EFBAtes
See Comments under D182203 Date Dw 11/02/93 Letter dated Jul 6-93 Rcvd Jan 31 1994

DATA EVALUATION RECORD

I. **CHEMICAL:** Phorate

II. **TEST MATERIAL:** AC 35,024 99% radiopurity

III. **STUDY TYPE:** Guideline Number 72-4 - Sheepshead Minnow Embryos and Larvae

IV. **CITATION:**

A.	Author:	Sousa, J. V.
B.	Year:	1991
C.	Title:	(AC 35,024)
D.	Laboratory:	Springborn Laboratories, Inc.
E.	Study No.	451.0889.6112.505
F.	SLI Report #:	90-7-3369
G.	Sponsor:	American Cyanamid Company
H.	Sponsor No.:	None
I.	MRID No.:	418038-06

V. **REVIEWED BY:**

Dennis J. McLane, Wildlife Biologist
Section 1, Ecological Effects Branch
Environmental Effects and Fate Division (H7507C)

Dennis McLane 4-15-92

VI. **APPROVED BY:**

Les Touart, Section Chief
Section 1, Ecological Effects Branch
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L. Touart 4/17/92

VII. **CONCLUSION:**

The study does not fulfill the guidelines due to high mortality in the controls <80% survived. This study cannot be repaired.

NOEC = 96 ppt
LOEC = 190 ppt
135 ng/L geometric mean

VIII. **BACKGROUND:**

Submitted in response to Registration Standard Requirements.

Total length 0.09le
LOEC = 0.09le
Williams Test
UPGraded

XI. **MATERIALS, METHODS, AND REPORTING REQUIREMENTS:**

A. Biological System

1. SEP (Standard Evaluation Procedure)
   -Acceptable Species -
   SLI - (Springborn Laboratories, Inc) yes
2. SEP-Source -
   SLI-"...fish were originally obtained from Cultured Aquatics, Northport, New York and maintained for several months."
3. Eggs from Adult Fish -
   a. SEP - Directly from hatcheries or commercial -sources
      SLI - no
   b. SEP-From wild populations of adult fish collected in the field -
   c. SEP-From brood fish culture in the laboratory - SLI- "fertilized embryos obtained from the sheepshead minnow culture unit maintained at SLI."
4. Embryo Exposure (Test Begins) -
   a. SEP-Eggs can be obtained from channel catfish, fathead minnows, sheepshead minnow, and bluegill by facilitating natural spawning either in the laboratory or a brood pond.
      SLI - "Natural spawning occurred overnight and eggs ($\leq$24 hours old) were collected on the day of test initiation."

   b. SEP- Although embryos are preferred to be started when less than 24 hours old. see a. above

   c. SEP-A minimum of 20 embryos are randomly selected per replicate cup with four replicates per concentration (80 embryos total). - SLI - "Thirty-five embryos were impartially selected and distributed to each of 28 embryo incubation cups, two of which were then suspended in each duplicate test aquarium per exposure concentration and the control(s). A total of 140 embryos were exposed to each treatment level and control."

   d. SEP-Water may flow directly over the embryos in the cup or the cups may be oscillated in the test solution by means of rocker arm apparatus driven by a low speed electric motor. SLI - "A rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the incubation cups in the test solutions."

e. SEP-Twenty-four hours after being placed in the incubation cups they should be counted and examined for dead or heavily fungused individuals, which should be discarded without disturbing the viable embryos. This counting and examination is repeated on a daily basis. The range of time-to-hatch in each cup is 7 days at 25°C for the sheepshead minnow.
SLI - "Dead embryos were counted daily until hatching was complete."

5. Post Hatch, Larval Fish

a. SEP-When hatching is about 99 percent completed or 48 hours after first hatch, live young fish should be counted.
SLI - "Hatching was deemed complete (exposure day 7) when no more than 10% unhatched viable embryos remained in any egg incubation cup." Raw data indicates that Day 4 live fry were sighted.

b. SEP-All of the normal and abnormal live fish should be released into the test chambers.
SLI "To initiate the 28-day post-hatch larval exposure, the surviving larvae in each incubation cup were released on test day 5 into screened retention chambers located in their respective exposure aquaria."

c. SEP-Fish numbers can be thinned to at least 30 per treatment.
SLI - "On test day 20, 13 post-hatch, the surviving larvae in each of the exposure aquaria were impartially thinned to 30 larvae per aquarium and released from the retention chamber into the respective aquarium."

d. SEP-A test should be terminated if the average percent of embryos (based on the number of embryos after thinning) that produce live fry for release into the test chambers in any control treatment is less than 50% or if the percent hatch in any control embryo cup is more than 1.6 times in another control embryo cup.

SLI - "Table 4. e. Survival of Organisms at
Hatch Controls A - 83%
B - 69%
Solvent Control
A - 76%
B - 79%

e. SEP-Test fish over two days old (post hatch swim-ups) must be fed live newly hatched brine shrimp.
See the following section, f. below concerning feeding.

f. SEP - Fish should be fed at least twice daily. SLI - "Larvae were fed live brine shrimp (*Artemia salina*) nauplii three times daily on weekdays and twice daily on weekends and holidays."

g. SEP-Time between feedings will be species specific, and must be based on a reliable hatchery feeding schedule.
See the above section, f. below concerning feeding.

h. SEP-Control and Treatment fish must receive equal amounts of food if growth is to be a meaningful endpoint.
Not reported.

i. SEP-Dead fish should be removed and recorded when observed.
SLI- "Behavior and appearance of larvae were observed and recorded daily, and larval survival was determined." The fate of the de dead fish were not reported. The SLI protocol states that, "If mortality is obviously occurring in any of the test aquaria, a thorough search for dead fry is made daily in those aquaria."

j. SEP-AT a minimum, the live fish should be counted (including those which are lethargic or gross abnormal in either swimming behavior or physical appearance) 11, 18, 25, and 32 days after hatching. See part i. above.

k. SEP-Fish should not be fed for at least 24 hours prior to termination day 32. SLI - "Larvae were not fed during the final 24 hours of study."

1. SEP-At termination, all live fish should be weighed (wet, blotted dry).
SLI- "The larvae were measured and weighed individually to calculate the mean standard deviation of total length and wet weight for each replicate vessel." Raw data indicates the fish were blotted dry before weighing.

6. Controls

a. SEP-A test is not acceptable if the average survival of the controls at the end of the test is less than 80 percent or if survival in any control chamber is less than 70 percent.

SLI-"Table 4. e. Survival of organisms at Hatch

Controls A - 83%
B - 69%
Mean - 77%
Solvent Control
A - 76%
B - 79%
Mean - 76%"

b. SEP-The relative standard deviation (RSD = 100x standard deviation divided by the mean) of weights of the fish that were alive at the end of the weights of the fish that were alive at the end of the test in any control test chamber must not be greater than 40%.
SLI - The highest percent was at the 96 ng A.I./L concentration. This was 26%.

$$\frac{0.06 \times 100}{21} = 0.26$$

c. SEP-A negative control (no toxicant or carrier) and a carrier control (when applicable) are required.
SLI-Both type were included for this study.

d. SEP-Regardless of the carrier used, the carrier concentration should be equal in each exposure concentration and carrier control. If they are not, the carrier concentration in the control (carrier) must be at least as high as that in any toxicant test chamber.
SLI-"The solvent control solution contained in the mixing chamber constituted the highest test concentrations (120-15 ng A.I./L)."

7. Data Endpoints
SEP-A record of the results of an acceptable test must include the number embryos hatched, time to hatch, mortality of embryos, larvae, and juveniles, time to swim-up, and if appropriate, measurement of growth, incidence of pathological or histological effects, and observations of other effects or clinical signs in each treatment.

SEP-The time to swim-up endpoints required by the guidelines was not statistically evaluated or presented in the report.

B. Physical System
1. Test Water
a. Saltwater fish
1) SEP-Natural saltwater (sterilized and filtered to remove particles 15 microns and larger).
SLI- Natural saltwater filtered through polypropylene core (20-and 5 micron). No mention was made of the sterilizing the water.

2) SEP-Natural seawater is considered to be of constant quality if the weekly range of salinity is less than six percent, and if monthly pH range is less than 0.8 of a pH unit;
SLI- Daily monitoring revealed 31°/oo with a range of 31-32°/oo for salinity and pH ranged from 7.7 - 8.1.

3) SEP-Salinity ≥15 parts per thousand during the test.
SLI-The test water salinity was 31 (range 31-32)

4) SEP-Water must be free of pollutants-
SLI-"IN compliance with EPA-GLP, routine analyses are also conducted on representative samples of the seawater for the presence of pesticides and PCB's. None of these compounds have been detected in any of the water samples analyzed monthly for total organic carbon (TOC) concentration.
SLI-"(1.4-6.0 mg/L November 1989 - April 1990)

2. SEP-Temperature - Temperature should be 30°C with 4 days to hatch or 25°C with 7 days to hatch for sheepshead minnow.

SLI- This study used 25°C temperature. The temperature did not vary over 2°C.

3. SEP-Photoperiod - A photoperiod of 18L/8D can be used with a light intensity of 400 to 800 Lux at the surface of the test solution.
SLI protocol - "Sixteen hours of light intensity of 20 - 70 footcandles (215.2 to 753.2 Lux) at the water surface were provided each day."

4. SEP- Dosing Apparatus - Intermittent-flow proportional diluters as described by Mount and Brungs or continuous-flow serial diluters, as described by Garton should be employed. A minimum of five toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.
SLI- "A modified intermittent flow proportional diluter, similar to that described by Mount and Brungs (1967) with a 50% dilution factor, was used to prepare and deliver the selected test concentration range of AC 35,024 to the exposure aquaria during the 35-day study."

5. SEP-Toxicant Mixing - A mixing chamber is recommend to assure adequate mixing of test material. Aeration should not be used for mixing. Separate flow splitter delivery tubes should run from this container to each replicate larval tank. Depending upon the apparatus used, a mixing chamber may not be required, but it must be demonstrated that the test solution is completely mixed before introduction into the test system. Flow splitting accuracy must be within 10 percent and should be checked periodically for accurate distribution of each tank.
SLI - "A 50-ml Hamilton gas tight syringe in conjunction with a Fraser mechanism, was calibrated to deliver 0.0348 mL of the 12.96 μg A.I./mL ^{14}C-AC 35,024 stock solution into the diluter system's chemical mixing chamber was positioned over a magnetic stirrer which was subsequently diluted (50%) to provide the remaining nominal exposure concentrations (120-15 ng A.I./L)"

6. SEP- Test Vessels - All test Tanks should be of either all glass or glass with stainless steel frame. Exposure vessels will vary in size according to the species under test. Generally, it is desirable to have a depth of water of at least 15 to 30 cm.

SLI - "Each glass test aquarium measured 39 x 20 x 25 cm with a 14.5 cm high standpipe which maintained a constantly exposure solution volume of 11L."

7. SEP - Embryo Cups - Embryo incubation cups should be made from 120 mL glass jars with the bottoms replaced with 40 mesh stainless or nylon screen. Cups can be oscillated vertically (2.5 to 4.0 cm) in the test water (rocker arm apparatus, 2 rpm motor) or placed in separate chambers with self-starting siphons. Both methods should insure adequate exchange of water and test material.
SLI - "Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® screen bottoms. A rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the incubation cups in the test solutions." The volume of a cylinder in diameter squared times the height. Hence, 200 cm^3 (5 cm^2 x 8) is the estimated volume of the container minus the container wall thickness.

8. SEP - Flow Rate - Flow rates to larval cups should provide 990 percent replacement in 8 to 12 hours. Flow rate must be capable of maintaining the toxicant level (concentration cannot drop below 20 percent with fish in the tank).

9. SEP - Aeration - Dilution water should be aerated vigorously insuring that dissolved oxygen concentration will be at or near 990 to 100 percent saturation. Test tanks and embryo cups should be aerated.
SLI - No mention was made of aeration, but the SLI protocol made the following reference:
"Aeration (with oil-free air) would be initiated as a last resort to raise and maintain the dissolved oxygen concentration at the desired level (≥ 3.8 mg/L)".

C. Chemical System

1. SEP - Concentrations - A minimum of five concentrations of toxicant and a control, (all replicated) are used in this chronic test. A solvent control is added if a solvent is utilized. "At a minimum, the concentration of toxicant must be measured in one tank at each toxicant level every week. Water samples should be taken about midway between top and bottom and sides of the tank. One concentration selected must adversely affect a life-stage and one concentration must not affect any life-stage.

SLI - " During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls on test days 0,6,14,21,28, and 35 for analysis of ^{14}C-AC 35,024. Each exposure solution sample was collected from the approximate midpoint of the aquarium with a volumetric pipet."..."Based on these results of the weekly solution analyses (radiometric), including day 19, the exposure solutions of phorate were defined as 190, 96, 47, 20 and 16 ng A.I./L."

2. SEP - Measurement of Other Variables
Dissolved oxygen must be measured at each concentration at least once a week. Natural seawater must maintain a constant salinity and not fluctuate more than six percent weekly or a monthly pH range of less than 0.8 of a pH unit.
SLI - "Dissolved oxygen concentration , pH and temperature were measured daily in each aquarium." In Table 1. where the D.O. was measured the lowest value was 3.8 mg/L or 55% saturation for the 120 ng A.I./L test level."

3. SEP - Solvents
If solvents other than water are necessary, they should be used sparingly and not to exceed 0.1 mL/L in a flow-through system.
SLI - "The solvent control solutions contained a concentration of acetone which equaled the solvent level in the highest AC 35,024 treatment level (0.0179 mL/L)." "Throughout the exposure period, solutions were clear, colorless, and contained no visible sign of insoluble test material (e.g. precipitate, film in solution surface."

D. Calculations

SEP - Data from these toxicity studies are of two types, continuous (i.e., length, weight) and discrete (i.e., number of fish hatched or surviving). In general, continuous data should be analyzed with the appropriate multiple comparison test. Dichotomous data should be analyzed using some form of a 2 x 2 contingency table.
SLI - "At the termination of the study, data obtained on organism survival at hatch, larval survival and larval growth (wet weight and total length) at test termination were statistically analyzed."

X. REVIEWER'S EVALUATION

A. The following items did not meet the guideline criteria:

1. No data was provided concerning the time to hatch other than the raw data or analysis. However, under Biological Observations the following statement was found: "No difference in development or time to hatch was evident in any of the treatment levels or control."
2. The author did not report if controls and treatment levels both received same amount of food.

3. The B replicate of the control group was less than 69% or 1% less than the required minimum of 70%. The control group average survival was 76% or 4% less than the required minimum of 80%.

4. The light intensity ranged from 215.2 to 753.2 lux. The lower end of the range is below the required minimum of 400 lux.

5. Table 1. where the D.O. was measured, the lowest value was 3.8 ng/L or 55% saturation for 120 ng A.I./L test level. The solvent control lower range also was low with values 3.9 mg/L or 57% saturation. Notes in the raw data indicate that ..."D.O. values once fell below 60% for more than 24 hours as stated in the protocol."

6. Natural saltwater (sterilized and filtered to remove particles 15 microns and larger) is required. SLI reported that natural saltwater was filtered through polypropylene core (20-and 5 micron). No mention was made of the sterilized water.

B. Verification of Statistical Analysis

1. Survival of organisms at hatch (test day 7)- Table 4. indicates a level 190 ng/L was statistically different from the pooled control data. The pooling of data because an ANOVA indicates there is no statistical difference is not acceptable procedure. EEB's analysis, which used SAS's contingency table chi-square, shows that the solvent control as well as the 190 ng/L. Therefore, the pooled control masked the difference shown at the 20 ng/L/.

2. Survival of larvae 28 days post-hatch-Table 4. indicates that all larvae survived.

3. Total Length-
EEB found that both the Dunnett's and Bonferroni T-Test both confirm the statistical differences between the solvent control and 190 ng/L level. However, in addition to this the Williams test shows a statistical difference at the 96 ng/L level.

4. Wet Weight-
EEB's statistical evaluation agrees with the report, the 190 ng/L level is different form the solvent control.

5. The reported geometric mean based on SLI LOEC and NOEC of 140 ng/L is incorrect. EEB calculations show that 135 ng/L is correct.

C.B. Conclusions

1. Categorization of Results

Invalid

Upgraded to core 1994 suppl. data submission

2. Rationale

The control survival was below acceptable limits indicating stress of the test organisms.

3. Reparability

N/A

4. Descriptive Conclusions

N/A

D.B. One Liner Completed

Yes

Table 4. Survival of organisms at hatch (test day 7) and survival, total length and wet weight of sheepshead minnow (*Cyprinodon variegatus*) larvae after 28 days post-hatch exposure to AC 35,024.

Mean Measured Concentration (ng A.I./L)		Survival of Organisms at Hatch (%)	Larvae (28 Days Post-Hatch) Larvae Survival (%)	Mean Total Length (S.D.) (mm)	Mean Wet Weight (S.D.) (g)
190	A	61	100	19 (1.8)	0.17 (0.04)
	B	57	100	20 (1.6)	0.17 (0.04)
	Mean	59[a]	100	20 (1.7)[b]	0.17 (0.04)[b]
96	A	80	100	21 (1.9)	0.19 (0.06)
	B	69	100	22 (1.0)	0.21 (0.04)
	Mean	74	100	22 (1.6)	0.20 (0.05)
47	A	71	100	23 (1.4)	0.21 (0.04)
	B	77	100	22 (1.3)	0.21 (0.04)
	Mean	74	100	22 (1.3)	0.21 (0.04)
20	A	66	100	22 (1.9)	0.19 (0.05)
	B	67	100	23 (1.1)	0.22 (0.04)
	Mean	66	100	22 (1.6)	0.21 (0.05)
16	A	77	100	22 (1.4)	0.17 (0.04)
	B	77	100	22 (1.6)	0.19 (0.04)
	Mean	77	100	22 (1.5)	0.18 (0.04)
Solvent Control	A	76	100	22 (1.4)	0.20 (0.03)
	B	79	100	22 (1.4)	0.20 (0.04)
	Mean	77	100	22 (1.4)	0.20 (0.04)
Control	A	83	100	23 (1.3)	0.22 (0.04)
	B	69	100	23 (1.3)	0.22 (0.04)
	Mean	76	100	23 (1.2)	0.22 (0.04)
Pooled Control		76	100		

[a] Significantly ($p \leq 0.05$) different as compared to the pooled control data.
[b] Significantly ($p \leq 0.05$) different as compared to the solvent control data.

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451 0889 6112 520

EMBRYO SURVIVAL AT THE COMPLETION OF HATCH : FATHEAD MINNOW

TEST DAY 7

DATA BY MB

DATE 5-3-90

CONC. (mg/L)	EGG CUP	TOTAL DEAD EGGS	DEAD FRY UH.	DEAD FRY SH.	TOTAL # DEAD FRY	LIVE DEFRMD. FRY	TOTAL LIVE FRY	TOTAL NUMBER EXPOSED	NUMBER UNACCNT. FOR	PERCENT (%) HATCH	ARC SIN √ TRANSFRM.			
230	1A	27	0	0	0	0	43	70	0	61.43	LA			
	1B	30	0	0	0	0	40	70	0	57.14				
120	2A	14	0	0	0	0	56	70	0	80.00				
	2B	22	0	0	0	0	48	70	0	68.57				
58	3A	20	0	0	0	0	50	70	0	71.43				
	3B	16	0	0	0	0	54	70	0	77.14				
29	4A	24	0	0	0	0	46	70	0	65.71				
	4B	23	0	0	0	0	47	70	0	67.14				
15	5A	16	0	0	0	0	54	70	0	77.14				
	5B	16	0	0	0	0	54	70	0	77.14				
Cont.	6A	12	0	0	0	0	58	70	0	82.86				
	6B	22	0	0	0	0	48	70	0	68.57				
Solv. Cont.	7A	17	0	0	0	0	53	70	0	75.71				
	7B	15	0	0	0	0	55	70	0	78.57				
A	B	C	D	E	F	G	H	I	J	K	L	M	N	

57/106
36/104
93
32/104
34/106
Control

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LARVAL SURVIVAL AT TEST TERMINATION

DATE 5-31-90

DATA BY MB

CONCENTRATION (ng/L)	REPLICATE	NUMBER OF LARVAE INITIALLY EXPOSED	NUMBER OF LARVAE SURVIVING	PERCENT SURVIVAL	ARC SIN $\sqrt{\%}$
230	1A	30	30	100.00	90.00
	1B	30	30	100.00	90.00
120	2A	30	30	100.00	90.00
	2B	30	30	100.00	90.00
58	3A	30	30	100.00	90.00
	3B	30	30	100.00	90.00
29	4A	30	30	100.00	90.00
	4B	30	30	100.00	90.00
15	5A	30	30	100.00	90.00
	5B	30	30	100.00	90.00
Control	6A	30	30	100.00	90.00
	6B	30	30	100.00	90.00
Solvent Control	7A	30	30	100.00	90.00
	7B	30	30	100.00	90.00