THE EFFECTS OF CONTINUOUS AQUEOUS EXPOSURE TO 78.01 ON HATCHABILITY OF EGGS AND GROWTH AND SURVIVAL OF FRY OF FATHEAD MINNOW (Pimephales promelas).

FM-3422

RESEARCH REPORT

SUBMITTED TO

3M COMPANY

ST. PAUL, MINNESOTA

REPORT #BW-78-6-195

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
June, 1978

ABSTRACT

Fathead minnow (Pimephales promelas) eggs and fry were continuously exposed to nominal 78.01 concentrations ranging from 20 to 1.3 µg/ℓ through 30 days post-hatch. Data were collected as percentage hatch of eggs, and survival, total length, and average wet weight of fry. Results of these data indicate that none of the above parameters were adversely affected by exposure to any of the 78.01 concentrations tested.

TABLE OF CONTENTS

SECTION I

INTRODUCTION

The objective of this study was to determine the effects of 78.01 on fathead minnow (Pimephales promelas) eggs and fry during continuous aqueous exposure. Exposures were initiated within 48-hours after egg fertilization and continued through 30 days post-hatch. The effects on egg hatchability and on survival and growth of fry were measured and would be used to make an estimate of the MTC (minimum threshold concentration). The MTC is virtually synonomous with the term MATC (maximum acceptable toxicant concentration) developed by Mount and Stephan (1967). Mount and Stephan's term, however, was estimated after the performance of full life-cycle chronic test where effects on reproduction and second generation fry were also measured.

Macek and Sleight (1977) and McKim (1977) described egg and fry investigations as being reasonably accurate short-term estimations of potential long-term chemical hazards to fish, and as being similar to those estimations derived from definitive chronic toxicity studies. In the majority of the studies reported by the authors and of those performed at this laboratory, the embryos and fry during early stages of development were generally the most sensitive stages to chemical exposure. Rarely was reproduction or survival and growth of second generation fry reduced at exposure levels lower than those that

reduced survival or growth of the first generation fry. The authors demonstrated that for the great majority of toxicants, the quicker and more economical egg and fry tests yielded estimates of safe concentrations very similar to those derived from chronic toxicity studies.

SECTION II

MATERIALS AND METHODS

The methodology for the egg and fry exposure closely followed that presented in "Proposed recommended bioassay procedure for egg and fry stages of freshwater fish" (U.S. EPA, 1972). The test material used, labelled 78.01, a white powder, was obtained January 23, 1978 from the 3M Company, St. Paul, Minnesota.

A. EXPOSURE SYSTEM

A modified, proportional diluter similar to that described by Mount and Brungs (1967) with a 0.50 dilution factor was used in this study. The diluent water was well water pumped to concrete reservoir where it was aerated before flowing to the exposure system through aged PVC pipe. The pH of this water ranged from 7.0-7.2; total hardness, 31-38 mg/ℓ as $CaCO_3$; alkalinity, 26-29 mg/ℓ as $CaCO_3$; and specific conductance, 149-170 µmhos/cm.

The diluter delivered five nominal 78.01 concentrations ranging from 20-1.3 µg/ℓ, control water (well water), and control water containing solvent (Dimethylsulfoxide, DMSO) to duplicate test aquaria. A 50 mℓ gas-tight syringe was used to deliver 46 µℓ of a 0.84 mg/mℓ stock solution of 78.01 in DMSO through a stainless steel needle and polyethylene tubing to the mixing chamber of the diluter. A mariotte bottle-volumetric tube

metering device was used to deliver DMSO into the solvent control aquaria at a concentration equal to the highest concentration of DMSO in the 78.01 exposure aquaria (24 mg/ℓ DMSO).

Each glass test aquarium measured 40 x 20 x 25 cm centimeters (cm) with a 19 cm high standpipe drain which maintained a constant test water volume of 15.2 ℓ. The diluter delivered 0.50 ℓ of test water to each aquarium 185 times per day, yielding a 90% test water replacement time of approximately 9 hours (Sprague, 1969). The aquaria rested in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test water temperature at 25°C.

B. EGG AND FRY EXPOSURE

On March 31, 1978, the exposure of fathead minnow eggs to 78.01 was initiated. Eggs used were from the brood stock of the U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota. Upon arrival at E G & G, Bionomics, the eggs were allowed to acclimate from 17.5°C to the test temperature of 25°C over a period of two hours. Sixty eggs were then randomly distributed to each of 14 egg cups which were then dipped in a 60 mg/ℓ malachite green solution for 15 seconds to prevent fungus growth. One egg cup was then suspended in each of the 14 test aquaria. Egg incubation cups were glass jars (5 cm O.D.,

8 cm high) with 40 mesh Nitex^{R} screen bottoms. An egg cup rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the egg cups in the test water.

Dead eggs were removed and counted daily until hatching was completed. Percentage hatch was calculated based on the number of live fry per aquarium after hatching was completed compared to the number of eggs per aquarium (60) at the initiation of the exposure. To initiate the 30-day fry exposure, forty fry were randomly selected from each egg cup and transferred to the respective aquaria.

Upon transfer of fry to the aquaria, the fry were fed newly hatched San Francisco Bay variety brine shrimp nauplii, ad libitum, three times daily throughout the exposure period. The aquaria were brushed and siphoned to remove excess food and fecal material three times per week. At 30 days post-hatch, the fry from each aquarium were anesthetized with MS-222 (tricaine methane-sulfonate) and percentage survival, mean total length, and mean wet weight were determined. The fry were measured individually to calculate a mean and standard deviation total length while each fry group (fry from one aquarium) was wet weighed to calculate a mean wet weight.

At the termination of the test, the fry from the control and the high concentration (20 µg/ℓ) were preserved in 10% buffered formalin while the fry from the other test aquaria were frozen.

Ten formalin preserved fry (5 from each replicate) from the control and the high concentration were sent to the Environmental Pathology Laboratories, Inc., Carolina, Rhode Island for histopathological examination of a transverse section of the nares and cephalic extension of the lateral line. The remaining preserved fry and frozen fry were sent to the 3M Company, St. Paul, Minnesota, May 31, 1978.

Temperature, dissolved oxygen concentrations, and pH were monitored daily, alternating between aquaria such that each aquarium was measured once each week. Temperature was measured with a mercury thermometer, dissolved oxygen with a YSI Model #54 dissolved oxygen meter and probe, while pH was measured with an Instrumentation Laboratory Model #175 portable pH meter and probe.

One thousand mℓ water samples were taken from each aquarium at the initiation of the test (day 0), when hatching was completed (day 4), and weekly thereafter (days 11, 18, 25 and 32) for determination of 78.01 concentration. Samples were taken with a beaker from a point approximately midway between the surface, bottom and sides of each aquarium, and stored in 1000 mℓ amber glass bottles with foil lined caps. All samples taken during the test were stored at room temperature and shipped on May 31, 1978 to the 3M Company, St. Paul, Minnesota.

C. STATISTICS

Means of measured biological parameters from duplicate aquaria were subjected to analysis of variance (Steel and Torrie, 1960, completely randomized block design, P=0.05). Data for percentage survival and percentage hatch were transformed to arc sin $\sqrt{\text{percentage}}$ prior to analysis.

SECTION III

RESULTS

Water quality parameters measured during the egg and fry exposure exhibited little variation between test days and test chambers. Mean measured dissolved oxygen was 9.0 ± 0.5 mg/ℓ and ranged from 8.2-10.2 mg/ℓ. Mean temperature was 25 ± 1°C and ranged from 23.5-26.5°C and pH ranged from 6.9-7.5.

The biological data generated in this study (Table 1) indicate that hatchability of eggs, and percentage survival, mean total length and mean wet weight of fry were not adversely affected at any 78.01 concentration tested.

Based on this datum, the MTC for fathead minnows and 78.01 is estimated to be >20 µg/ℓ based on nominal concentrations.

006404

SECTION IV

REFERENCES

Macek, K.J. and B.H. Sleight, III. 1977. Utility of toxicity tests with embryo and fry of fish in evaluating hazards associated with chronic toxicity of chemicals to fishes. Symposium Proceedings, ASTM, Memphis, Tennessee, October, 1976: 137-146.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Bd. Can. 34: 1148-1154.

Mount, D.I. 1968. Chronic toxicity of copper to fathead minnow (Pimephales promelas, Rafinesque). Water Res. 2: 215-223.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 20-29.

Mount, D.I. and C.E. Stephen. 1967. A method for establishing acceptable toxicant limits for fish, malathion and the butoxyethanol ester of 2,4-D. Trans. Amer. Fish. Soc. 96: 185-193.

Sprague, J.B. 1969. Measurements of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water

Res. 3: 793-831.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York: 481 pp.

U.S. EPA. 1972. Proposed recommended bioassay procedure for egg and fry stages of freshwater fish: 7 pp.

SECTION V

TABLE

Table 1 -- Percentage hatch of eggs, percentage survival, mean and standard deviation total length, and mean wet weight of fathead minnow (Pimephales promelas) fry exposed to 78.01 for 30 days post-hatch.

Nominal concentration (µg/ℓ)	Replicate	Hatch (%)	30 Days Survival (%)	30 Days Mean length (mm)	30 Days Mean weight (mg)
20	A	92	98	21(2)	64
	B	87	98	21(2)	66
10	A	93	92	21(3)	60
	B	92	88	23(2)	79
5.0	A	88	100	20(4)	58
	B	92	92	21(2)	69
2.5	A	97	90	21(3)	65
	B	95	95	21(2)	64
1.3	A	90	92	21(3)	65
	B	92	92	21(3)	70
control	A	93	82	21(3)	69
	B	92	92	21(2)	71
solvent control	A	92	82	20(3)	64
	B	93	90	20(4)	66

SUBMITTED BY:

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
June, 1978

PREPARED BY:

Jerry W. Dean

Aquatic Biologist

Stephen J. Ells

Aquatic Toxicologist

APPROVED BY:

George A. Cary

Director, Aquatic Biology