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Early Life Stage Final Report  
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Early Life Stage Toxicity of Acrylonitrile  
to Fathead Minnows (Pimephales promelas)  
in a Flow-Through System



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### SUMMARY

A flow-through 30 day early life stage toxicity study of Acrylonitrile (AN) to fathead minnows (Pimephales promelas) was conducted to estimate the Maximum Acceptable Toxicant Concentration (MATC) limits. A one-liter proportional diluter system was used to maintain constant test concentrations. Exposure concentrations of Acrylonitrile were determined through the use of gas chromatographic methods. The mean measured levels of Acrylonitrile were 0.34 mg/l, 0.44 mg/l, 0.86 mg/l, 1.8 mg/l and 3.6 mg/l.

The survival of fathead minnow fry was significantly reduced in the highest levels (7.6 mg/l, 1.8 mg/l and 0.86 mg/l) of exposure to Acrylonitrile. Growth of the fry, as measured by standard length and weight, was the most sensitive measured parameter in the Acrylonitrile study. At all concentrations  $\geq 0.44$  mg/l, there was a significant decrease in growth of the fathead minnows after 30 days of exposure. At the 0.34 mg/l concentration, one of two duplicate exposures showed a significant effect upon growth; therefore a "no effect" concentration could not be established. Based on this data, it is our opinion that the upper limit of the MATC for a 30 day exposure of Acrylonitrile to fathead minnows is 0.34 mg/l.

## INTRODUCTION

Monsanto Chemical Company contracted the Aquatic Toxicology Division of Analytical BioChemistry Laboratories, Inc., to conduct a dynamic 30 day early life stage (egg-fry) bioassay with fathead minnows (Pimephales promelas) exposed to Acrylonitrile. The primary objective of the study was to estimate Maximum Acceptable Toxicant Concentration (MATC) limits for Acrylonitrile using what is believed to be the most critical and sensitive life stage (embryo to the juvenile stage) of the fathead minnows, (1, 2). This was achieved by measuring the effects of Acrylonitrile on hatchability, survival, growth, behavior and morphological changes of the embryos and fry. This is determined by comparison between the control and exposure concentrations. The study was authorized by A. F. Werner, Monsanto Chemical Company, in a letter dated June 3, 1980 and was conducted from October 6 to November 10, 1980.

The term Maximum Acceptable Toxicant Concentration (MATC) (3) as used in this report is the concentration limit of which the lower value is the concentration that causes no observable deleterious effect for any of the parameters measured during the study. The upper value is the concentration that produces at least one deleterious effect of the measured parameters (4). A deleterious effect is one that is a statistically significant ( $P=0.05$ ) reduction from the control for the parameter being measured.

## METHODS AND MATERIALS

The biological methods used for this early life stage study with fathead minnow are basically those described in: Recommended Bioassay Procedure for Egg and Fry Stages of Fresh Water Fish (5) and ASTM Standard Practice for Conducting Toxicity Tests on the Early Life Stages of Fishes (4). The study was conducted following the methods outlined in ABC Protocol No. 7809, as approved by A. F. Werner, Monsanto Chemical Company.

### I. Test Fish

The fathead minnow eggs used for the initiation of the test were obtained from the U.S.D.I. Columbia National Fisheries Research Laboratory in Columbia, Missouri. These eggs were less than 18 hours old and from adults that had been identified to species using the taxonomic keys developed by Eddy (6). The fathead minnow stock culture as well as the test system was maintained at 25°C ( $\pm 2^\circ\text{C}$ ) with a 16 hour daylight photoperiod. During acclimating, acclimation and test periods, the fish received a mixed diet of live newly hatched brine shrimp nauplius (Artemia salina) and ground commercial fish food (Rangen's) at least twice daily ad libitum.

### II. Test System

A one-liter proportional diluter system described by Mount and Brungs (7), with the modifications of McAllister et al. (8), was

used for the intermittent introduction of Acrylonitrile to duplicate control and exposure aquaria. Clear 4 mil polyethylene curtains supported by a wooden framework completely enclosed the diluter system. Fumes of Acrylonitrile were vented from under the enclosure to the outside of the laboratory building by means of an exhaust fan. Flow-splitting chambers divided each of the five Acrylonitrile concentrations and one control four ways into duplicate test aquaria with replicate growth chambers in each test aquarium (Figure 2). The glass aquaria measured 35 X 30 X 30 cm with a water depth of 24 cm. Each test aquaria was divided by a glass partition to provide space for two growth chambers which measured 23 X 15 X 30 cm and had stainless steel screening (40 mesh) attached to one end (Figure 1-B). Aerated well water (40 l) was delivered to the glass aquaria at an average rate of 100 ml/minute/aquarium, an amount which was sufficient to replace the 25 liter test volume at least 5.5 times in a 24 hour period. The test aquaria were immersed in a circulating water bath held at 25°C (±1°C) by electronically controlled submersible heating elements.

The fathead minnow eggs were incubated in cups suspended in the treatment and control water (Figure 1-A). Egg cups were made from 4.5 cm OD glass tubing with stainless steel screening (40 mesh) attached to the bottom with silicon sealer. To insure exchange of water, the egg cups were oscillated in the test solution and/or water by means of a rocker arm apparatus driven by a 4 r.p.m. electric motor (9).

### III. Test Compound

The Acrylonitrile standard was received on May 15, 1980, in good condition. The sample upon receipt was observed to be a clear liquid and was stored in the dark at 4°C. The stock solutions were prepared as needed on a weight:volume basis by dissolving in deionized water and were delivered to the diluter from a Mariotte bottle enclosed in aluminum foil. During the study, a new stock solution of Acrylonitrile was prepared every two to four days.

### IV. Statistical Analyses

The design of the early life stage study was a complete block design. Measured parameters in the duplicate exposure chambers were analyzed at least once during the study, using two-way analysis of variance with an interaction model to determine if significant differences existed between duplicates (4). If the analysis showed no significant differences ( $P=0.05$ ) or interactions, duplicate data were pooled. The pooled growth data was subjected to one-way analyses of variance. If analysis showed a significant difference, duplicate data was not pooled and considered as two separate data sets. When treatment effects were indicated followed by a significant F-test or the means, a multiple means comparison test, Tukey's protected Least Significant Difference (LSD), was used. Treatment effects on percent survival data were transformed using the following equation (10).

$$\text{Angle} = 2 \arcsin \sqrt{\frac{X}{N}}$$

where, N = number of organisms tested  
X = number of organisms hatched or alive.

This was followed by analysis of variance and a least significant difference test. Those concentrations exhibiting decreased survivability were not used in any further ANOVA programs so as not to bias the results. All differences we considered significant at P=0.05 (95% confidence level).

#### V. Test Procedure - Biological

Before initiating the biological portion of the study, the test solution was allowed to flow through the test aquaria for a 24 hour equilibration period. The test concentration was confirmed by gas chromatographic analysis before introducing the eggs. Days for analytical confirmation (water residue days) were numbered from 0 to 35 beginning with the initiation of the study.

The biological phase of the study was initiated on October 10, 1980, by distributing 50 randomly selected fathead minnow eggs into each of two incubator cups per duplicate exposure aquaria, i.e. 200 eggs per concentration. Egg mortality, as discerned by a distinct change in coloration, was recorded daily and dead eggs were removed to prevent fungal growth. When hatching commenced the approximate percent of embryos hatching in each incubation cup was recorded daily until hatching was completed. The date of complete hatch was designated as "day 0" for growth sampling periods (growth days). The eggs were exposed to Acrylonitrile for 5 days before the complete hatch date (Table 3). When hatching was complete, surviving fry were impartially reduced to four groups of 20 fry each per growth chamber. Survival was monitored at least weekly by visually inspecting each growth chamber, and behavioral or physical changes were recorded.

From the beginning of hatch until day 30 of growth, all fry were fed live brine shrimp nauplii in combination with a standard commercial fish food (Rangen's) 2 to 4 times a day ad libitum. All aquaria were siphoned daily to remove fecal material, excess food and any biological growth on the glass or stainless steel screen. At 30 days post hatch all surviving fish were killed in ice water and immediately measured for standard length, blotted and weighed.

#### VI. Test Procedure - Chemical and Physical

Nominal concentrations were determined by placing a known stock solution of Acrylonitrile in the Mariotte bottle and allowing the diluter to equilibrate for twenty-four hours. The final concentration was then analytically confirmed. Concentrations were

corrected by adjusting the amount of the toxicant aliquot by raising or lowering the standpipe in the Mariotte bottle. Because the diluter was designed to have a 50 percent dilution factor, serial dilutions of the high concentration were used to estimate all lower nominal concentrations.

Water quality parameters of dissolved oxygen, pH and ammonia were measured on water residue days 0, 1, 5, 10, 15, 20, 30 and 35 during the study. Two hundred milliliter test water samples, collected in 250 ml glass beakers, were taken from the control, low concentration and highest concentration with surviving eggs or fry. Measurements of these selected parameters were determined with a Corning pH-millivolt meter and selective ion probes. Temperature in control chamber 3D was monitored continuously using a remote platinum-resistance sensor and strip chart recorder.

Analysis of all water samples for Acrylonitrile (AN) were accomplished by gas-liquid chromatography as outlined in the Monsanto Industrial Chemicals Company procedure entitled, "Determination of Acrylonitrile in Water for Environmental Fate Studies."

Representative 100 ml volumes of water samples were collected in 100 ml volumetric flasks directly from the test aquaria. If necessary, dilutions were made by adding an appropriate aliquot of the solution to a 100 ml volumetric flask and diluting to volume with ionized water. For an internal standard, 50  $\mu$ l of a 1.00 mg/ml Proptonitrile (PN) stock solution was added to each 100 ml sample solution for GC analysis. Standards were also prepared in this manner to contain 0.5  $\mu$ g AN.

Direct aqueous injections of samples and standards were done within 6 hours of sampling. Between sampling and injection, all samples and standards were stored in a refrigerator at 4-10°C. A Varian Model 3700 gas chromatograph equipped with a Thermionic Specific Detector (TSD) was employed with the following parameters:

Column: 20' X 2 mm I.D. stainless steel  
Packing: 100/120 mesh Porapak Q (Waters Associates)  
Carrier Gas: Helium, 30 ml/minute  
Column Temperature: 115°C  
Injector Temperature: 170°C  
Detector Temperature: 250°C  
Detector Settings: Bias Voltage 64  
                    Beam Current 480  
                    H<sub>2</sub> Pressure 10 psi

Linearity was established for each day's injections by calculating a relative response factor (R) for each standard injected using the equation

$$R = \frac{A_s}{V_s} \cdot \frac{V_p}{A_p}$$

where,  $C_A$  = concentration of AN in  $\mu\text{g/ml}$ ,  
 $C_P$  = concentration of PN in  $\mu\text{g/ml}$ ,  
 $A_A$  = height of AN peak,  
and  $A_P$  = height of PN peaks.

Example calculation of R for 0.1  $\mu\text{g/ml}$  AN standard, injected October 6, 1980, 4:10 p.m.:

$$R = \frac{(0.1)(146)}{(46)(0.5)} = 0.635$$

An average relative response factor ( $R_{ave}$ ) was calculated for each set of standards by averaging the R obtained for all standards in that set. The concentrations of AN in the samples were calculated by using the equation:

$$C_A = \frac{(R_{ave})(A_A)(C_P)(\text{Dilution Factor})}{(A_P)}$$

where,  $C_A$  = concentration of AN in the sample in  $\mu\text{g/ml}$ ,  
 $R_{ave}$  = average relative response factor,  
 $A_A$  = peak height of AN,  
 $C_P$  = concentration of PN in the sample in  $\mu\text{g/ml}$   
and  $A_P$  = peak height of PN.

Example calculation of  $C_A$  for sample L, Day 10/11/80, 10:30 p.m. injection:

$$C_A = \frac{(0.589)(73)(1.5)(20)}{(116)} = 3.71 \mu\text{g/ml AN.}$$

Accuracy of the method was determined by recoveries of AN spiked into control aquarium water. Analysis of spiking levels of 0.25  $\mu\text{g/ml}$  AN and 4.0  $\mu\text{g/ml}$  AN, and blind spikes yielded average recoveries of 106%, 104% and 92.5%, respectively.

Precision of the method was determined by analyzing triplicates of the high fortification and treatment level 3 for each sample day. Precision results are included in the raw data.

Because AN residues were present in some control samples, a reagent blank of well water was included with each set. The concentration of AN in the controls were calculated although it should be noted that the response for these residues were lower than that of the standard range.

## RESULTS

The test concentrations of Acrylonitrile were determined on later residue days 0, 1, 5, 10, 20, 30 and 35 through the use of gas chromatographic methods outlined in the text of this report. The results are summarized in Table 4. The mean measured concentrations of Acrylonitrile were: 0.34 mg/l, 0.44 mg/l, 0.86 mg/l, 1.8 mg/l and

3.6 mg/l. These values represented 136%, 88%, 86%, 90% and 90% of the expected nominal values, respectively. The Acrylonitrile stock was analyzed at each water residue sample day and results tabulated in Table 5. The mean stock concentration was 70% of the expected nominal concentration. The difference was attributed to the volatility of Acrylonitrile. The measured stock concentration remained consistent throughout the study.

Low levels of Acrylonitrile were found to be present in the control water throughout the first ten days of the study. Again, the volatility of the compound resulted in contamination of the control water due to agitation during the dilution cycle. To remedy this situation, the control water portion of the diluter face was separated from the toxicant mixing area of the proportional diluter and the flow splitting box was covered with polyethylene plastic. As a result, levels of Acrylonitrile in the control water steadily declined from water residue days 1 to 20. Levels of Acrylonitrile in the control water remained below the detectable limit from water residue day 20 until termination of the study.

Water quality parameters of dissolved oxygen, pH and ammonia were measured in the control, low concentration and highest concentration with surviving fish on water residue days 0, 1, 5, 10, 15, 20, 25, 30 and 35 during the study (Table 6). The dissolved oxygen concentrations ranged from 5.4 mg/l to 8.3 mg/l which represents 64 to 99% saturation and was considered adequate for testing (11). The ammonia concentrations remained constant and consistent with the control. Temperature, which was monitored continuously in growth chamber 3D, did not fluctuate by more than  $\pm 1^{\circ}\text{C}$  in any 24 hour period.

Mean percentage hatch in the control aquaria was 69 while the percentages in the exposure aquaria ranged between 46 and 87 (Table 7 and Figure 3). There was no apparent difference between the appearance of the control eggs and those continuously exposed to Acrylonitrile. A multiple means comparison was used for the parameter of percentage hatch but, because the incidence of fungus in various egg cups was not uniform in frequency, hatchability would not be a reliable indicator of toxicant effect.

The survivability of fathead minnow fry exposed to 0.86 mg/l, 1.3 mg/l and 3.6 mg/l of Acrylonitrile was significantly lower than the control after 30 days (Table 7 and Figure 3). There was no abnormal behavior observed prior to death of the fry which is an unusual characteristic which may be peculiar to Acrylonitrile. Survival of fathead minnows at concentrations less than 0.86 mg/l was not significantly affected but scattered mortality in all concentrations except the control was observed during the study. To eliminate disease as a cause of observed mortality, one fathead minnow fry was removed on November 6, 1980, from the 0.86 mg/l exposure for disease investigation. Pathological examination revealed that no disease was present. Results of this investigation have been included in the raw data.

As seen in Figure 1, each test tank is composed of four chambers, A, B, C and D with A and B (AB) being distinct from C and D (CD). In the comparison of growth, as measured by length and weight between the

control and treated aquaria, a significant difference was revealed. The analysis of duplicate chambers AB showed that all concentrations caused a statistically decreased growth, as measured by standard length and weight, of the fathead minnow fry after 30 days of exposure (Table 7 and Figures 1, 5). However, the analysis of duplicate chambers CD showed that 0.34 mg/l of acrylonitrile did not significantly decrease growth (Table 7 and Figures 1, 6). Neither of the above programs included the concentrations 0.30 mg/l, 1.8 mg/l or 3.0 mg/l in the analyses because the percent survival at these levels was found to be significantly reduced.

Subsequent to the typical analysis of variance mentioned above, the same programs were run with one major change. The analysis was run comparing the exposure concentration from one duplicate to another duplicate of the same duplicate. For example, an analysis of growth variance as measured by standard length, was done comparing exposure concentrations of duplicate CD to the AB control. It turned out that this variation made little difference in the results as already presented.

Based on the data for this 30 day fathead minnow early life stage study the Maximum Acceptable Toxicant Concentration (MATC) limits as defined cannot be estimated for Acrylonitrile. But the results of growth and length analysis for the low concentration of 0.34 mg/l gave a significant difference in the AB chambers and a significant difference in the CD chambers. It is our opinion that the low concentration of 0.34 mg/l would be an estimate of the upper limit of the MATC at 30 days of exposure to Acrylonitrile.

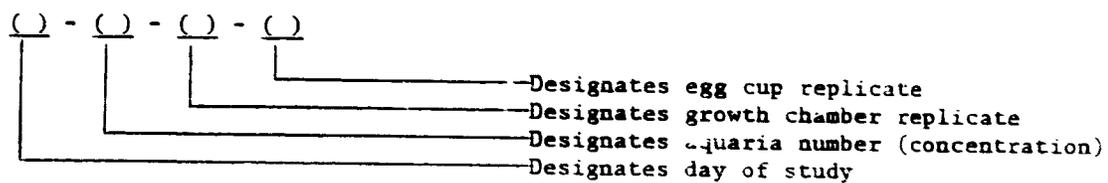
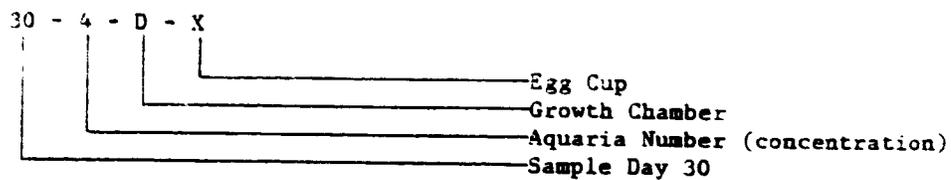
The study was conducted following the intent of the Good Laboratory Practice Regulations (13) and the final report was reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit. Original raw data was provided to Monsanto Chemical Company, with a copy retained at Analytical BioChemistry Laboratories.

Table 1: Chemical characteristics of well water at ABC's Aquatic Bioassay Laboratory.

<u>Parameter</u>	<u>Concentration</u>
Dissolved Oxygen	9.3 ppm
pH	8.2
Hardness (CaCO <sub>3</sub> )	255 ppm
Alkalinity (CaCO <sub>3</sub> )	368 ppm
Conductivity	50 μmhos/cm
Total Ammonia (NH <sub>3</sub> )	<0.05 ppm
NO <sub>3</sub> -N	0.15 ppm
Ortho-Phosphate	0.10 ppm
Aluminum	<0.01 ppm
Arsenic	<0.001 ppm
Cadmium	<0.001 ppm
Chromium	0.001 ppm
Cobalt	<0.001 ppm
Copper	<0.01 ppm
Iron	0.012 ppm
Lead	0.009 ppm
Mercury	<0.0001 ppm
Nickel	0.0157 ppm
Zinc	<0.01 ppm
Measured organophosphorus pesticides	a
Measured organochlorine pesticides plus PCB's	a

<sup>1</sup>See Raw Data Report for individual analyses.

TABLE 2: Tank code example.



Day of Study

0, 1, 15, 30, 60

(These numbers are not in the code for daily observations.)

Aquaria Identification

Control (1), 2, 3, 4, 5, 6

Growth Chamber

A, B, C, D

Egg Cups

X, Y

(These letters are not in the code after the eggs are removed from cups.)

TABLE 3. Hatching success of fathead minnow eggs continuously exposed to Acrylonitrile.

EGG Cup Code	No. eggs per cup	Day 1 Cumulative Hatch	Day 2 Cumulative Hatch	Day 3 Cumulative Hatch (Approximate %)	Day 4 Cumulative Hatch (Approximate %)	Day 5 Cumulative Hatch (Approximate %)
Control-B-X	50	--	--	5	95	100 <sup>b</sup>
B-Y	50	--	--	10	90	100
D-X	50	--	--	2	95	100
D-Y	50	--	--	6	95	100
2-A-X	50	--	--	2	90	100
A-Y	50	--	--	2	10	100
C-X	50	--	--	--	99	100
C-Y	50	--	--	--	95	100
3-A-X	50	--	--	--	95	100
A-Y	50	--	--	--	95	100
C-X	50	--	--	--	60	100
C-Y	50	--	--	2	90	100
4-A-X	50	--	--	10	95	100
A-Y	50	--	--	6	95	100
C-X	50	--	--	--	90	100
C-Y	50	--	--	20	90	100
5-B-X	50	--	--	2	80	100
B-Y	50	--	--	--	90	100
D-X	50	--	--	4	80	100
D-Y	50	--	--	2	60	100
6-B-X	50	--	--	1	95	100
B-Y	50	--	--	0	60	100
D-X	50	--	--	2	60	100
D-Y	50	--	--	2	60	100
				0	50	100

<sup>a</sup>Day 5 is the date of complete hatch.

<sup>b</sup>100 percent hatch values indicate that all of the eggs which are capable of hatching have hatched.

NOTE: See Table 2 for description of egg cup and tank code.

TABLE 4: Concentrations of Acrylonitrile during the 30 day early life stage study with fathead minnows.

Aquaria No.	Nominal Concentration (mg/l)	Measured Concentration (mg/l)										Mean (±S.D.) or (% recovery)
		Day 0	Day 1A	Day 1B	Day 5	Day 10	Day 20	Day 30	Day 30	Day 30	Day 30	
Control	---	0.045	0.037	0.041	0.023	0.016	<0.015	<0.012	0.020	<0.026	(±0.013)	
Level #1	0.25	0.32	0.38	0.21	0.21	0.38	0.40	0.40	0.39	0.34	(±0.082)	
Level #2	0.5	0.39	0.45	0.45	0.45	0.44	0.50	0.41	0.41	0.44	(±0.034)	
Level #3a	1.0	0.79	0.94	0.77	0.80	0.87	1.0	0.74	0.94	0.86	(±0.095)	
b	1.0	0.82	0.94	0.78	0.80	0.89	1.0	0.58	0.96	0.85	(±0.13)	
c	1.0	0.82	0.93	0.78	0.82	0.89	1.1	0.75	0.95	0.88	(±0.11)	
Level #4	2.0	1.4	1.6	1.8	2.1	1.3	2.4	2.0	2.1	1.8	(±0.38)	
Level #5	4.0	3.0 <sup>b</sup>	3.4	3.5	3.7	3.3	3.3	4.0	4.3	3.6	(±0.42)	
Low Spike	0.25	0.26 (104%)	0.27 (108%)	0.27 (108%)	0.26 (104%)	0.27 (108%)	0.26 (104%)	0.26 (104%)	0.27 (108%)	0.27 (108%)	(±0.0053)	
Blind Spike	<sup>a</sup>	0.89 (89%)	0.38 (84%)	0.82 (90%)	0.60 (100%)	0.49 (82%)	0.34 (97%)	1.1 (110%)	2.3 (92%)	---		
High Spikea	4.0	4.1 (103%)	4.0 (100%)	4.1 (103%)	4.2 (105%)	4.1 (103%)	4.0 (100%)	3.8 (95%)	5.5 (138%)	4.2 (105%)		
b	4.0	4.1 (103%)	4.0 (100%)	4.1 (103%)	4.0 (100%)	4.1 (103%)	4.1 (103%)	3.9 (98%)	4.2 (105%)	4.2 (105%)		
c	4.0	4.3 (108%)	4.1 (103%)	4.2 (105%)	4.1 (103%)	4.1 (103%)	4.2 (105%)	4.2 (105%)	4.4 (110%)	4.2 (105%)		
Reagent Blank	---	<0.011	<0.011	<0.010	<0.013	<0.013	<0.015	<0.012	<0.013	<0.012	<0.012	

<sup>a</sup>The following nominal concentrations were used for blind spikes; 1.0, 0.45, 0.91, 0.60, 0.60, 0.35, 1.0 and 25 mg/l, respectively for the above sample days.

<sup>b</sup>Percent recovery are included in the parenthesis. The average recoveries for the high, low and blind spike was 104%, 108% and 93%, respectively.

TABLE 5: Concentrations of Acrylonitrile in stock solutions during the 30 day early life stage study with fathead minnows.

<u>Water Residue Day/Date</u>	<u>Acrylonitrile (mg/ml)</u>	<u>Percent of Nominal Concentration</u>
0 (10/6/80)	5.21	54
1A (10/7/80)	6.56	68
1B (10/7/80)	6.64	69
5 (10/11/80)	6.80	71
10 (10/16/80)	6.39	67
20 (10/26/80)	7.14	74
30 (11/5/80)	7.91	82
35 (11/10/80)	<u>7.28</u>	<u>76</u>
Mean	6.74	70
Standard Deviation	0.79	8.2

Nominal Stock Concentration = 9.6 mg/ml.

TABLE 6: Water quality measurements during the Acrylonitrile early life stage study with fathead minnows.

Study Day	Water Quality											
	Control				Low Concentration <sup>c</sup>				High Concentration <sup>c</sup>			
	Temp. <sup>a</sup> °C	D.O. <sup>b</sup> mg/l	pH <sup>c</sup>	NH <sub>3</sub> <sup>d</sup> mg/l	Temp. <sup>a</sup> °C	D.O. <sup>b</sup> mg/l	pH <sup>c</sup>	NH <sub>3</sub> <sup>d</sup> mg/l	Temp. <sup>a</sup> °C	D.O. <sup>b</sup> mg/l	pH <sup>c</sup>	NH <sub>3</sub> <sup>d</sup> mg/l
0	25	7.9	7.9	0.39	25	7.8	7.9	0.40	25	7.9	8.0	0.38
1	24	8.2	7.9	0.40	24	8.2	7.9	0.40	24	8.3	8.0	0.38
5	25	8.0	8.0	0.45	25	8.0	8.0	0.50	25	8.1	8.0	0.40
10	24	6.2	7.9	0.36	24	7.2	8.0	0.40	24	7.4	8.1	0.38
15	25	7.5	7.9	0.35	25	7.5	7.9	0.40	25	7.9	8.0	0.45
20	25	7.3	8.0	0.25	25	7.0	8.0	0.20	25	7.0	8.0	0.54
25	25	6.8	7.9	0.22	25	6.6	7.9	0.28	25	6.7	7.9	0.30
30	25	6.3	7.8	1.0	25	6.0	7.8	0.70	25	5.4	7.8	1.0
35	24	7.9	7.8	0.45	24	7.6	7.8	0.39	24	7.3	7.8	0.64

<sup>a</sup>Temperature - Monitored continuously using a remote platinum resistance sensor and strip chart recorder.

<sup>b</sup>Dissolved oxygen concentrations - Dissolved Oxygen Probe used with a YSI Model 54A Dissolved Oxygen System.

<sup>c</sup>pH - pH Probe (Corning Model 476022) used with a Corning Model 125 pH and mV meter.

<sup>d</sup>Ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with a Corning Model 125 pH and mV meter.

<sup>e</sup>Highest concentration with surviving fry.

TABLE 7. Mean percentage hatch of eggs, mean survival, standard lengths and wet weights of fathead minnow fry continuously exposed to Acrylonitrile.

Aquaria Identification	Mean Measured Concentration of Acrylonitrile (mg/l)	Mean Hatch (%)	Survival (%)	Mean Standard Length <sup>a</sup> (mm)		Mean Standard Length (mm)		Mean Wet Weight (g)	
				AB	CD	AB	CD	AB	CD
Control	0.026	69	81	22.2 <sup>b</sup> (±1.98)	21.2 (±1.5)	0.21 (±0.052)	0.19 (±0.038)		
Level #1	0.34	87 <sup>a</sup>	75	20.5 (±1.72) <sup>a</sup>	21.3 (±1.5)	0.16 (±0.042) <sup>a</sup>	0.19 (±0.040)		
Level #2	0.44	62	85	20.8 (±1.33) <sup>a</sup>	20.1 (±1.6) <sup>a</sup>	0.17 (±0.06) <sup>a</sup>	0.15 (±0.039) <sup>a</sup>		
Level #3	0.86	69	54 <sup>a</sup>	19.8 <sup>c</sup> (±0.85)	20.1 <sup>c</sup> (±1.1)	0.12 <sup>c</sup> (±0.018)	0.15 <sup>c</sup> (±0.027)		
Level #4	1.8	62	15 <sup>a</sup>	18.2 <sup>c</sup> (±1.59)	---	0.098 <sup>c</sup> (±0.014)	---		
Level #5	3.6	46 <sup>a</sup>	0 <sup>a</sup>	---	---	---	---		

<sup>a</sup>Standard length is measured from tip of head to the base of the caudal fin.

<sup>b</sup>Standard deviations are shown in parentheses.

<sup>c</sup>Values not included in multiple means comparison.

<sup>a</sup>Denotes values significantly different (P=0.05) from the control using one way analysis of variance (ANOVA) and Fisher's protected least Significant Difference (LSD) multiple means comparison.

FIGURE 1: Photographs of test system with egg incubation cups and growth chambers.

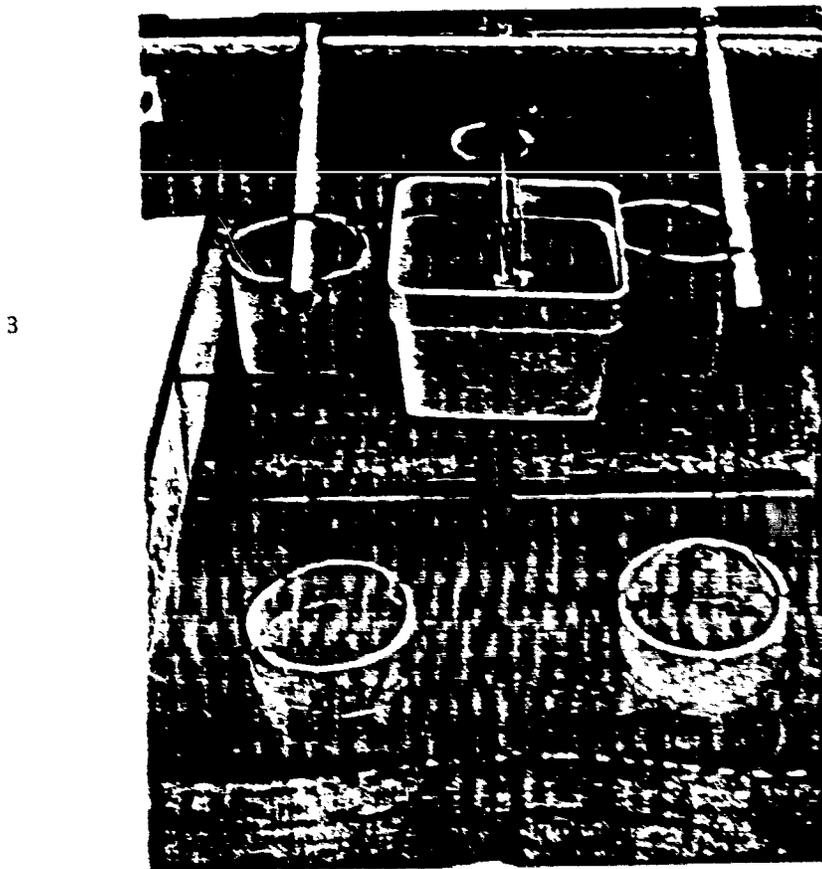
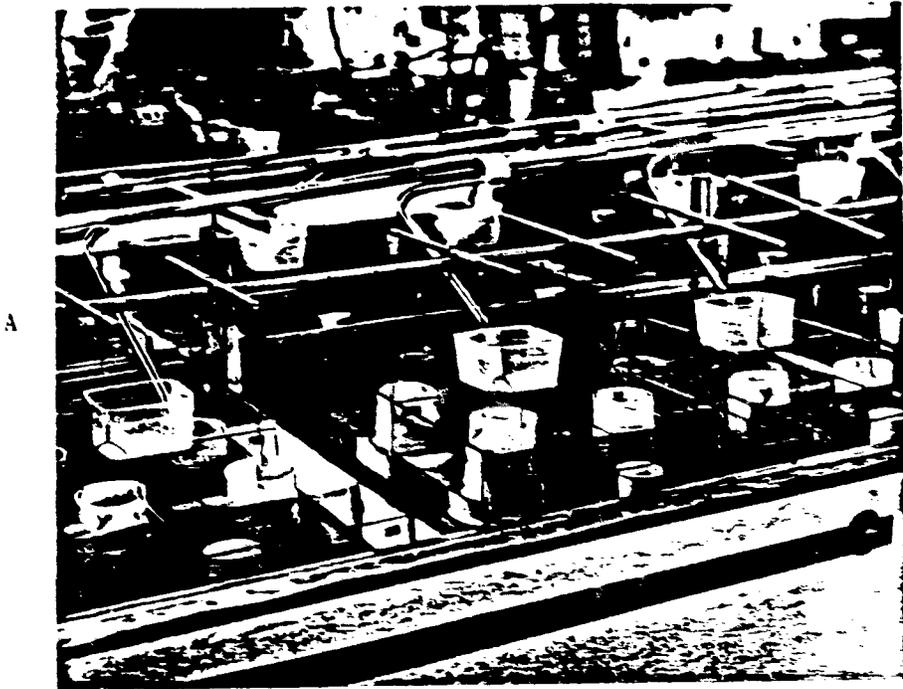


FIGURE 2: Early life stage toxicity testing system schematic and terminology.

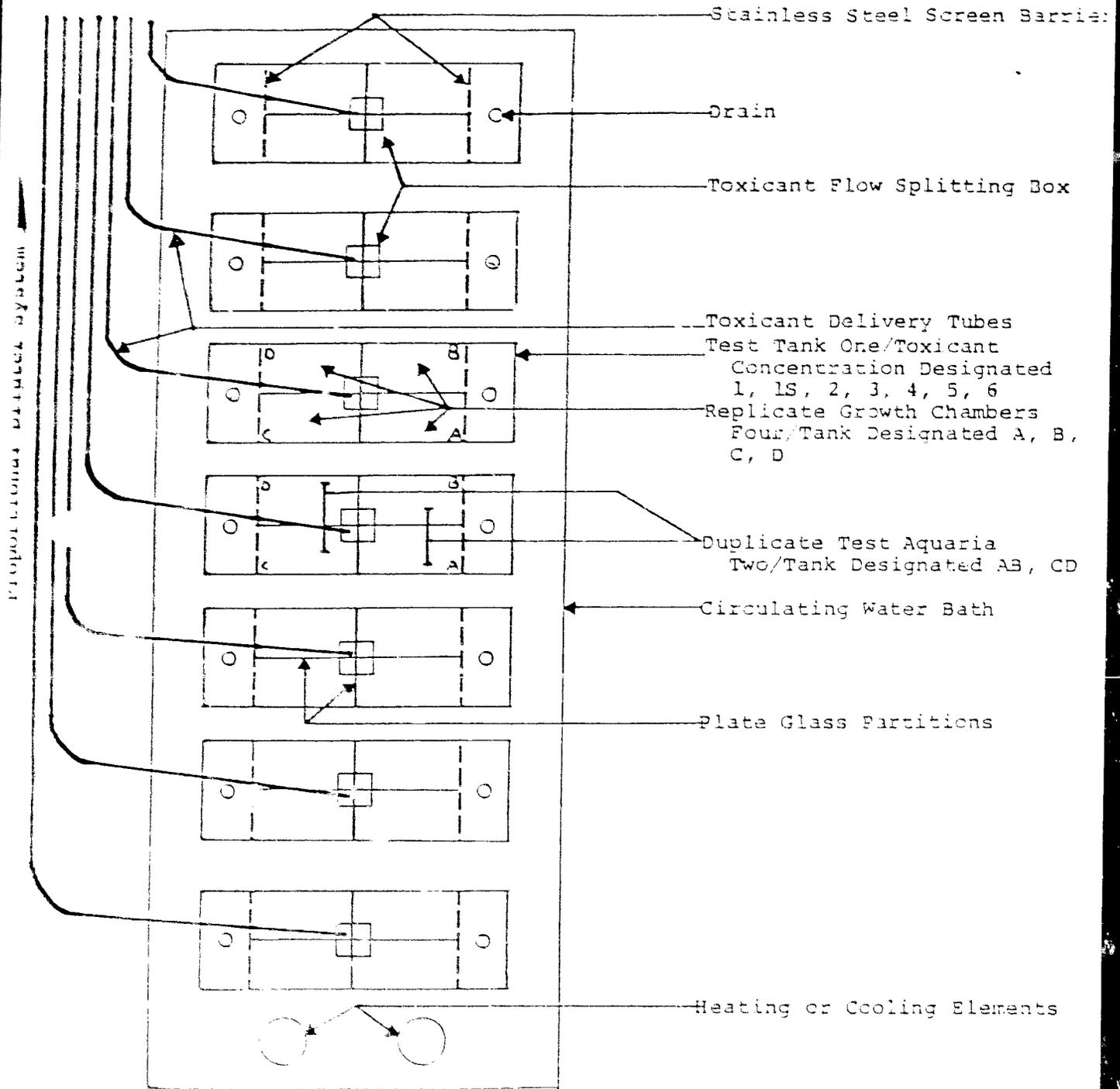


FIGURE 3: Mean percent egg hatch and mean percent survival of fathead minnows exposed to Acrylonitrile.

 Denotes values significantly different ( $P=0.05$ ) from the control.

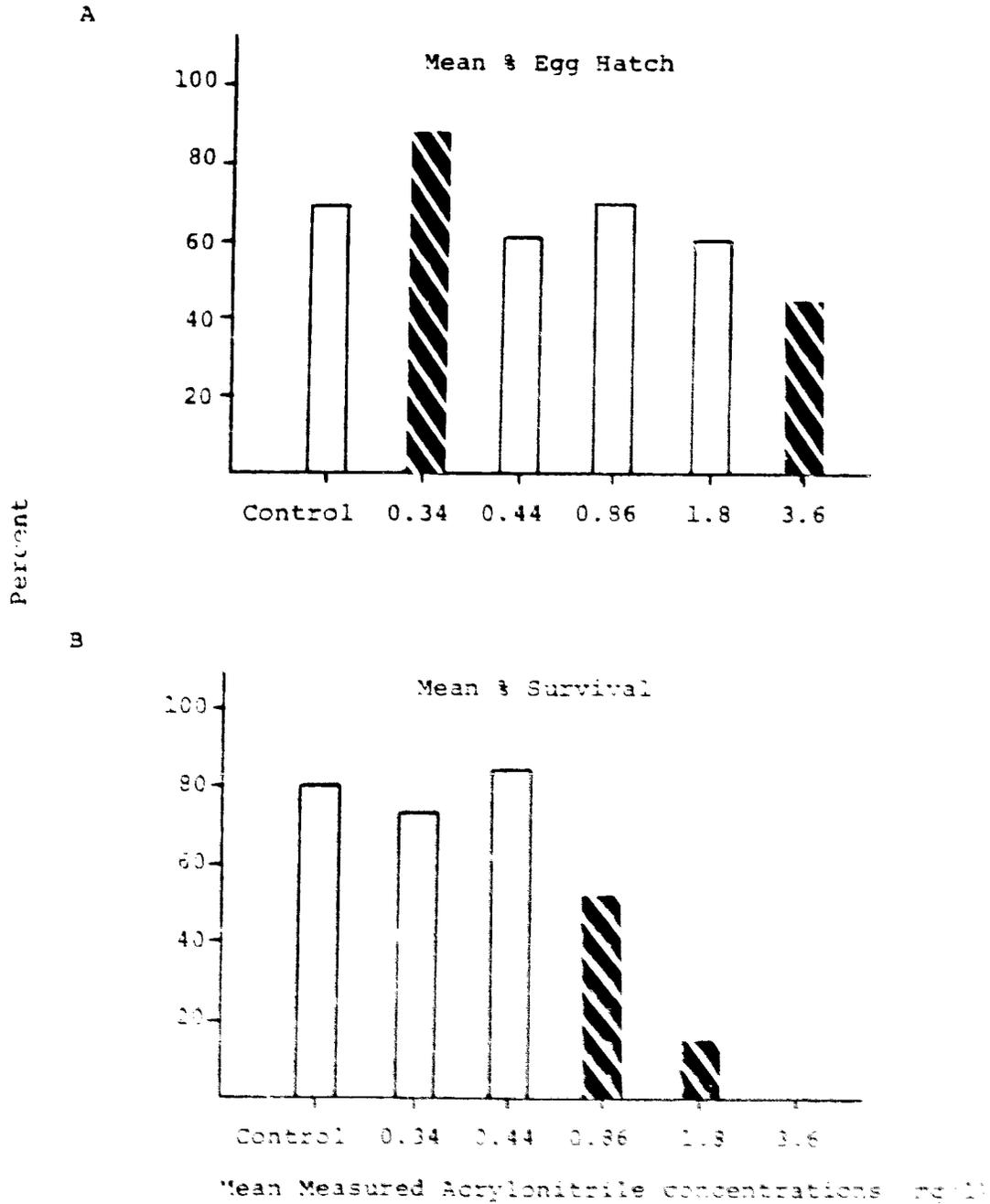
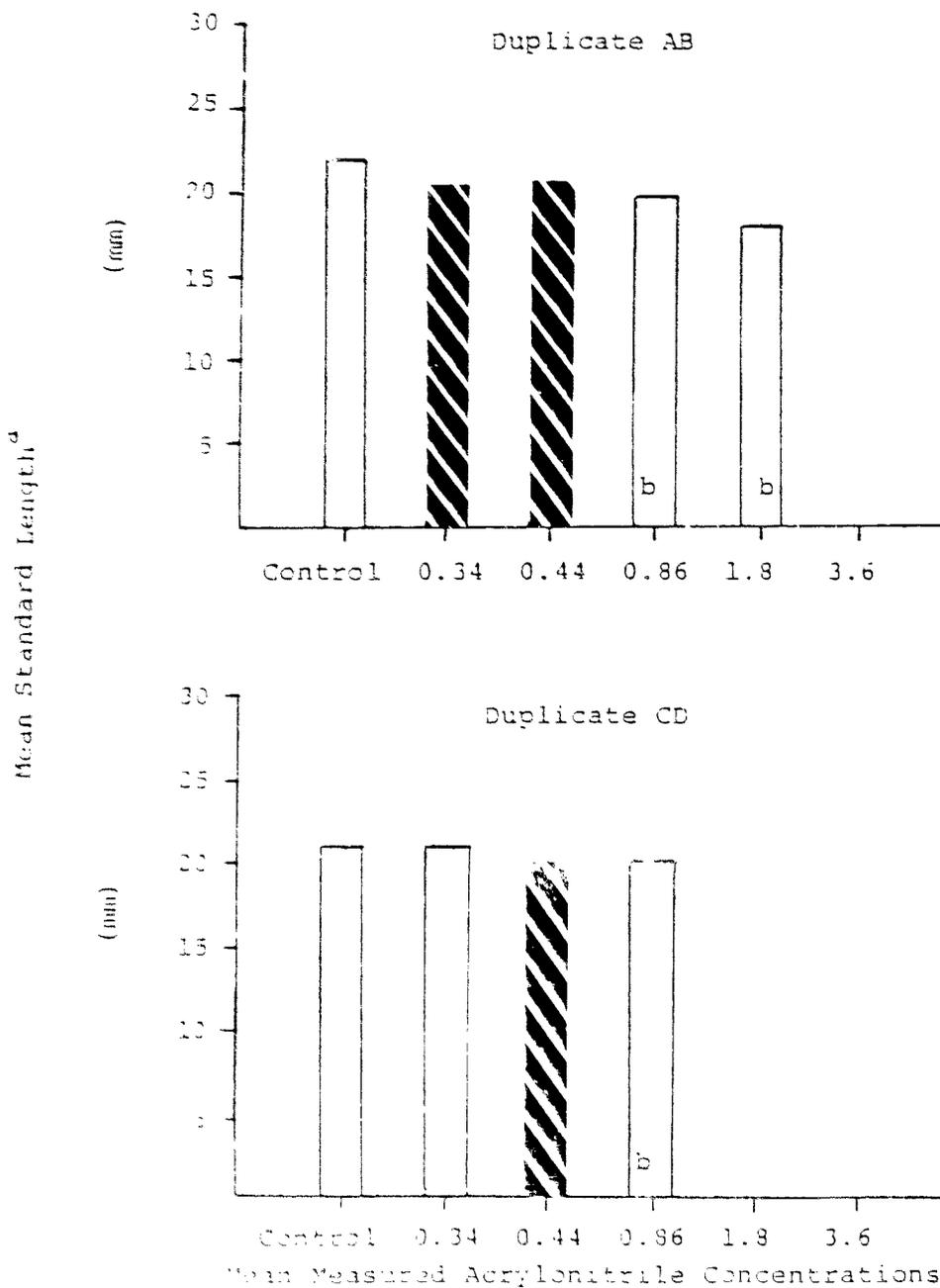


FIGURE 4: Mean standard length of fathead minnow fry after 30 days of exposure to Acrylonitrile.

 Denotes values significantly ( $P=0.05$ ) different from the control.

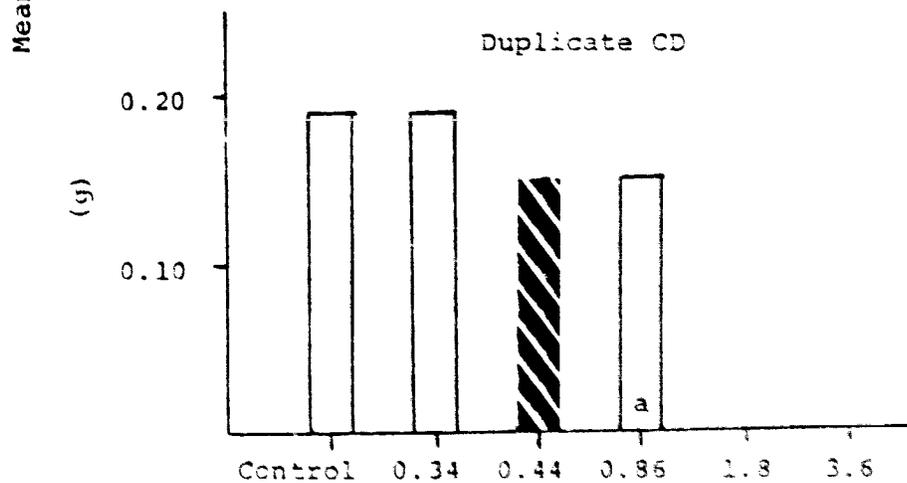
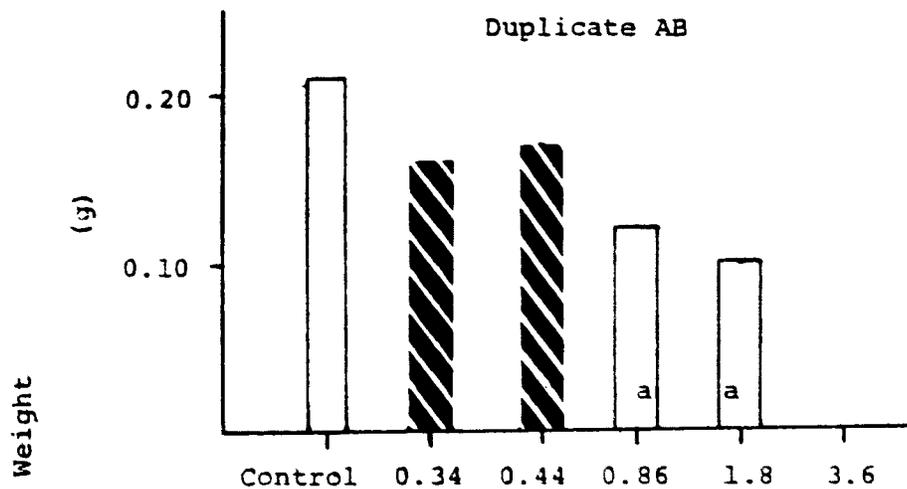


<sup>a</sup>Standard length is from the apex of the head to the caudal peduncle.

<sup>b</sup>Values not included in multiple means comparison.

FIGURE 5: Mean total wet weight of fathead minnow fry after 30 days of exposure to Acrylonitrile.

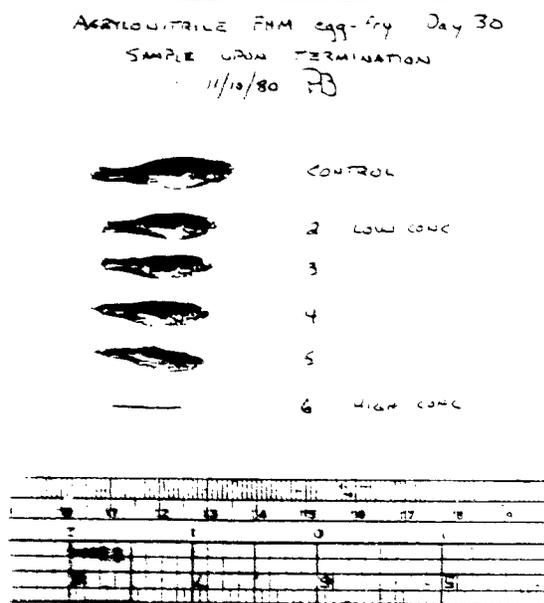
 Denotes values significantly different (P=0.05) from the control.



Mean Measured Acrylonitrile Concentrations (µg/l)

<sup>a</sup>Values not included in multiple means comparison.

FIGURE 6: A comparison of growth between control and treated groups of fathead minnows after 30 days of exposure to Acrylonitrile.



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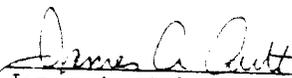
Quality Assurance Statement for Final Report #25673 entitled, "Early Life Stage Toxicity of Acrylonitrile to Fathead Minnows (Pimephales promelas) in a Flow-Through System," for Ms. A. F. Werner, Monsanto Chemical Company, St. Louis, Missouri.

In accordance with ABC Laboratories intent that all studies conducted by our facilities meet or exceed the criteria promulgated by the Good Laboratory Practice regulations for Non-clinical Laboratory Studies (21 CFR, Part 58) to assure adherence to protocol specifications and methods, the above named report was reviewed by a member of our Quality Assurance Unit.

In-progress study inspections were conducted on October 8, 10, 13, 17, 20, 22, 24, 27, 31 and on November 3, 1980.

A final inspection of all data and records on November 20, 1980, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by the Aquatic Toxicology Division of ABC Laboratories, Inc.

If you should have any questions concerning this statement or the function of our Quality Assurance Unit, please contact me at your convenience.

  
James A. Ault 12/1/80  
Quality Assurance Officer Date

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