EFFECTS OF AROSURF® MSF ON A VARIETY OF AQUATIC NONTARGET ORGANISMS IN THE LABORATORY

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ABSTRACT. Ninety-six-hour static acute toxicity tests were conducted in the laboratory to determine the effects of a 47 ml/m^2 (50 gal/acre) application of Arosurf® MSF (2 mole ethoxylate of isostearyl alcohol) on *Fundulus similus*, *Palaemonetes pugio*, *Palaemonetes paludosus*, *Uca* spp., *Procambarus* spp., *Gammarus* spp., *Asellus* spp., *Streptocephalus seali*, *Physa* spp., *Laeonereis culveri* and an unidentified amphipod. Test temperatures of 20–27°C were based on the ambient water temperature for the season of the year when the desired life stages were more abundant. No acute toxicity was observed with any of the organisms exposed to this concentration of Arosurf MSF.

INTRODUCTION

Arosurf® MSF (monomolecular surface film), 2 mole ethoxylate of isostearyl alcohol, has been registered for control of immature mosquitoes in all classes of water by the U.S. Environmental Protection Agency (EPA). Before registration, a number of mosquito control programs in Florida were using this product under a Florida experimental use permit. One stipulation of the permit required research studies be conducted to determine the effects of this material on a variety of aquatic organisms that normally occur in or adjacent to the fresh or saltwater habitats in which the larvicide might be used.

Arosurf MSF has been shown to be effective for control of many mosquito species in a variety of aquatic habitats (Levy et al. 1980, 1981, 1982a, 1982b; Mulla et al. 1983, Takahashi et al. 1984). Limited laboratory studies have been conducted on the effects of Arosurf MSF on nontarget organisms. No acute effects were observed from one 0.68-ml/m^2 treatment of Arosurf MSF on a species of frog or 2 freshwater fish species exposed for 6 months or to 4 saltwater fish species exposed for 7 days (Webber and Cochran 1984). Webber (1983) conducted 96-h acute toxicity tests with Arosurf MSF at 46.8 ml/m^2 on 3 species of fish without noticeable detrimental effects. There were no effects on mayfly naiads, adult diving beetles and ostracods after field evaluations at 1 gal/acre (Mulla et al. 1983). Field evaluations of Arosurf MSF at 1 gal/acre by Takahashi et al. (1984) indicated that corixids, notonectids, clam shrimp and a species of adult beetle were acutely affected but all, except for clam shrimp, had recovered to pretreatment population levels by day 3. Their sampling methods did not indicate detectable mortality in copepods, mayfly naiads, chironomid larvae or 4 species of beetle larvae.

In this paper we report the results of static (test solutions and test organisms were placed in test chambers and kept there for the duration of the test) laboratory studies to determine the acute toxic effects of Arosurf MSF on a wide variety of aquatic organisms, excluding members of the class Insecta. The criteria for determining acute effects in these organisms was lack of movement or reaction to gentle prodding. The maximum dosage tested was 47 ml/m^2 (50 gal/acre), 100 times the maximum Arosurf MSF label recommended application rate for mosquito control.

MATERIALS AND METHODS

Static toxicity tests to determine the acute effects of Arosurf MSF on specific indicator organisms were conducted in the laboratory utilizing methods recommended by the American Society for Testing and Materials (ASTM Standard E 729-1980).

The organisms used in conducting test were longnose killifish [*Fundulus similus* (Baird and Girard)], grass shrimp (*Palaemonetes pugio* Holthius), freshwater shrimp [*Palaemonetes paludosus* (Gibbs)], fiddler crab (*Uca* spp.), crayfish (*Procambarus* spp.), freshwater amphipod (*Gammarus* spp.), freshwater isopod (*Asellus* spp.), fairy shrimp (*Streptocephalus seali* Ryder), snail (*Physa* spp.), polychaete, [*Laeonereis culveri* (Webster)] and an unidentified amphipod.

All but 4 organisms were tested in 600-ml Pyrex® beakers containing 400 ml of water. When testing polychaetes, 100 ml of sterilized beach sand was added to the water to provide a substrate for burrowing. Three organisms were tested in 6-liter rectangular glass aquarium jars: longnose killifish, grass shrimp and fiddler crabs were tested with 4, 2 and 1 liters of water, respectively. The fiddler crab jars contained 600 ml of sand at one end to provide a beach above the water level. Containers were suspended in a water bath that utilized a Haake® (Haake Inc. Saddle Brook, NY) open bath immersion circu-

lator for heating or a Ladau® (Brinkmann Instruments Inc. Westbury, NY) cooling coil to regulate and maintain the water temperatures (±0.5°C) during tests.

Waters used for a diluent in preparing test solutions came from a deep well (alkalinity and hardness of ca. 150 ppm) for freshwater tests or from the Gulf of Mexico for saltwater tests. Sea water was filtered through a 10-μm sieve and the salinity lowered to 15–17 ppt with deionized water. The pH values for both fresh and saltwater tests ranged from 7.7 to 8.3. All water was aerated and treated with ultraviolet irradiation for 24 h before testing. Water quality was monitored during all tests for dissolved oxygen and pH; salinity was also monitored in saltwater tests, and hardness and alkalinity in freshwater tests.

Equal numbers of treatment and control containers were used in all tests. Arosurf MSF was pipetted directly onto the water surface of treatment containers at an application rate of 47 ml/m^2 based on the surface area of the water in the test containers. A 12L:12D photoperiod was maintained using fluorescent lighting during all tests. Test water temperatures were selected for each organism based on conditions during their peak natural abundance. Most organisms were collected during the summer months when water temperatures were relatively high and were tested at 27°C. Amphipods and isopods only occurred in abundance during the winter months; therefore, the water temperature for these tests was maintained between 20–22°C.

All organisms were held for 96 h posttreatment. The number of organisms and replications varied among organisms (Table 1). The number of organisms utilized per replication was based on loading (organism numbers that would not result in stress or unacceptable levels of dissolved oxygen) and/or cannibalism. Mortality counts were conducted at 24 h intervals with dead organisms removed at each count. Control mortality exceeding 5% rendered a test invalid and results were discarded. The treatment mortality was adjusted by use of Abbott's formula (Abbott 1925) when control mortality occurred and was exceeded by treatment mortality.

Some organisms were field-collected at the desired size or stage of development (Tables 1 and 2) for conducting the toxicity tests, whereas others (grass shrimp, saltwater amphipods, snails and fairy shrimp) required specific rearing techniques to obtain the desired developmental stage.

Special handling requirements (such as fed-nonfed, diet, number/container and techniques of transfer to minimize mortality) for each of the organisms were determined by preliminary nonchemical tests (Table 1). Grass shrimp and amphipods were fed during the toxicity test, whereas all other organisms were fed only during the pretest acclimation period.

RESULTS AND DISCUSSION

Water quality parameters measured during each test were within the standards prescribed for conducting static acute toxicity tests (Environmental Protection Agency 1975, ASTM Standard E 729-1980). Dissolved oxygen contents were above 60% saturation at 48 h posttreatment and above 40% saturation at 96 h. Change in both the fresh and saltwater pH values were within 0.2 units for any given test. Salinities during saltwater tests normally increased by 1 ppt at 96 h posttreatment due to evaporation.

Ninety-six-hour static acute toxicity tests of Arosurf MSF (47 ml/m^2) did not acutely affect any of the life stages at which organisms were tested (Table 2). The highest treatment mortality observed was 3.3% in fiddler crabs. Eight percent of the crabs used in this test escaped to the water bath with equal numbers escaping from both treatment and control containers, indicating that treatment had no affect on their ability to climb the glass sides of the container. Grass shrimp and fiddler crab were observed on occasions to be physically caught in the globule of excess Arosurf MSF. Arosurf is a monomolecular film and after the film has covered the water surface, the excess (as in this extremely heavy dosage) forms a globule that collects in some area of the container. Upon probing, it was evident that the organisms were alive, and by the next observation period they had escaped from the globule.

The organisms tested were not dependent on the air-water interface during any stage of their life cycle. However, snails and fiddler crabs did move through the interface without apparent deleterious effects during the period of time that tests were conducted. Takahashi et al. (1984) indicated that, with the exception of clam shrimp, the organisms affected by Arosurf MSF were insects that have a ventral plastron which may be affected by the reduction in surface tension. This is supported by observations by Mulla et al. (1983), who indicated that dead adult mosquitoes and chironomids occurred on the water surface of treated ponds.

The data herein list a number of organisms that were not acutely affected by even excessive applications of Arosurf MSF. Studies conducted to date indicate that this material primarily affects surface dependent insects including mosquitoes.

Table 1. Test criteria of organisms utilized in static acute toxicity testing.

Common name	Scientific name	Natural habitat	Acclimation period	Food source	Mean length (cm)	Replicates/ test	Organisms/ replicate
Longnose killifish	*Fundulus simulus*	Estuary	14 days	Tetra-Min®	3.40	10	4
Freshwater shrimp	*Palaemonetes paludosus*	Lake	7 days	TetraMin	1.50	84	1
Grass shrimp	*Palaemonetes pugio*	Estuary	<72 h	Brine shrimp	0.70	10	20
Fiddler crab	*Uca* spp.	Estuary	14 days	TetraMin	0.25[2]	10	12
Crayfish	*Procambarus* spp.	Roadside ditches	1 days	TetraMin	1.00	84	1
Amphipod	Order Amphipoda	Estuary	Colonized	TetraMin	0.20	20	5
Amphipod	*Grammarus* spp.	Freshwater marsh	14 days	TetraMin	0.30	20	5
Isopod	*Asellus* spp.	Freshwater marsh	14 days	TetraMin	0.33	20	10
Fairy shrimp	*Streptocephalus seali*	Temporary pond	Colonized	Marine[1] diet	0.30	10	5
Snail	*Physa* sp.	Roadside ditches	Colonized	TetraMin	0.10[2]	16	5
Polychaete	*Laeonereis culveri*	Estuary	28 days	Mosquito larvae	—	16	5

[1] Marine Invertebrate Diet®—Hawaiian Marine Imports, Inc. P.O. 21867, Houston, TX 77218.
[2] Mean width of animal.

Table 2. Ninety-six-hour acute static toxicity test of Arosurf® MSF (47 ml/m^2) against selected nontarget organisms.

Organism	Life stage	Salinity ppt	Temp. °C	Adjusted % mortality[1]
F. similis	Juvenile	15–16	27	0.0
P. pugio	Zoea	17–19	27	0.0
P. paludosus	Juvenile	0	27	0.0
Uca spp.	Juvenile	16–17	27	3.3
Procambarus spp.	Juvenile	0	27	0.0
Amphipod	Immature	15–16	22	0.0
Gammarus spp.	Immature	0	20	1.9
Asellus spp.	Immature	0	21	0.5
S. seali	Post-nauplius	0	27	0.0
Physa spp.	Immature	0	27	0.0
L. culveri	Adult	15–16	27	0.0

[1] Treatment mortality adjusted by Abbott's formula when control mortality occurred and was exceeded by treatment mortality.

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