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INVESTIGATIONS ON THE MOSQUITO CONTROL POTENTIAL OF FORMULATIONS OF AROSURF®MSF AND CONVENTIONAL LARVICIDES[1]

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Research by Levy et al. (1984) has indicated that formulations of Arosurf® MSF (Monomolecular Surface Film)[2] and commercial preparations of *Bacillus thuringiensis* var. *israelensis (B.t.i.)* (Teknar®, Bactimos® and Vectobac®) can significantly improve the mosquito-controlling efficacy when compared to the formulation components. Formulations of Arosurf MSF and Teknar, Bactimos or Vectobac were shown to kill larvae, pupae and emerging adults with application rates at or below label recommendations for each product. These Arosurf MSF-*B.t.i.* formulations are expected to be effective against ovipositing females of certain species and organophosphate and organochlorine resistant mosquitoes while producing no adverse impact upon the environment.

The following is a report of additional tests that were conducted to determine the potential of Arosurf MSF as a formulation component for enhancing the efficacy of conventional chemical mosquito larvicides.

Laboratory bioassays were conducted against immature stages of *Culex quinquefasciatus* Say with formulations of Arosurf MSF and diesel oil No. 2, or diesel oil No. 2-isopropanol, or Abate®4-E (temephos) to determine the efficacy of each series of formulations when compared to the efficacy of the formulation components. Tests with diesel or diesel-isopropanol formulations were conducted against 10 third or fourth instar larvae while bioassays with Abate 4-E were conducted against mixed groups of 5 fourth instar larvae and 5 pupae.

Bioassays were performed in 400 ml glass beakers containing 250 ml of reverse osmosis (RO) water (3 replications/formulation). Diesel base formulations were applied to the surface of the water in each beaker with a microsyringe while formulations of Abate 4-E were applied with a glass pipette as an agitated (Bamix [M100] biomixer, Biospec Products, Bartlesville, OK 74003) water-base suspension.

Prior to application of the test formulations, larvae were fed 2 drops of finely ground rabbit chow-RO water suspension. All bioassays were conducted in a room maintained at 26–27°C (ambient) and 80% RH.

Cumulative mean percentage mortality of larvae or larvae and pupae was recorded at 24 hr post-treatment intervals and was the main criterion used to evaluate the efficacy of the formulations and their components. Results were statistically analyzed using "z" and "t" tests.

Additional tests were conducted to determine the mixing compatibility and sprayability of the various formulations at recommended application rates for each product. Preliminary field evaluations on the efficacy of several of the experimental formulations were also conducted on an operational basis to corroborate the laboratory evaluations.

Bioassays were conducted with formulations of Arosurf MSF and diesel oil No. 2 since diesel oil combined with spreading agents such as Triton®X-207 has been a standard larviciding/pupiciding formulation for many years (Hester et al. 1979). Tests against 3rd and 4th instar larvae of *Cx. quinquefasciatus* (Table 1) with formulations of Arosurf MSF and diesel oil applied at and below label recommendations for each product indicated that the 2 materials are compatible when mixed, and can produce a significantly higher rate of larval/pupal mortality in 24 hr, as well as effective delayed mortality, than either of the formulation components. Previously, Levy et al. (1982) showed that little or no Arosurf MSF-induced kill of 1st-4th instar larvae of *Cx. quinquefasciatus* would occur within

[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.

[2] Arosurf®MSF (= ISA-20E = Arosurf®66-E2) is an EPA registered mosquito larvicide and pupicide produced by Sherex Chemical Company, Inc., P. O. Box 646, Dublin, OH 43017.

Table 1. Efficacy of formulations of Arosurf® MSF and diesel oil No. 2 against larvae of *Culex quinquefasciatus*.

Formulation	Total dosage (gal/acre)	Larval instar	Days old	Cumulative mean percentage mortality of larvae, pupae, and/or emerging adults at indicated post-treatment time period[1]			
				24 hr	30 hr	48 hr	72 hr
Arosurf MSF + diesel	1.16*	4th	7	63.3	83.3	93.3	93.3
Arosurf MSF + diesel	1.29**	3rd	5	43.3	—	70	96.7
		4th	7	76.7	80	96.7	96.7
		4th	9	86.7[2]	—	100	—
Arosurf MSF + diesel	2.15*	4th	7	80	86.7	90	93.3
Arosurf MSF + diesel	2.28**	3rd	5	66.7	—	93.3	100
		4th	7	90	96.7	100	—
		4th	9	93.3[3]	—	—	—
Arosurf MSF + diesel	3.26**	3rd	5	90	—	96.7	100
		4th	9	100	—	—	—
Arosurf MSF	0.26	3rd	5	13.3	—	30	36.7
		4th	7	10	10	28.3	64.3
		4th	9	16.7	16.7	36.7	73.3
Diesel	1.03	3rd	5	0	—	3.3	6.7
		4th	7	0	0	0	0
		4th	9	0	—	0	10
Diesel	2.02	3rd	5	16.7	—	20	23.3
		4th	7	0	0	0	10
		4th	9	10	—	23.3	33.3
Diesel	3.00	3rd	5	43.3	—	50	63.3
		4th	9	3.3	—	26.7	36.7

* Formulation includes 0.13 gal Arosurf MSF/acre.
** Formulation includes 0.26 gal Arosurf MSF/acre.
[1] No control mortality.
[2] 93.3% mortality at 26 hr post-treatment.
[3] 100% mortality at 26 hr post-treatment.

24 hr of treatment (i.e., 0–37% mortality). The data (Table 1) indicated that susceptibility of *Cx. quinquefasciatus* to the Arosurf MSF-diesel oil formulations increased with larval instar (i.e., 3rd (5 day old) to 4th (9 day old) and age within an instar (i.e., 5 to 7 day old 4th instar). This general trend in larval susceptibility was observed for Arosurf MSF and diesel at the 1.03 and 2.02 gal/acre levels but was not observed at the 3 gal/acre rate. Formulations of Arosurf MSF and diesel oil applied at lower than recommended Arosurf MSF application rates (i.e., 0.13 gal/acre) also produced significantly higher levels of larval mortality in 24 hr when compared to the formulation components (Table 1). A direct relationship between diesel oil concentration and larvicidal action was also observed.

Bioassays with isopropanol-diesel oil No. 2-Arosurf MSF formulations resulted in 90–93% mortality of 4th instar *Culex* larvae at all application rates in 24 hr and 100% mortality in 48 hr; however, few or no larvae were killed with the diesel oil-isopropanol mixtures (Table 2). Field trials by Levy et al. (1980) against mixed populations of larvae and pupae of *Culex* spp. breeding in sewage treatment systems have indicated the significance of 2-propanol (isopropanol) and other alcohol solvents in the enhancement of larvicidal action of certain monomolecular surface films. Additionally, bioassays against larvae of *Cx. quinquefasciatus* with over 50 types of Arosurf MSF-alcohol/solvent formulations have indicated that the addition of various concentrations of isopropanol to Arosurf MSF can result in a significant increase in larvicidal activity in 24 hr when compared to Arosurf MSF alone (Levy et al., unpublished data). This was also shown in bioassays with acetone-Arosurf MSF formulations and suggested that the use of certain solvents as diluents for monomolecular surface films on bioassays against immature mosquitoes may lead to erroneous conclusions.

These bioassays suggest that diesel oil or diesel oil-isopropanol formulations of Arosurf MSF can produce rapid larvicidal action as well as persist beyond 24 hr to kill larvae or pupae that survived the initial exposure. The data also suggest that the effective per acre application rate of diesel oil No. 2 in the Arosurf MSF formulations is lower than the recommended 3–7 gal/acre application rates for diesel oil larviciding/pupiciding formulations currently in use in operational mosquito control programs under 24C state registrations.

Table 2. Efficacy of diesel oil No. 2-isopropanol formulations of Arosurf® MSF against fourth instar larvae of *Culex quinquefasciatus*.[1]

Formulation	Total dosage (gal/acre)	Cumulative mean percentage mortality of larvae, pupae, and/or emerging adults at indicated post-treatment time-period[2]	
		24 hr	48 hr
Arosurf MSF + mixture of 50% diesel-50% isopropanol	1.29*	90	100
Arosurf MSF + mixture of 50% diesel-50% isopropanol	2.28*	93.3	100
Arosurf MSF + mixture of 50% diesel-50% isopropanol	3.26*	90	100
Arosurf MSF	0.26	13.3	23.3
50% diesel-50% isopropanol	1.03	0	0
50% diesel-50% isopropanol	2.02	0	6.7
50% diesel-50% isopropanol	3.00	3.3	10

* Formulation includes 0.26 gal Arosurf MSF/acre.
[1] Larvae 9 days old when treated.
[2] No control mortality.

Diesel oil or diesel oil-isopropanol mixed together satisfactorily with Arosurf MSF at all concentrations with simple hand-shaking without separation of the formulation components being noted over a 24 hr period. However, the compatibility of the components after prolonged storage and the effect of long term storage on the resulting mosquito-controlling efficacy of the combined products is not known. Field trials to determine the operational mosquito-controlling efficacy of these formulations are in progress.

Water-base formulations of Abate 4-E and Arosurf MSF produced similar efficacy against 4th instar larvae and pupae of *Cx. quinquefasciatus* when compared to the formulation components (Table 3). The data showed the larvicidal and pupicidal efficacy of the formulations when the 2 components were mixed at rates recommended for each product. Results of bioassays indicated that under certain situations when mixed immature stages were encountered, Arosurf MSF can be formulated with Abate 4-E for application at lower than recommended rates (i.e., 0.13 gal/acre) when surface film control is targeted for pupal populations only. Under these conditions, the low recommended application rate for Abate 4-E (i.e., 0.5 fl oz/acre) appears to be satisfactory for rapid control of larval populations while the lower than label rate of Arosurf MSF appeared to persist for a sufficient period of time to eliminate all pupae and emerging adults that were not affected by the Abate.

In general, tests showed that there would be a significant benefit in formulating diesel oil or Abate 4-E with Arosurf MSF for control of mixed stages of immatures. Arosurf MSF is compatible with diesel oil or technical Abate 4-E and is also compatible with Abate 4-E as a water-base suspension if high sheer agitation is utilized when the components are being mixed.

Preliminary field trials in roadside ditches and flooded fields with paddle-agitated water-

Table 3. Efficacy of water-base formulations of Arosurf® MSF and Abate® 4-E against mixed populations of fourth instar larvae and pupae of *Culex quinquefasciatus*.[1]

Water-base formulation (dosage-active ingredient)[2]	Cumulative mean percentage mortality of larvae, pupae, and/or emerging adults at indicated post-treatment time period			Cumulative percentage adult emergence
	24 hr	48 hr	72 hr	
Arosurf MSF + Abate 4-E (0.26 gal/acre + 1.0 fl oz/acre)	66.7	100	—	0
Arosurf MSF + Abate 4-E (0.13 gal/acre + 0.5 fl oz/acre)	56.7	100	—	0
Abate 4-E (1.0 fl oz/acre)	53.3	53.3	—	46.7
Abate 4-E (0.5 fl oz/acre)	36.7	36.7	—	63.3
Arosurf MSF (0.26 gal/acre)	10	83.3	100	0
Control	0	6.7	6.7	93.3

[1] Abate 4-E is an organophosphate larvicide of American Cyanamid Company.
[2] All water-base formulations applied at a total application rate of 5.02 gal/acre.

base tank mixes of Arosurf MSF and Abate 4-E applied at application rates of 5–6 gal/acre from a roadside ditch truck gave 100% control of mixed larval and pupal populations of *Cx. nigripalpus* Theobald, *Psorophora columbiae* Dyar and Knab and *Aedes taeniorhynchus* Wiedemann in 24 or 48 hr. Abate can also be easily mixed with Arosurf MSF with minimal agitation (e.g., hand shaking) for application of the technical products at recommended rates. The efficacy of these formulations in hand sprayers has also been demonstrated under field conditions. Observations of rapid kill of larvae in areas that were far removed from the points of application indicated the Arosurf-induced surface spreading of Abate 4-E throughout the habitat prior to solubilization.

Further laboratory and field evaluations are planned to determine the operational feasibility, environmental safety and cost-effectiveness of the Arosurf MSF and diesel-base or Abate 4-E formulations.

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OBSERVATIONS OF THE BITING ACTIVITY OF MOSQUITOES AT A FLOODED DAMBO IN KENYA[1]

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The biting habits of mosquitoes associated with flooded dambos (Ackermann 1936) in Kenya may be of importance in understanding their role in the ecology of Rift Valley fever (RVF) virus and other viruses. No studies of the biting habits of mosquitoes in East Africa have specifically dealt with the dambo associated species (Haddow 1960), several of which have been incriminated as vectors of RVF (Linthicum et al. 1983). Rift Valley fever is primarily a cattle and sheep disease with man becoming infected when in close association with these animals (W.H.O. 1982). In areas of Kenya, where the disease occurs, herdsman graze their cattle in and around dambo formations. The purpose of this study was to determine: (1) the mosquito species feeding on man and cattle, and (2) the time(s) of the diel with which mosquito blood feeding activity occurred.

The study was conducted on a ranch at the northeastern margin of the Athi plains, 7 km SE of Ruiru, Thika District, Central Province, Kenya (1°12's; 37°E), at an elevation of 1500 m. The dambo was situated in an area of bushed grasslands along the Kamiti River (ecological zone III, Pratt et al. 1966). It showed zonal differentiation that is typical of a dambo (Mackel 1974, Linthicum et al. 1983) with predominantly sedge (*Cyperus immensus* C.B. Clarke) in the seepage zone with the grass *Digitaria abyssinica* (A. Richard) Stapf in the remainder of the site. On April 26–27, 1983, overflow from the Kamiti River flooded the study site. A generation of mosquitoes emerged from the flooded dambo which contained standing water until May 9, 1983.

A continuous 96 hr human bait collection (1000 hr May 10–1000 hr May 14, 1983) and a continuous 48 hr biting collection at a single calf (1100 hr May 12—1100 hr May 14, 1983) were made. For the human bait collection 2 men (part of a 4 man rotating team) were positioned

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