Toxicity of a Linear Alkylate Sulfonate Detergent to Larvae of Four Species of Freshwater Fish

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The current use of linear alkylate sulfonate (LAS) as the active ingredient in detergent formulations has prompted considerable investigation on their toxicity to aquatic animals. Several investigators have demonstrated acute and chronic effects of LAS on several species of fish and invertebrates under varying environmental conditions and during different stages of development (SWISHER et al. 1964, MARCHETTI 1965, PICKERING 1966, ARTHUR 1970, PICKERING and THATCHER 1970, HOKANSON and SMITH 1971). Previous work has established that the larval stages of rainbow trout, bluegills, and fathead minnows are the most vulnerable to LAS. MARCHETTI (1965) did a series of acute mortality tests on eight developmental stages of the rainbow trout and found that the end of the alevin stage was the most sensitive to surfactants. During a complete life cycle exposure of fathead minnows to LAS, PICKERING and THATCHER (1970) found that newly hatched larvae were more sensitive than other stages in the life cycle. HOKANSON and SMITH (1971) also found that bluegill yolk-sac larvae were more sensitive to LAS than the embryos or larvae following yolk-sac absorption.

If larval stages of other fish species are the most sensitive to LAS, the laborious and costly long-term exposures involving all developmental stages in the life cycle could be eliminated, and LAS concentrations that had no discernable effects could be determined by shortened exposures with newly hatched larvae. This study was designed to determine the 96-hr LC50 for LAS and the LAS concentrations that had no measurable effect on the 30-day standing crop of larval northern pike (Esox lucius), white sucker (Catostomus commersoni), smallmouth bass (Micropterus dolomieu), and fathead minnow (Pimephales promelas).

MATERIALS AND METHODS

Physical System

A continuous flow serial diluter (MOUNT and WARNER 1965) and exposure tanks described by ARTHUR (1970) were used for all larval fish exposures. The test tanks held approximately 12.5 liters of water, and the mean water retention time was approximately 4 hr. All tanks were siphoned daily Monday through Friday to remove debris.

Water was obtained directly from Lake Superior and used without prior treatment. The water temperatures for the exposures were 15° ± 1° C except for the fathead minnows where a temperature of 23° ± 1° C was used.

Total hardness and pH determinations were made on all tanks, one tank per week (mean total hardness = 43.0 mg/liter as $CaCO_3$, range 36.0-48.0; pH = 7.2-7.9. Dissolved oxygen was measured in a different tank each day, Monday through Friday (mean D.O. = 8.5 mg/liter, range 5.6-10.0).

The LAS detergent formulation used in this study was supplied by Procter and Gamble Co. and had the following percentage composition: LAS, 14.0; alcohol-ethoxylate oxide condensate, 2.3; sodium soap, 2.5; sodium tripolyphosphate, 48.0; sodium silicate, 9.7; sodium sulfate, 15.4; moisture and miscellaneous, 8.1. Stock LAS detergent solutions were prepared by dissolving 50 g of formulation in 10 liters of demineralized water.

For the 30-day studies, aliquots from one duplicate tank at each concentration were collected daily, Monday through Friday. One milliliter of formalin was added to each composite bottle, and the samples were refrigerated between daily collections to prevent degradation of the LAS. The composited weekly samples were brought to room temperature before analysis. Analyses were alternated weekly between duplicate test chambers. Standard aqueous LAS (4.045%) from the New York Soap and Detergent Association (lot 2-3) was used as the analytical standard against which the composited test concentrations were determined. The methylene blue procedure was used for determining the LAS test concentrations (AMERICAN PUBLIC HEALTH ASSOCIATION 1971). Measurements of the LAS test concentrations are shown in Table 1. The control water contained negligible methylene blue active substances (MBAS), and LAS added

TABLE 1

Measured LAS detergent concentrations during the four separate larval fish exposures (mg/liter)

Duplicate test chambers	Northern pike	Fathead minnow	White sucker	Smallmouth bass
1	5.9 ± 0.2[a] (4)[b]	5.0 ± 0.3 (4)	6.3 ± 0.05 (4)	5.8 ± 0.35 (4)
2	2.4 ± 0.1 (4)	2.6 ± 0.150(4)	2.5 ± 0.05 (4)	2.3 ± 0.05 (4)
3	1.2 ± 0.05 (4)	1.1 ± 0.10 (4)	1.2 ± 0.05 (4)	1.2 ± 0.05 (4)
4	0.5 ± 0.05 (4)	0.5 ± 0.05 (4)	0.5 ± 0.05 (4)	0.5 ± 0.01 (4)
5	0.3 ± 0.01 (4)	0.2 ± 0 01 (4)	0.3 ± 0.01 (4)	0.2 ± 0.05 (4)
6 (control)	0.02 ± 0.005(4)	0.01 ± 0.005(4)	0.02 ± 0.005(4)	0.02 ± 0.01 (4)

[a]Standard error.

[b]Number of analyses.

to control water was completely recoverable. Tests showed that this LAS formulation did not degrade in refrigerated water containing formalin (ARTHUR 1970).

Biological System

The 96-hr LC50 values were calculated from mortality during the first 4 days of the 30-day exposures as outlined by the AMERICAN PUBLIC HEALTH ASSOCIATION (1971).

In the 30-day exposures the toxic effects on survival and growth were measured. Size at test termination was determined by wet weight measurements on the surviving fish in each duplicate tank. In each tank an estimate of the 30-day standing crop, as defined by BEVERTON and HOLT (1957), per original larva was obtained by multiplying the observed proportion of survivors at 30 days by the average wet weight at that time. This calculation is numerically equivalent to the total weight of live fish at the end of 30 days divided by the original number of exposed larvae. A one-way analysis of variance in conjunction with Dunnett's test (DUNNETT 1955) was utilized to test for any significant reduction of standing crop from control.

All of the larval fish used in this study were obtained from embryos incubated and hatched at the same test temperatures used in the LAS exposures. Larvae of four species were transferred to the LAS exposure system 2-3 days after hatching. The northern pike and white sucker eggs were hand stripped in the laboratory. Adult northern pike from Cow Horn Lake, Itasca County, Minnesota, and adult white suckers from Greenwood Lake, Cook County, Minnesota, provided the eggs necessary for the experiments. Smallmouth bass eggs were collected from nests in Two Island Lake, Cook County, Minnesota; fathead minnow eggs were obtained from National Water Quality Laboratory brood stock.

Fifty northern pike and white sucker larvae, 25 smallmouth bass larvae, and 15 fathead minnow larvae were exposed in each duplicate tank. Each species was exposed individually for 30 days over a 1-year period. Live brine shrimp were fed twice daily to the young northern pike, white suckers, and smallmouth bass. The fathead minnows were fed Glencoe Mills[1],[2] a dry trout

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[1]Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

[2]Glencoe Mills, Inc., Glencoe, Minnesota.

starter twice daily. In addition, a live mixed culture of zooplankton and phytoplankton was fed once a day to the fathead minnows.

RESULTS AND DISCUSSION

The 96-hr LC50 values (mg/liter LAS) were similar for all species tested: northern pike, 3.7; white sucker, 4.0; smallmouth bass, 3.7; and fathead minnow, 3.4.

The effect of 30 days' exposure to LAS detergent on standing crop for each experimental concentration for each of the four fish species tested is shown in Figure 1. The plot is of the ratio of the average experimental to the average control standing crop by species (expressed as a percentage change) and LAS concentration. The dotted line in Figure 1 indicates the level below which all values were statistically different ($P \leq 0.05$) from controls.

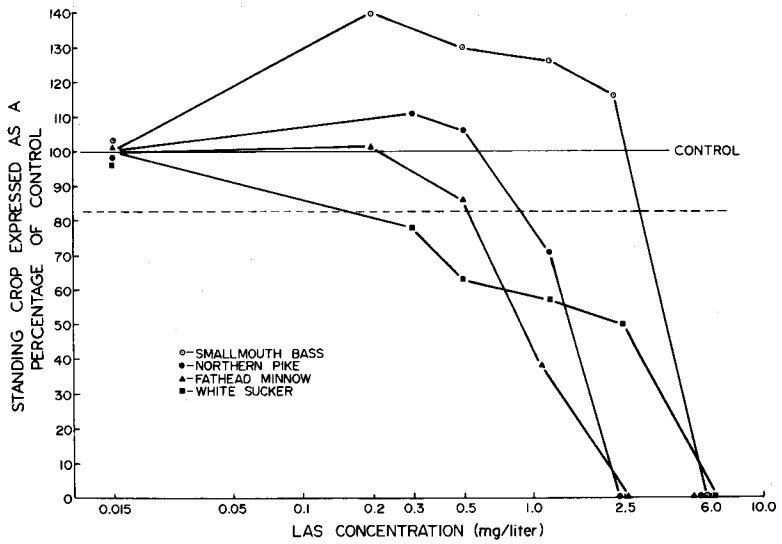

Figure 1. Ratio of experimental to control standing crop for four species of larval fish exposed for 30 days to various LAS concentrations.

Smallmouth bass were the most resistant to LAS detergent during the 30-day exposure, and significant reductions in standing crop were observed at concentrations above 2.3 mg/liter. The smallmouth bass 30-day standing crop at LAS concentrations of 0.2-2.3 mg/liter was significantly greater than the controls. Northern pike and fathead minnows were more sensitive than the smallmouth bass to LAS and showed a significantly reduced standing crop at concentrations exceeding 0.5 mg/liter. The white sucker was the most sensitive of the four species tested, and standing crop was significantly reduced at all LAS concentrations tested.

Water hardness has been shown to affect the toxicity of LAS during acute toxicity tests. HOKANSON and SMITH (1971) showed an increased sensitivity of bluegill larvae to LAS in hard water (300 mg/liter as $CaCO_3$) versus soft water (15 mg/liter as $CaCO_3$). TOVELL et al. (1974) recently showed that the anionic detergent sodium lauryl sulfate (SLS) was more acutely toxic to goldfish and rainbow trout in hard water than in soft water. They also observed a more rapid uptake of SLS into the fish in the harder diluent waters. However, a comparison of the 30-day toxicity of LAS to larval fathead minnows in the present soft water study with the chronic toxicity of LAS to fathead minnows in hard water (PICKERING and THATCHER 1970) indicated that hardness seemed to play a minor role in LAS chronic toxicity, although other differences in test conditions between laboratories may have contributed to this observation. PICKERING and THATCHER (1970) exposed fathead minnows through one complete life cycle to LAS in hard diluent water (200 mg/liter $CaCO_3$). They found that the concentration of LAS that had no effect on survival, growth, or reproduction was between 0.6 and 1.2 mg/liter. In our 30-day exposure the concentration of LAS that had no significant effect on larval fathead minnow standing crop was between 0.5 and 1.1 mg/liter in a soft diluent water (45 mg/liter $CaCO_3$).

The fact that several investigators, cited previously, have found the larval stages of fish to be the most sensitive to LAS coupled with the similarity of the LAS concentrations causing no measurable effects on fathead minnows, as determined by both the 30-day larval exposures in this study and the complete life cycle exposure of PICKERING and THATCHER (1970), strengthens the assumption that larval fish exposures alone may be useful in establishing LAS water concentrations acceptable for fish.

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