DATA EVALUATION RECORD
ESTUARINE FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4(A)

1. **CHEMICAL:** Pirate® AC 303,630 Shaughnessey #: 129093

2. **TEST MATERIAL:** CL 303,630 (AC 303,630) Purity: 94.5%

3. **CITATION:**

Authors: McElwee, Cindy, Ward, G. Scott, and Wisk, Joseph D.
Title: Toxicity of AC 303,630 During the Early Life-Stages of Sheephead Minnow (*Cyprinodon variegatus*) Under Flow-Through Test Conditions
Study Completion Date: January 26, 1994
Laboratory: Toxikon Environmental Sciences, 106 Coastal Way, Jupiter, Florida 33477
Laboratory Report ID: J9201015
Sponsor: American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ 08543-0400
MRID No.: 434928-20
DP Barcode: D210808

4. **REVIEWED BY:** William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: William Evans **Date:** 10/11/96

5. **APPROVED BY:** Ann Stavola, Section Chief, Section 5
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: Ann Stavola **Date:** 11/5/96

6. **CONCLUSIONS:** Disolved oxygen (DO) level was consistently maintained between 47 and 65% saturation from day 7 in the solvent controls and test chambers throughout the experiment. Only 4 of the replicates maintained a DO saturation level between 70 - 75%. To ensure that toxicity tests are carried out under optimal conditions to test organisms EPA Guideline criteria require that DO concentration must be above 75% saturation. ASTM guidelines recommend maintaining DO levels above 60% saturation. Raw data on daily fish survival counts may reveal that true toxicological effects first appeared before day 7. However, this raw data was not submitted, and it is doubtful that such data could discount the inflence of the low DO levels on observed effects throughout the experiment. This study is therefore not scientifically sound and does not meet guideline requirements. It is classified as invalid.

7. **ADEQUACY OF THE STUDY:**

A. Classification: Invalid

B. Rationale: Toxicity tests must be carried out under optimal conditions. The dissolved oxygen (DO) level was maintained between 47 and 65% saturation from day 7 throughout the experiment. For further information refer to page 121 of the Ecological Effects Rejection Rate Analysis (EPA-738-R-94-035).

C. Reparability: N/A

8. **MAJOR GUIDELINE DEVIATIONS:**

1. Raw data on daily counts was not submitted. As the dissolved oxygen (DO) level was consistently below 60% saturation after day 7, daily counts for fish survival must be submitted.

2. Salinity measured in 1 replicate of dilution control daily ranged from 16 to 21 ‰ (31% fluctuation). Salinity fluctuation should not exceed 6%.

3. The disolved oxygen (DO) level was maintained above 51% saturation. Guideline criteria require that DO concentration must be above 75% saturation. ASTM guidelines recommend maintaining above 60% saturation.

4. Replicate data was not included for hatching time or time to swim-up.

9. **MATERIALS AND METHODS:**

A. **Biological System:**

Guideline Criteria	Reported Information
Species: An estuarine fish species, preferably a silversides species or sheepshead minnow (Cyprinodon variegatus).	Test species tested was the sheepshead minnow (*cyprinodon variegatus*)
Source	Aquatic BioSystems, Fort Collins, CO
Age at beginning of test: Embryos 2 to 24 hours old.	Fertilized eggs were distributed within 24 hours of fertilization.

Guideline Criteria	Reported Information
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.	20 embryos per embryo chamber were distributed to each replicate, 4 replicates per concentration. All embryo hatchling (> 30 fish per treatment) were transferred into screen retention chambers.
Post Hatch: % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	Hatching success was > 50% in each control and the greatest % hatch was 1.2 times that of another cup.
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish were fed 2-3 times a day up to 1 day before test termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Survival was monitored daily after hatching. However, raw data on daily counts was not submitted.
Controls: Avg. survival at end of test must be ≥ 80%. Survival in any control chamber must not be < 70%.	Control survival ranged from 93 to 97% in control and solvent controls.
Controls: Negative control and carrier control (when applicable) are required.	Solvent control of 6.0 µl Dimethylformamide (DMF)/L was used.

Comments:

B. **Physical System:**

Guideline Criteria	Reported Information

Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture; 2) Natural seawater should have weekly range of salinity less than 6 ‰ (parts per thousand (g/Kg)), monthly pH range less than 0.8 pH units; 3) Salinity should be ≥ 15 ‰ 4) Water must be free of pollutants.	1) Dilution water was natural saltwater, carbon treated and adjusted to 20% salinity with municipal freshwater that aerated, carbon filtered, and reaerated. 2&3) Salinity ranged from 16 to 21 ‰, and monthly pH ranged 8.0 to 8.3. 4) Water was free of pollutants.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For sheepshead minnow, either 25°C or 30°C is recommended.	Test temperature ranged from 24.3 to 30.6°C.
Photoperiod: Recommend 16L/8D.	16L/8D photoperiod was maintained.
Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	A modified proportional vacuum-siphon diluter volumetrically calibrated to provide a 50% dilution. 5 toxicant concentrations with dilution factor of 0.5 was used.
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) Mixing chamber was used. 2) Aeration was not used for mixing. 3) An average high nominal concentration of 30 µg ai/L was provided to each chamber. 4) Flow splitting accuracy was within 5% of desired volumes.
Test Vessels: All glass or glass with stainless steel frame.	24 liter glass tanks for the test chambers.

Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	60 mm diameter glass petri dishes encircled by 350 µm Nitex mesh.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.	8 volume additions were provided every 24 hrs. DO was maintained above 51% saturation.
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	Dilution water was aerated.

Comments: No comments.

C. **Chemical System:**

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	5 replicated concentrations plus solvent control were used. - Toxicant conc. was measured in each replicate once each week. - One concentration adversely affected a life stage and one concentration did not.
Other Variables: 1) DO must be measured at each conc. at least once a week; 2) Natural seawater must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range < 0.8 pH units.	1) DO was measured at initiation and once a week thereafter. 2) Salinity measured in 1 replicate of dilution control daily ranged from 16 to 21 ‰ (31% fluctuation). pH values ranged from 8.0 to 8.3.

Guideline Criteria	Reported Information
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Solvent: Dimethylformamide (DMF) Concentration was maintained at 0.006 mL/L

Comments:

10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Data Endpoints must include: - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.	Data endpoints included: - Number embryos hatched - Mortality of embryos, larvae, and juveniles - Measurement of growth - Incidence of pathological or histological effects - Observations of other effects. Data Endpoints not included: - Time to hatch - Time to swim-up
Raw data included? (Y/N)	No. Replicate data was not included for hatching time or time to swim-up. Raw data on daily fish survival counts are also needed as the dissolved oxygen (DO) level was consistently below 60% saturation after day 7.

Effects Data:

Toxicant Conc. (µg/L)		Per cent Hatch		Time to Hatch		Survival (37 days)		Total Length (mm)		Wet weight (mg)	
Nom.	Meas.	A	B	A	B	A	B	A	B	A	B
Ctrl	<0.51	90	98			32	38	18	18	182.0	155.3

Toxicant Conc. (µg/L)		Per cent Hatch		Time to Hatch		Survival (37 days)		Total Length (mm)		Wet weight (mg)	
Solv	<0.51	90	88			34	35	17	17	153.6	134.9
1.9	1.08	88	93			35	33	17	17	148.3	I35.1
3.8	2.00	90	78			32	23	17	18	135.7	161.4
7.5	4.21	93	80			35	30	17	17	128.0	128.3
15	8.72	83	83			32	28	17	17	132.0	132.7
30	18.6	83	68			8	8	18	19	169.2	172.6

a Replicate data on time to hatch was not submitted. Study claimed that hatching begun on day 5 and was complete in all but the 60 µg/L level by day 6.

Toxicity Observations: Hatching success throughout the test ranged from 84% at the 3.8 µg/L level to 20% at the 60 µg/L level.

Statistical Results:

Statistical Method: Analysis of variance (ANOVA) - Dunnett's Test

NOEL: 8.72 µg/L LEL: 18.6 µg/L

MATC: 12.7 µg/L

Most sensitive endpoint: Reduction in hatching success an survival.

Comments: None

11. **Reviewer's Statistical Results:** The reviewer verified the results of the statistical analysis.

Statistical Method: As this study has been classified as Invalid a statistical analysis was not performed.

NOEL: LEL: MATC:

Most sensitive endpoint: Reduction in hatching success and survival.

Comments: DO level was consistently maintained between 47 and

65% saturation from day 7 in the wolvent controls and test chambers throughout the experiment. Only 4 of the replicates maintained a DO saturation level between 70 -75%. To ensure that toxicity tests are carried out under optimal conditions to test organisms EPA Guideline criteria require that DO concentration must be above 75% saturation. ASTM guidelines recommend maintaining DO levels above 60% saturation. Raw data on daily fish survival counts may reveal that true toxicological effects first appeared before day 7, and the influence of low oxygen levels on the observed biological effects cannot be discounted. However, this raw data was not submitted, and this study is therefore classified as invalid.

12. **COMPLETION OF ONE-LINER FOR STUDY**: YES