first proposed by Garrett and White (1977). Since then, this control agent had been tested on various species in the genera *Anopheles, Aedes, Culex, Psorophora* and *Uranotaenia* both in the laboratory and field (White and Garrett 1977, Levy et al. 1980a, 1981, 1982a; Mulla et al. 1983), and against all stages of mosquitoes (Levy et al. 1982a).

These reports propose ISA-20E as a promising mosquito control agent because: (1) The ability to kill mosquitoes appears to be high, depending on species, characteristics of the habitat treated, application rate, formulation of the agent, and certain weather factors (especially wind). (2) The mammalian toxicity appears to be low (acute oral LD_{50} rats + 20,000 mg/kg) and the agent is nonirritating to eyes and skin (Levy et al. 1980a, 1981, 1982b). (3) It apparently does not create resistance in mosquitoes. (4) It is cost-competitive due to its current cost, long shelf-life, and low rates of application needed (0.04–0.48 ml/m²) depending on factors listed in #1. (5) It is biodegradable. (6) It apparently causes little or no harm to plants, fish and wildlife. Previously published accounts referring to effects of this agent on nontarget organisms in mosquito habitats have been made through critical observations and few have quantitative data. Our objective was to quantify the acute lethal effects of this mosquito control agent on aquatic nontarget arthropods and also on indigenous populations of *Aedes* and *Culex* mosquitoes.

MATERIALS AND METHODS

TEST SITE. The experimental plots were 6.1 × 30.5 × 0.4 m deep, located 30 km NW of Bakersfield, CA. The plots were oriented in a north-south plane, and flooded in alternate cycles with water from the California Aqueduct via underground pipe. Vegetation was predominantly hybrid bermuda pasture grass and the surrounding environment was arid, flatland under row crop agriculture such as cotton and sugar beets.

The plots were filled to a depth of ca. 30 cm and initially produced synchronous larval populations of mixed *Aedes melanimon* Dyar and *Aedes nigromaculis* (Ludlow). Mixed instars of *Culex tarsalis* Coquillett and *Culex quinquefasciatus* Say appeared about 1 week later.

TREATMENT. The ISA-20E was semiviscous technical grade liquid which we sprayed undiluted from a plastic hand-pump sprayer. The material was dispersed in each plot while walking around the perimeters to get uniform coverage. We used 3 treatment rates, 0.25, 0.5, and 1.0 ml/m², and 1 or 2 untreated controls in separate plots. Efficacy studies were conducted concurrent with applications on 6/2/82 (Test #1) and on 6/29/82 (Test #2).

MOSQUITO SAMPLING. Mosquito evaluations were by dip sampling, using 400 ml dippers. For the *Aedes* spp., 40 dip counts were recorded just prior to treatment and daily thereafter through pupation. The difference between pretreatment and last posttreatment count totals (corrected with Abbott's formula) was used to provide percent mortalities.

For the *Culex* spp. tests, 40 dips per plot were taken before treatment and daily thereafter for

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[1] Polyoxyethylene (2) isostearyl ether (2 mole ethoxylate of isostearyl alcohol) manufactured as a cosmetic ingredient under Arosurf 66–E2® by Sherex Chemical Company, Inc. Dublin, OH.

3 days. Normal receding of water levels precluded further sampling. Dip samples were preserved in alcohol for later counting. Each posttreatment day's counts were compared to the pretreatment count to derive a daily percent mortality, with Abbott's correction for control plot counts.

NONTARGET SAMPLING. Four sampling methods were employed to capture nontarget arthropods in different habitats: (1) 23 × 40 × 6 cm deep white enameled collection pans placed under water at the bottom of the plots to collect free swimming macroorganisms dying in the water after treatment. (2) Trapping with modified minnow traps to capture nekton. (3) Dipping with standard 400 ml mosquito dippers to collect organisms near the surface. (4) Area sampling with a 0.1 m^2 square (Test #1) and a 0.045 m^2 plastic cylinder (Test #2) area sampler to capture benthos. On each sampling day, 3 (Test #1) or 4 (Test #2) area samples, 20 dipper, 4 trap and 10 pan samples were collected from each plot. A sample set was taken just prior to treatment and one taken on each of 3 posttreatment days in all control and treated plots. Descriptions and use of the square area sampler (only outer unit used in this study) and the modified minnow trap are as described by Takahashi et al. 1982. The use of the cylinder area sampler was identical to that of the square.

In addition to the sampling methods, during the 2nd test, 25 adult *Notonecta unifasciata* Guerin (Hemiptera: Notonectidae) were contained in separate 0.028 m^3 screened cages and placed in plots subsequently treated at 0.5 ml/m^2. One cage was placed in the control plot and 3 in separate locations of the treatment plot.

High and low water temperatures at 10 cm below the surface were recorded daily. Winds were measured at daily intervals with a Dwyer hand-held wind meter 1.5 m (5 ft) above ground level. An indicator oil used to check for persistence of the ISA-20E (Levy et al. 1980b) was not used because: (1) The possibility that mortality in nontarget organisms could exist after initial exposure. (2) The oil does not indicate persistence when the film pressure exerted by ISA–20E falls below that of the indicating oil (Levy et al. 1980b). (3) Even when the oil indicated persistence, sometimes poor control is achieved (Levy et al 1982b).

RESULTS—MOSQUITOES

Water temperatures ranged from a low of 17°C ($\bar{x}$ = 18) to a high of 33°C ($\bar{x}$ = 31) during test 31 and from a low of 20°C ($\bar{x}$ = 21) to a high of 34°C ($\bar{x}$ = 30) during test #2. Although winds were predominantly from the NW at 2–13 km/h with gusts of 24 km/h, we recorded wind directions from the south at 5 km/h within minutes of one in the opposite direction, and also periods of calm.

Aedes spp. populations were fairly high. Average pretreatment counts for all larval test plots were 4.7 per dip, and for all pupal test plots were 3.9 per dip.

ISA–20E was moderately to very effective against third and fourth instar mixed population of *Ae. nigromaculis* and *Ae. melanimon* at 0.5 and 1.0 ml/m^2 (Table 1), averaging 88% and 96% respectively, based on dip count reduction. When applied to *Aedes* spp. pupae, ISA–20E was very effective at the 3 rates used; based on these unreplicated tests—mortality ranged from 92 to 100%.

Culex spp. populations were lower than *Aedes*. There was an average of 1.9 immatures (all stages) per dip for overall pretreatment counts.

Results of the unreplicated *Cx. quinquefasciatus* and *Cx. tarsalis* tests are summarized in Table 2. Mortality was 100% at the 1.0 ml/m^2 rate and the population of mixed stages was completely eliminated for the 3-day period of the test. However, at 0.25 ml/m^2 and 0.5 ml/m^2 population reduction was much less, with considerable survival each day at both rates.

RESULTS—NONTARGET ORGANISMS

COLLECTION PANS. Relatively large numbers of clam shrimp, *Eulimnadia sp.*, (Conchostraca: Limnadiidae) and corixids, *Corisella* spp., (Hemiptera: Corixidae) were found dead in collection pans placed on the bottom of treated plots. For example, after control mortality was subtracted, the 1.0 ml/m^2 rate yielded 247 dead clam shrimp and 515 dead corixids per 10 pans

Table 1. Summary of ISA-20E vs *Aedes* spp.[1] immatures in 0.017 ha field plot tests.

Test date	Stage treated	Rate (ml/m^2)	Percent[2] mortality
6–15–82	larvae	0.25	50
6–15–82	larvae	0.25	78
8– 3–81	larvae	0.5	79
8–10–81	larvae	0.5	98
6–15–82	larvae	0.5	87
8–10–81	larvae	1.0	97
6–15–82	larvae	1.0	94
8–19–81	pupae	0.25	92
8–19–81	pupae	0.5	99+
8–12–81	pupae	1.0	100

[1] *Ae. melanimon* (average 74%) and *Ae. nigromaculis* (average 26%), III and IV instar.

[2] Percent reduction between pretreatment and last posttreatment counts (until emergence or depletion of population); 40 dips per plot.

Table 2. Summary of ISA-20E vs *Culex*[1] spp. immatures in 0.017 ha field plot trials, 6/29/82.[2]

Rate (ml/m²)	Percent mortality[3] 1 day	2 days	3 days
0.25	78	85	87
0.5	92	77	49
1.0	100	100	100

[1] *Cx. tarsalis* and *Cx. quinquefasciatus*, all instars.
[2] One treatment each rate.
[3] 40 dips each plot each day. Percent reduction between pretreatment total counts and each day's posttreatment counts, corrected for control plots counts.

Table 3. Cumulative number dead[1] clam shrimp (*Eulimnadia* sp.) and Corixidae (*Corisella* spp.) per 10 collection pans in plots treated with ISA-20E (Test #2).

Rate (ml/m²)	Cumulative mortality posttreatment day 1	2	3
		Clam Shrimp	
0.25	0	0	0
0.5	160	457	566
1.0	247	588	570
		Corixids	
0.25	187	187	187
0.5	160	171	167
1.0	515	595	599

[1] Control mortality in any day subtracted from treatment numbers, if latter was lower, then 0 was posted.

(0.92 m²) in 24 hr and mortality had increased in 48 hr (Table 3). The lowest rate appeared to cause no mortality to clam shrimp but resulted in 187 per 10 pans mortality in corixids.

TRAPS. Results from trap sampling data (Fig. 1) show a decline in corixid populations at all treatment rates on the first posttreatment day. Subsequently, the population increased substantially in the 0.25 ml/m² plot and back to pretreatment levels in the 0.5 and 1.0 ml/m² plots on posttreatment day 3. There was a substantial decrease in corixid mortality in traps on days 2 and 3 (Fig. 1) indicating that ISA–20E has a short term effect upon this nontarget

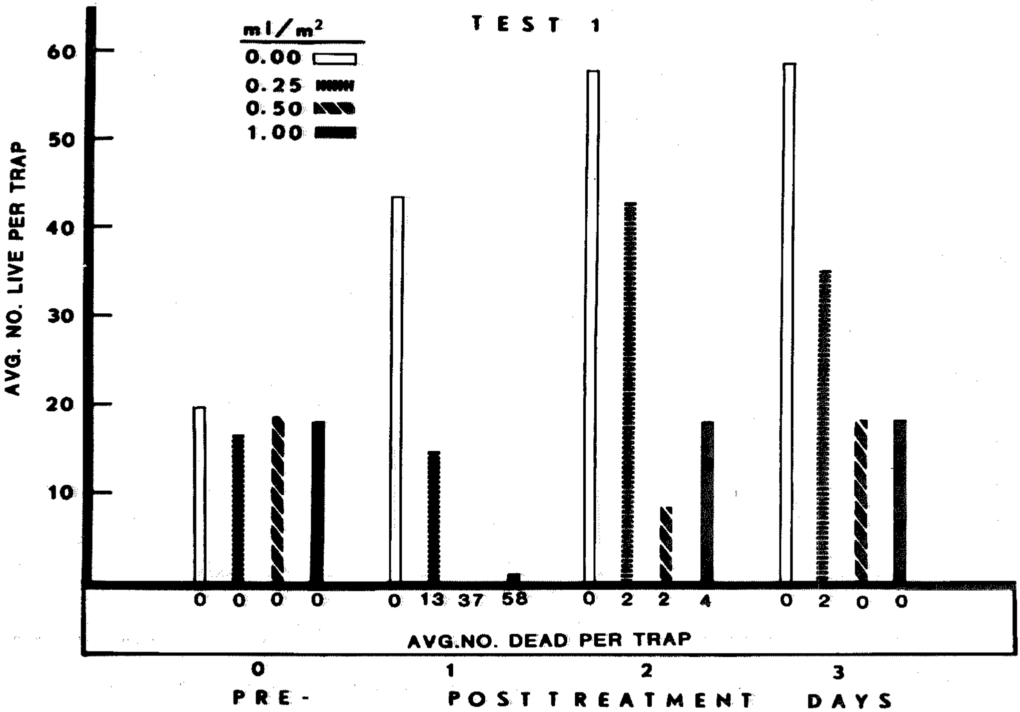

Fig. 1. Mean number of live and dead Corixidae (*Corisella* spp.) captured per trap-day before and after plots were treated with ISA–20E (Test no. 1).

group. On the first posttreatment day, there was a slight decline in populations of *N. unifasciata* (Fig. 2) at the 0.25 and 0.5 ml/m² rates followed by stable levels on days 2 and 3. At the 1.0 ml/m² rate, the notonectid population declined drastically on posttreatment days 1 and 2 followed by a resurgence on day 3. As with corixids, notonectid mortality also decreased in traps after the first posttreatment day. At the 1.0 ml/m² rate, *Tropisternus* spp. beetle adult populations also declined (days 1 and 2) and recovered (day 3). Mortality at this rate was 36% only on day 1. There was no discernible decrease at the other 2 rates using the sampling method. Mayfly naiads (*Callibaetis* spp.) showed little effect due to treatment and what mortality there was had no apparent relationship to rate of application.

DIP SAMPLES. Results of dip sampling counts indicate that little or no mortality occurs in copepods, mayfly naiads (*Callibaetis* spp.), chironomid larvae and the beetle larvae, *Tropisternus* spp., *Enochrus* sp., *Laccophilus* spp. and *Hygrotus* sp. when exposed to the rates of ISA–20E used in this study. At all treatment rates the first posttreatment sample numbers were substantially higher than the pretreatment samples for most of the above nontargets. In addition, generally sample numbers progressively increased on days 2 and 3.

AREA SAMPLES. Chironomids were the only organisms captured in large enough numbers to report. Chironomid larvae appear unaffected by any of the rates of ISA–20E used. All posttreatment sample numbers were progressively and substantially higher than pretreatment samples. In test #1, at the 1.0 ml/m² rate, sample increases for days 1, 2, 3 were 2.7x, 8.3x and 11.1x the pretreatment sample; respective control samples were 2.4x, 4.2x and 5.2x. In test #2, similar increases occurred.

CAGE. The treatment cages placed in the 0.5 ml/m² rate plot yielded an average of 40% mortality in *N. unifasciata* adults in 48 hr.

DISCUSSION AND SUMMARY

ISA–20E was effective against mixed 3rd and 4th instars of *Ae. nigromaculis* and *Ae. melanimon* at rates of 0.5 and 1.0 ml/m² and less effective at 0.25 ml/m². Against the *Aedes* spp. pupae it was effective at all 3 rates. Sensitivity of pupae was observed in other species by Levy et al. (1980a, 1981, 1982a). We also observed that when surviving treated *Aedes* spp. were placed in emergence cages in their respective treated plots, 2/22 and 5/16 successfully emerged in the 0.5 ml/m² plots but none (out of 20 and 45) emerged in the 1.0 ml/m² plots. When ISA–20E was applied to mixed populations and instars of *Cx. tarsalis* and *Cx. quinquefasciatus* there was 100% mortality at the 1.0 ml/m² rate. At the 0.25 and 0.5 ml/m² rate there was less mortality but these results are preliminary.

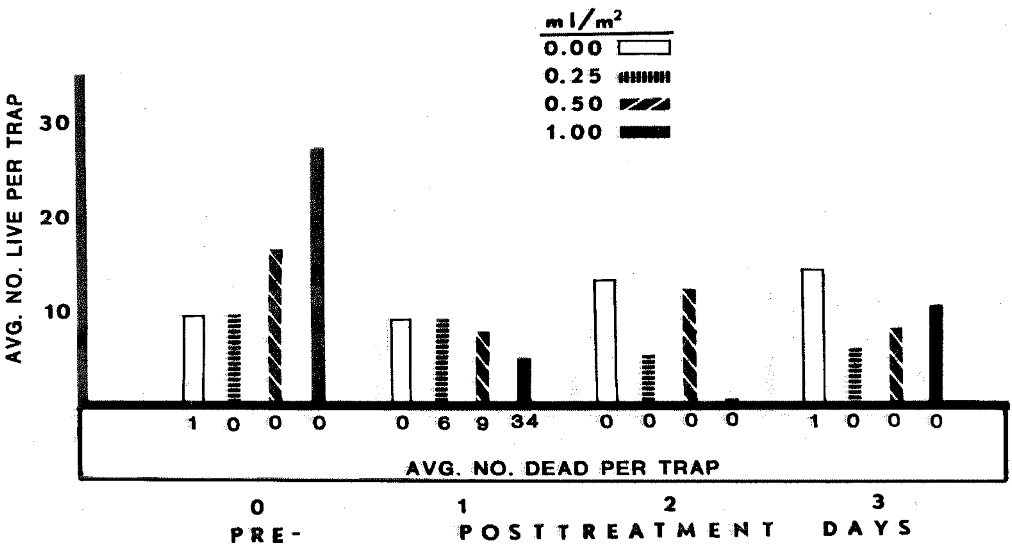

Fig. 2. Mean number of live and dead Notonectidae (*Notonecta unifasciata*) captured per trap-day before and after plots were treated with ISA–20E (Test no. 2).

The aquatic nontarget arthropods which were killed to some extent by a 0.25 ml/m^2 application rate were corixids (primarily *Corisella* spp.) and notonectids (*N. unifaciata*). Those affected by higher rates were the above plus clam shrimp (*Eulimnadia* sp.) and the beetle adult *T. lateralis*. Those which showed little or no effect were copepods (*Cyclops* spp., *Diaptomus* spp.), mayfly naiads (*Callibaetis* spp.), chironomid larvae and beetle larvae.

It is not surprising that chironomid larvae would be unaffected since they generally reside in the benthic substrate. Chironomid pupae which were not sampled in this study need to be tested for effects, because they come to the surface just prior to adult emergence. Except for clam shrimp, the nontarget insects which we could discern to have been affected all have ventral plastrons produced by setae (notonectid) or hydrofuge pubescence (corixid and hydrophilid). Although we did not test this directly, it is reasonable to postulate that plastron surfaces are affected by ISA–20E because of its inherent ability to reduce surface tension in water. Corixids especially make extensive use of plastron surfaces created by hydrofuge pubescence (Usinger 1963, Pennak 1978). Dytiscid beetle samples were too small to detect differences in treatments; a few dead beetles were observed in treated traps.

Most accounts of the effects of ISA–20E on nontarget organisms are from critical observation and indicate that ISA–20E is relatively safe to nontarget species (Levy et al. 1980b, 1981, 1982b; Mulla et al. 1983). White and Garrett (1977) indicated some mortality to gerrid and ". . . species intimately associated with the air-water interface . . ." Levy et al. indicated adverse effects of "surface orientation" to some Gerridae, Hydrometridae and Chironomidae under "certain environmental conditions," apparently wind. Mulla et al. (1983) observed "large numbers" of dead adult chironomids and mosquitoes on the surface of treated ponds.

We have presented quantitative data which indicates that ISA–20E causes acute lethal effects to several nontarget species, including some which are mosquito predators, at rates of 0.25 to 1.0 ml/m^2. Therefore we recommend that these effects be weighed before applying this material.

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