oped to the L_3 stage and started to move out of thoracic muscles, 34 to 60% mortality was observed in *Ae. aegypti* and 18% in *An. quadrimaculatus* by 12 days postinfection (Table 1).

In the remaining species of mosquitoes studied, only prelarvae were found. No developing larvae were observed in females of these species and none of the mosquitoes died during the 12 days postinfection. Usually 1 or 2 developing larvae in one out of 10 females were observed in *An. albimanus* and both strains of *Cx. salinarius*.

Two important aspects were apparent from these studies. First, these studies showed that the microfilariae of *B. patei* developed comparably in *Ae. aegypti* LVP and VBS strains. *Aedes aegypti* LVP strain supported development of *B. pahangi*, *B. malayi* and *D. immitis* (Macdonald and Ramachandran 1965). *Aedes aegypti* VBS strain also supports development of *B. pahangi* and *B. malayi* (Nayar, unpublished data). Additionally, the microfilariae of *B. patei* did not develop, but remained as prelarvae in the *Aedes aegypti* VBR strain. Secondly, *An. quadrimaculatus* in addition can successfully support development and transmit several other parasites, such as *Dirofilaria immitis*, *D. uniformis*, *D. tenuis*, *Brugia pahangi*, *Plasmodium falciparum*, *P. vivax*, *P. gallinaceum* and *P. berghei* (cf. Nayar and Sauerman 1975).

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EFFECT OF LOW TEMPERATURE ON THE MOSQUITO LARVICIDE AND PUPICIDE AROSURF®MSF (MONOMOLECULAR SURFACE FILM) AND ADOL®85 (INDICATOR OIL): PHYSICAL EVALUATIONS[1, 2, 3]

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Arosurf®MSF (Monomolecular Surface Film) is the designation for the two mole ethoxylate of isostearyl alcohol. The efficacy of this product for use in the control of natural populations of mosquito larvae and pupae as well as the effective use of the oleyl alcohol indicator oil Adol®85 to monitor field persistence of Arosurf MSF have been reported by Levy et al. (1980a, 1980b, 1981, 1982a, 1982b, 1982); however, most of their field trials were conducted against mosquitoes breeding in warm water (i.e. temperatures of ca. 14–35°C). Since spontaneous spreading/respreading of Arosurf MSF on the water surface is essential for the maintenance of a stable monomolecular film for effective mosquito control, and has been demonstrated in laboratory bioassays and field trials in warm water habitats of Florida, bioassays were conducted to determine if this product would spread satisfactorily on water at temperatures typically encountered in northern areas where snow pool mosquitoes are severe pests. Similar evaluations were conducted to determine if accurate Adol 85 indicator oil readings (Levy et al. 1980b) would result when used in conjunction

[1] Arosurf®MSF (= ISA-20E = Arosurf®66–E2); Adol®85 (= Adol®).

[2] Arosurf®MSF and Adol®85 are products of Sherex Chemical Company, Inc., P. O. Box 646, Dublin, OH 43017.

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

with Arosurf MSF in cold water. These data could then be used to determine the feasibility of using Arosurf MSF as a mosquito larvicide and pupicide against cold water species (e.g. boreal *Aedes*).

A series of laboratory tests to determine the effect of low water and ambient temperatures on the rate of spreading of Arosurf MSF and Adol 85 were conducted in stainless steel trays (68.6 × 25.4 cm) or enamelled tubs (35 cm diam) containing well water purified by reverse osmosis filtration (7,570 and 2,000 ml, respectively). Baby powder (Vaseline Intensive Care®) was evenly sprinkled over the surface of the water to visualize spreading of the Arosurf MSF or Adol 85. Water was precooled in a container with ice cubes to reach the desired test temperatures of 2, 4, 5 or 10°C. A heated water standard (32°C) was used to evaluate the comparative spreading potential of the 2 products. The rate of movement of the powder on the surface of the water after point source application of a product was recorded with stopwatches* and was the main criterion used to evaluate the relationship between water and/or product temperature and the spreading potentials of the Arosurf MSF and Adol 85. The proper functioning of the indicator oil before and after Arosurf MSF application was also determined at each temperature. In another series of tests several alcohol formulations of Arosurf MSF were evaluated to determine if the spreading potential of this product could be enhanced.

STAINLESS STEEL PAN TESTS. Powder (0.15 g) was sprinkled over the water at the desired temperature and a drop (0.02 ml) of Arosurf MSF or Adol 85 was dispensed onto the surface of the water in a central position ca. 0.64 cm from the narrow side of the tray with a Pharmaseal® Stylex® syringe (1 cc) equipped with a Stylex needle (27 gauge × 1/2). The time required for the powder to be completely pushed (compacted) to the opposite side of the tray by each of the products was determined at water temperatures of 2, 5 and 32°C (3 replications/temperature). The ability of Adol 85 (0.02 ml application rate) to indicate effective mosquito-controlling film pressure (i.e. bead) on the surface of water treated with Arosurf MSF (0.25 ml/m²) was also determined at each of the test temperatures.

ENAMELLED TUB TESTS. Arosurf®MSF and formulations of Arosurf MSF and 2-propanol or 2-ethyl butanol were applied to water (4, 10 and 32°C) that was evenly covered with 0.1 g powder. Each formulation was applied to the water at a rate of ca 0.1 ml/m² with an "0" gauge needle that was dipped 0.64 cm into a formulation and then redipped into the water in the center of the pan to the same level. The comparative spreading rates (spreading radius − 17.5 cm) was recorded at each temperature (3 replications/formulations). The effectiveness of the Adol 85 indicator oil was also determined for each Arosurf MSF formulation.

Results of spreading tests are presented in Table 1. In general, results indicated that 2 and 27°C Arosurf MSF will spread over the surface of 2°C water to form a mosquito-controlling surface film; however, the rate of movement will be dependent on the water and Arosurf MSF temperatures, i.e., spreading rates will increase as the water and/or film temperatures increase. Although the viscosity and opaqueness of Arosurf MSF significantly increases as the temperature decreases from 32 to 2°C (Table 2), refrigerator tests at 2°C indicated that the product could be satisfactorily sprayed from a hand-activated pump sprayer or compressed air sprayer at low ambient temperatures. It should be noted that the melting point of Arosurf MSF is ca. −5°C (range of −3 to −7°C) (Sherex Chemical Company, personal communication), thereby suggesting that the product should have satisfactory operational and storage characteristics at ambient temperatures approaching freezing.

Enamelled tub tests indicated that enhanced Arosurf MSF spreading rates would result from the addition of 5 or 10% 2-propanol or 2-ethyl butanol (v/v) (Table 1). Also, 2-propanol and 2-ethyl butanol have melting points of −89.5 and −114°C respectively, and therefore would act to depress the melting point as well as lower the viscosity of the Arosurf MSF as a function of alcohol concentration (Table 2). Therefore, the data suggest that the addition of alcohols to Arosurf MSF can produce more flowable (sprayable) formulations at temperatures as low as 2°C, as well as provide a means for a more rapid establishment of a mosquito-controlling surface film in certain cold water habitats. Bioassays (unpublished data) with these alcohol-Arosurf MSF formulations (27°C) against larvae and pupae of *Culex* and *Aedes* spp. indicated that the addition of the alcohols did not inhibit the mosquito-controlling efficacy of the product. Field trials against mosquitoes with formulations of a monomolecular surface film and 2-propanol and 2-ethyl butanol have been reported by Levy et al. (1980).

Delayed larval mortality is to be expected when Arosurf MSF is used to control mosquitoes breeding at low ambient and water temperatures (2°C). Low temperature has been reported by Reiter (1978) as a major factor

* These stopwatches were accurate to 0.1 and 0.01 sec for stainless steel trays and enamelled tub tests, respectively.

Table 1. Effect of low temperature on the spreading rates of Arosurf® MSF and Adol® 85

Formulation[1]	Average time in seconds (range) for formulation to move to perimeter of test container at designated water temperture (°C).				
	2	4	5	10	32
			Stainless steel trays		
Arosurf MSF	6.30 (6.2–6.4)	—	5.16 (5.0–5.3)	—	4.77 (4.7–4.8)
Arosurf MSF (2°C)	8.13 (8.0–8.3)	—	—	—	—
Adol 85	8.36 (8.2–8.5)	—	7.16 (7.1–7.2)	—	5.07 (5.0–5.1)
			Enamelled tubs		
Arosurf MSF	—	3.06 (3.01–3.12)	—	2.62 (2.50–2.73)	2.31 (2.11–2.45)
Arosurf MSF/5% 2-propanol	—	2.82 (2.73–2.96)	—	2.52 (2.46–2.59)	2.11 (2.05–2.16)
Arosurf MSF/10% 2-propanol	—	2.71 (2.66–2.75)	—	2.36 (2.27–2.44)	2.13 (2.08–2.22)
Arosurf MSF/5% 2-ethyl butanol	—	2.80 (2.78–2.82)	—	2.54 (2.48–2.59)	2.09 (2.06–2.11)
Arosurf MSF/10% 2-ethyl butanol	—	2.65 (2.61–2.72)	—	2.40 (2.33–2.46)	2.06 (2.01–2.12)

[1] Formulations were tested at 27°C unless otherwise indicated; technical grade (99%) 2-propanol (= isopropyl alcohol) or 2-ethyl butanol used in alcohol base formulations.

contributing to delayed larvicidal action of Arosurf MSF. Decreased metabolic rate (hence, activity) and development of larvae, and increased dissolved oxygen concentrations of water have been shown to occur at low water temperatures. Therefore, less frequent larval surface film contacts and increased cuticular respiration could greatly enhance larval survival in low temperature habitats treated with Arosurf MSF. Additional factors such as high wind, increased product viscosity and decreased spreading pressures could also contribute to the enhancement of delayed larvicidal action at low ambient and water temperatures.

The spreading rate trend of 27°C Adol 85 on 2, 5 and 35°C water (Table 1) was similar to that observed with Arosurf MSF, and therefore indicated that false positive indicator oil readings would not occur on cold water that was not treated with Arosurf MSF. Accurate indicator oil readings (i.e. the beading of a drop of Adol 85 to show high film pressure) was observed on water treated with Arosurf MSF at all test temperatures; however, the indicator oil bead became increasingly viscous and opaque as the temperature was lowered to 2°C.

In a 30 ml plastic dispensing bottle, Adol 85 became a whitish solid after 1 hr exposure to 2°C in a refrigerator. This test indicated that Adol 85 could not be satisfactorily applied to back check mosquito habitats treated with Arosurf MSF at low ambient temperatures. This was expected since the melting point of Adol 85 has been reported by Sherex Chemical Company (personal communication) as ranging from 3–7°C. However, additional refrigerator tests indicated that the addition of 25% 2-propanol or 2-ethyl butanol to Adol 85 (v/v)

Table 2. Relationship between temperature and viscosity for Arosurf® MSF and two alcohol-base formulations.[1]

Arosurf MSF		Arosurf MSF/5% 2-Propanol		Arosurf MSF/10% 2-Propanol	
Temperature (°C)	Viscosity (CPS)	Temperature (°C)	Viscosity (CPS)	Temperature (°C)	Viscosity (CPS)
2	435	2	150	1	141
3	280	4	145	5	112
5	210	7	120	11[2]	100
7	175	10[2]	100	15[2]	98
10	145	15[2]	124	21	42
15	118	19	88	27	29
20	107	21	64	32	23
28	45	28	35		
32	38	32	28		

[1] Viscosity determined by Sherex Chemical Company, Inc., Dublin, OH with a Brookfield Synchrolectric viscometer—Model LVF (#1 and #2 spindles at 30 rpm).

[2] Stratification/flocculation observed at indicated temperatures.

produced indicator oil formulations that were dispensable (flowable) and effective in monitoring the presence or absence of Arosurf MSF after being held in 30 ml dispensing bottles for over 1 week at 2°C, even though some precipitates and stratification/flocculation of the formulation components were observed. Solubilization of the precipitates and disappearance of the stratification/flocculation occurred upon return of the indicator oil formulations to room temperature. Tests with additional alcohols as well as other solvent formulations are planned to determine if improved indicator oils or Adol 85 formulations can be developed.

Laboratory data have indicated that the viscosities and/or spreading rates of Arosurf MSF and Adol 85 on a mosquito habitat or in storage was affected by low ambient and water temperatures. Therefore, the following recommendations are suggested to insure the effective operational utilization of the products: (1) point source introduction of Arosurf MSF on a mosquito habitat should be avoided—product should be evenly sprayed over the surface of the water whenever possible; (2) further evaluations of alcohol-Arosurf MSF blends should be conducted to determine if improved product formulations can be developed; (3) alcohol-base Adol 85 indicator oil formulations should be used to achieve satisfactory flowability for use in back checking a mosquito habitat for Arosurf MSF persistence; and (4) bulk products should be stored indoors whenever possible—outdoor storage at near freezing temperatures should be avoided, particularly in respect to Adol 85.

In summary, results of physical evaluations have indicated that it is feasible to use Arosurf MSF or Arosurf MSF-alcohol formulations to control mosquito larvae and pupae at low ambient and water temperatures. Also, alcohol-base-Adol 85 formulations can be used to monitor post-treatment persistence of Arosurf MSF in mosquito habitats. Field trials with Arosurf MSF and Adol 85, and alcohol formulations of these products are planned in several low temperature geographical areas of the USA and overseas.

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A DOUBLE-SIPHON *HAEMAGOGUS EQUINUS* LARVA

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The occurrence of double-siphon mosquito larvae appears to be very rare and we have been unable to find any report of this type of larva in the literature. We report here the occurrence of a *Haemagogus equinus* Theobald larva with two siphons.

On April 21, 1983 four conventional ovitraps (Fay and Eliason 1966) were set between 1 and 2 meters high on branches of shrubs at Orange Hill, Tobago. This was part of a routine trapping program for the collection of *Aedes berlini* Schick eggs. The paddles were removed on April 28, 1983 and were returned to the laboratory. After a 3-wk period of drying, the four paddles containing 142 eggs were flooded in a