

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

1. **CHEMICAL:** Naled Shaughnessey #: 034401
2. **TEST MATERIAL:** Technical Purity: 93.6%
3. **CITATION:** Machado, Mark W. 1993. Naled Technical - The Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) During an Early Life-Stage Exposure. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Valent U.S.A. SLI Report No. 93-4-4740. MRID No. 42986401.

4. **REVIEWED BY:**

Allen W. Vaughan
Entomologist
Ecological Effects Branch (7507C)
U.S.E.P.A.

Signature: *Allen W. Vaughan*
Date: 04-21-94

5. **APPROVED BY:**

Norman J. Cook
Section Head
Ecological Effects Branch (7507C)
U.S.E.P.A.

Signature: *Norman J. Cook*
Date: 04-25-94

6. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirement for an early life-stage study with estuarine fish. Survival at hatch averaged 27.5% for the four control and solvent control treatments, with the highest value being 37.1% in one solvent control. All controls must have survival at hatch $\geq 50\%$ for the study to be acceptable.

7. **ADEQUACY OF THE STUDY:**

- A. Classification: Invalid
- B. Rationale: Per cent hatch in controls < 50%.
- C. Reparability: None

8. **MAJOR GUIDELINE DEVIATIONS:**

- No adverse effects noted at any concentration in the definitive test;
- Per cent hatch based on count of viable embryos after 3 days of exposure; should be based on total number of embryos introduced into incubation cups.



9. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: An estuarine fish species, preferably a silversides species or sheepshead minnow (<i>Cyprinodon variegatus</i>).	Test species is sheepshead minnow.
Source	Sea Plantations, Inc., Salem, MA.
Age at beginning of test: Embryos 2 to 24 hours old.	Embryos $\leq$ 24 hours old at test initiation.
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.	70 embryos per replicate cup, 2 reps. per concentration. 30 fish/treatment; 2 reps.
Post Hatch: % of embryos that produce live fry must be $\geq$ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	Per cent hatch ranged from 83.33-96.55% in controls, based on count of viable embryos on day 3 of exposure.
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 live brine shrimp nauplii three times daily.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Larval survival was estimated at least twice weekly. Per cent survival was determined at test termination (28 days post-hatch).
Controls: Avg. survival at end of test must be $\geq$ 80%. Survival in any control chamber must not be $<$ 70%.	Avg. survival in controls $>$ 80%.
Controls: Negative control and carrier control (when applicable) are required.	Test included negative and solvent controls.

Comments: Reported per cent survival at hatch ranged from 68.75 to 100% across all controls and treatments, because calculations were based on counts of viable embryos on day 3 of exposure. Actual per cent survival at hatch, based on number of embryos at test initiation, ranged from 21.4 to 48.6%. Because all control cups had less than 50% survival at hatch, the test should have been terminated at that point.

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‰, monthly pH range less than 0.8 pH units; 3) Salinity should be ≥ 15 parts per thousand; 4) Water must be free of pollutants.	Dilution water was natural seawater, filtered in the laboratory; salinity range 30 to 32 parts per thousand; pH range 7.7 to 8.0. Previous chemical analyses for presence of pesticides, PCB's, and toxic metals showed no toxic 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.	Temperature range of 23°C to 25°C for duration of test.
Photoperiod: Recommend 16L/8D.	16L/8D.
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.	Intermittent flow proportional diluter. Five toxicant concentrations with dilution factor ≈ 0.5 , plus control and solvent control.

Guideline Criteria	Reported Information
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%.	Mixing chamber positioned over magnetic stirrer; no aeration; flow splitting accuracy was within 10%.
Test Vessels: All glass or glass with stainless steel frame.	All glass and silicone sealant.
Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	Glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex screen bottoms.
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.	90% replacement time of approx. 5.5 hours. DO maintained at > 75% 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.	Test tanks and embryo cups were not 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.	- Five concentrations plus control and solvent control, all replicated twice. - Toxicant conc. measured in all tanks on test days 0, 8, 15, 22, 29, and 37. - No adverse effects noted at any concentration.
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.	DO, salinity, and pH were measured daily in each aquarium. Salinity and pH were within acceptable limits.
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Solvent control equal to conc. of solvent in highest treatment level (18 µl/L, acetone).

Comments: No adverse effects noted at any concentration.

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.	Number of embryos hatched; mortality of embryos and larvae; measurement of growth; behavior and appearance of larvae.

Guideline Criteria	Reported Information
Raw data included? (Y/N)	No

Effects Data:

Toxicant Conc. ($\mu\text{g/L}$) ¹		Per cent Hatch		Time to Hatch ²		Survival (37 days)		Total Length (mm)		Wet weight (gm)	
Nom.	Meas.	A	B	A	B	A	B	A	B	A	B
Ctrl		89	83			80	93	23	23	.18	.19
Solv		87	93			100	93	24	23	.23	.18
3.1	2.4	100	88			93	100	25	22	.23	.15
6.2	4.4	85	69			80	100	23	23	.18	.17
12	7.8	85	85			100	100	22	24	.18	.19
25	16	90	96			100	100	24	24	.21	.21
50	40	97	83			100	87	23	21	.20	.13

¹ Measured concentration of naled equivalents (naled plus DDVP).

² Time to hatch was not measured.

Toxicity Observations: No adverse effects reported.

Statistical Results:

Statistical Method: Williams' Test

NOEL: 40 $\mu\text{g/L}$ LEL: Not determined

MATC: Not determined

Most sensitive endpoint: Not determined

Comments: None

11. Reviewer's Statistical Results:

Statistical Method: N/A

NOEL: N/A LEL: N/A MATC: N/A

Most sensitive endpoint: N/A

Comments: Since the study was invalid, the data subject to analyses were meaningless. EEB did not validate the analyses.

12. COMPLETION OF ONE-LINER FOR STUDY:

One-liner completed 04/07/94.