DATA EVALUATION RECORD PAGE 1 OF

CASE: GS0103 PHORATE FRSTR

CONT-CAT: 01 GUIDELINES: 72-4

MRID: 158335

Surprenant, D. (1986) The Toxicity of [Carbon-14]-AC35,024 to Rainbow Trout (Salmo gairdneri) Embryos and Larvae: Report No. BW-86-3-1-1968. Unpublished study prepared by Springborn Bionomics, Inc. 57 p.

REVIEW RESULTS:

VALID_____ INVALID ✓ INCOMPLETE_____

GUIDELINE: SATISFIED ___ PARTIALLY SATISFIED ____ NOT SATISFIED ✓

DIRECT RVW TIME = 8 START DATE: 6/23/88 END DATE 6/24/88

REVIEWED BY: Ann Stavola

TITLE: Aquatic Biologist

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SIGNATURE: Ann Stavola DATE: 6/24/88

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE: Douglas J. Urban DATE: 10/11/88

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Invalid SUN

4-3-92: Denis pointed out that recent memo dated 1/20/91 has upgraded this study to "core" based on the review of the second addendum (MRID 41695-01) from American Cyanamid's. ! — So this study was entered into the Phorate database today " Sem.

1. Chemical: Phorate

2. Test Material: Technical Grade, 92.1% ai

3. Study/Action Type: Fish Early-Life Stage Rainbow trout (*Salmo gairdneri*)

4. Study ID: MRID 158335 Suprenant, D. (1986) The toxicity of (Carbon-14)-AC35,024 to rainbow trout (*Salmo gairdneri*) embryos and larvae. Repori No. BW-86-3-1-1968. Unpublished study prepared by Springborn Bionomics, Inc.

5. Reviewed By: Ann Stavola
Aquatic Biologist
HED/EEB
Signature: Ann Stavola
Date: 7/25/88

6. Approved By: Douglas Urban
Supervisory Biologist
HED/EEB
Signature: Douglas Urban
Date: 10/11/88

7. Conclusions:

The study is not scientifically sound and does not fulfill the data requirement for a fish chronic toxicity test with technical phorate. The mortality of the control embryos was excessively high.

8. Recommendations:

A new study is required.

9. Background:

A fish early life stage study was required in the Phorate Registration Standard, 1984.

10. Materials and Methods:

a. Test Animals - Species: Rainbow trout (Salmo gairdneri); Source: Unfertilized eggs and sperm were individually packed and shipped under refrigeration from Mount Lassen Trout Farms, Red Bluffs, CA.

b. Dosage - Stock solution: labeled phorate (40 uCi/mg) mixed with nonlabeled phorate (92.1% ai) in acetone for a stock solution containing 278 ug ai/mL of phorate.

c. Test System - Modified proportional diluter with a 0.5 dilution factor. The diluter delivered 0.5 L test water to each aquarium at an average rate of 147 times a day. This equals 6.7 aquarium volumes per 24-hours, with a 90% replacement time of 8 to 9 hours.

- Nominal concentrations: 0.31, 0.62, 1.2, 2.5, and 5.0 ug/L plus control and solvent control.

- Measured concentration: measured on day 0 and weekly thereafter until test ended. 0.22, 0.47, 1.1, 1.9, and 4.2 ug/L.

- Dilution water: well water supplemented with Town of Wareham untreated well water and aerated. Soft quality, pH 7.0 to 7.6.

d. Study Design - Protocol followed:

- American Cyanamid protocol #980-85-183.

- Types of test chambers: Glass aquaria, 39 x 20 x 25 cm with constant water volume of 11 L. Embryo cups--glass jars 5 x 8 cm high with 16 mesh Nitex screen bottoms.

- Number of organisms per test concentration: 50 embryos per cup, 2 cups per aquarium, 2 aquaria per concentration (200 embryos per concentration).

- After hatching: 20 larvae per aquarium, 2 aquaria per concentration (40 larvae per concentration).

- Length of study: 88 days (60 days posthatch and 44 days post-swimup).

- Photoperiod: Embryos and and swim-up larvae were shielded from fluorescent light and sunlight by black polyethylene. Older larvae exposed to 16 hours light.

- Test temperature: 10 to 13 °C.

e. Test Procedure - Eggs and sperms were received at 4 °C and gradually warmed to 7°C over 30-minute period prior to initiating fertilization. After eggs and sperms were mixed together, the hardening period was extended to 2 hours as the temperature was gradually raised to 12 °C. After water hardening was completed, the embryos were impartially distributed to each of 28 embryocups. The cups were gently oscillated in the aquaria with a rocker arm apparatus. Dead embryos were counted and removed daily until hatching was complete except during days 7 to 12 when the eggs were particularly susceptible to disturbances. Hatching was considered to be complete when no more than 5 unhatched viable embryos remained in any cup. The 60-day posthatch larvae exposure period began when the surviving larvae from each of the 2 embryo cups in each aquarium were combined. Twenty larvae were impartially selected. Larvae were fed live brine shrimp nauplii 2 to 3 times daily when larvae reached swimup stage (about 9-13 days posthatch). Aquaria were cleaned periodically to remove organic matter. Larvae were counted twice a week.

f. Analytical Methods - DO, pH, and temperature were measured in each aquarium on day 0, then they were measured daily in one replicate aquarium of each concentration. Each aquarium was monitored on alternate days. Phorate concentrations were measured on day 0 and then weekly until the test ended. Total hardness was measured weekly.

g. Statistical Analyses - Percent survival of hatched embryos = $\frac{\text{No. of live larvae/incubation cup after hatching}}{\text{No. of embryos/cup on day 0}}$

The data from the solvent controls and controls were analyzed by a one-way ANOVA. As no statistical differences were found, all control and solvent control data were pooled.

Differences in percent survival were determined after arcsine transformation of the data. Williams' procedure was used to compare the results.

The MATC was calculated by taking the geometric mean of the limits set by the LOEC that showed a statistically significant effect and the highest test concentration that showed no statistically significant difference from the control (NOEC).

11. Reported Results:

DO, pH, and total hardness did not vary significantly throughout the 88 days of the study. The mean values for

DO and pH were respectively 9.6 to 10.1 mg/L and 6.6 to 7.7. Temperature was 10 to 13 °C except for 1 day when a malfunction caused the temperature to increase by 20 °C. Temperature returned to normal within 24 hours. (The data table for mean temperature reports the average temperature as 12 ± 1 °C.)

The mean measured concentrations are given in Table 2.

Table 2. Measured concentrations of 14C-AC35,024 in water during the 88-day exposure of rainbow trout (Salmo gairdneri) embryos and larvae.

Nominal Concentration (ug/L)	Mean Measured Concentration (ug/L)	Range	N
Control	< 0.063		28
Solvent control	< 0.063		28
0.31	0.22 (0.08)[a]	0.16-0.44	28
0.62	0.47 (0.13)	0.35-0.85	28
1.2	1.1 (0.3)	0.79-1.9	28
2.5	1.9 (0.5)	0.95-3.5	28
5.0	4.2 (1.0)	3.6-8.5	30

[a]Standard deviation in parentheses.

No insoluble material was observed in any aquarium.

Table 4 (attached) provides a summary of the percent survival at hatch, percent survival at 60 days posthatch and mean total length and mean wet weight at 60 days posthatch for each concentration. Also attached are charts depicting these parameters.

12. Study Author's Conclusions/Quality Assurances (QA) Measures:

a. Larval length was the most sensitive indicator of the toxicity of phorate to rainbow trout larvae.

b. No adverse effects regarding survival of eggs and larvae or weight of larvae.

c. MATC was $\geq$ 1.9 ug/L $\leq$ 4.2 ug/L based on the effect on total length.

QA Statement - "The data contained in this report were audited by the QA unit to assure compliance with the protocols, standard operating procedures, and the pertinent EPA Good Laboratory Practice Regulations."

13. Reviewer's Evaluation:

a. Test Procedures - EEB's Standard Evaluation Procedure (SEP) for Fish Early Life-Stage was used as a basis for evaluating the procedures used in this study. The major problem with their test procedure was the malfunction in temperature. The SEP states that the temperature should not deviate more than 2°C from the appropriate temperature, but they report it ranged normally from 10 to 13°C, except for one day when it reached 20°C. Unfortunately, their data table contradicts the written statement, as the table indicates the temperature was 12 $\pm$ 1 °C. These fluctuations in temperature may have been responsible for the high rate of mortality in the control and solvent control embryos.

b. Statistical Analysis - As they did not submit any raw data (number of embryos per cup, number of larvae per replicate, individual weights and lengths) the reported results could not be verified.

c. Results/Discussion - The SEP states that "a test should be terminated if the average percent of embryos . . . that produce live fry for release into the chambers in any control treatment is less than 50 percent . . . ." Their report that only 29 to 47 percent of the control embryos had survived at hatch indicates the study is unacceptable.

d. Conclusions

1) Classification - Invalid

2) Rationale - Less than 50 percent survivability of control embryos and wide range of temperature fluctuations.

3) Reparability - None; a new study is needed.

upgrade to "Core" in 1991

Table 4. Survival of organisms at hatch and survival, total length and wet weight of rainbow trout (Salmo gairdneri) larvae exposed to 14C-AC35,024 for 60 days post hatch.

Mean measured concentrations (µg/L)		Organism Survival at Hatch (%)	Larvae (60 days post-hatch)		Mean
			Larvae Survival (%)	Mean total length (S.D) (mm)	Wet weight (mg)
control	A	47	90	43(3)	0.82 (0.16)
	B	30	95	42(3)	0.78 (0.15)
Solvent control	A	44	75	44(3)	0.81 (0.14)
	B	29	100	43(3)	0.74 (0.16)
0.22	A	44	90	42(4)	0.77 (0.25)
	B	31	100	42(3)	0.72 (0.19)
0.47	A	35	85	44(3)	0.86 (0.20)
	B	36	85	44(2)	0.85 (0.14)
1.1	A	41	85	43(3)	0.82 (0.19)
	B	34	85	43(3)	0.83 (0.18)
1.9	A	31	85	44(2)	0.85 (0.14)
	B	35	95	42(2)	0.76 (0.15)
4.2	A	28	85	40(1)	0.74 (0.12)
	B	36	70	39(3)	0.72 (0.24)

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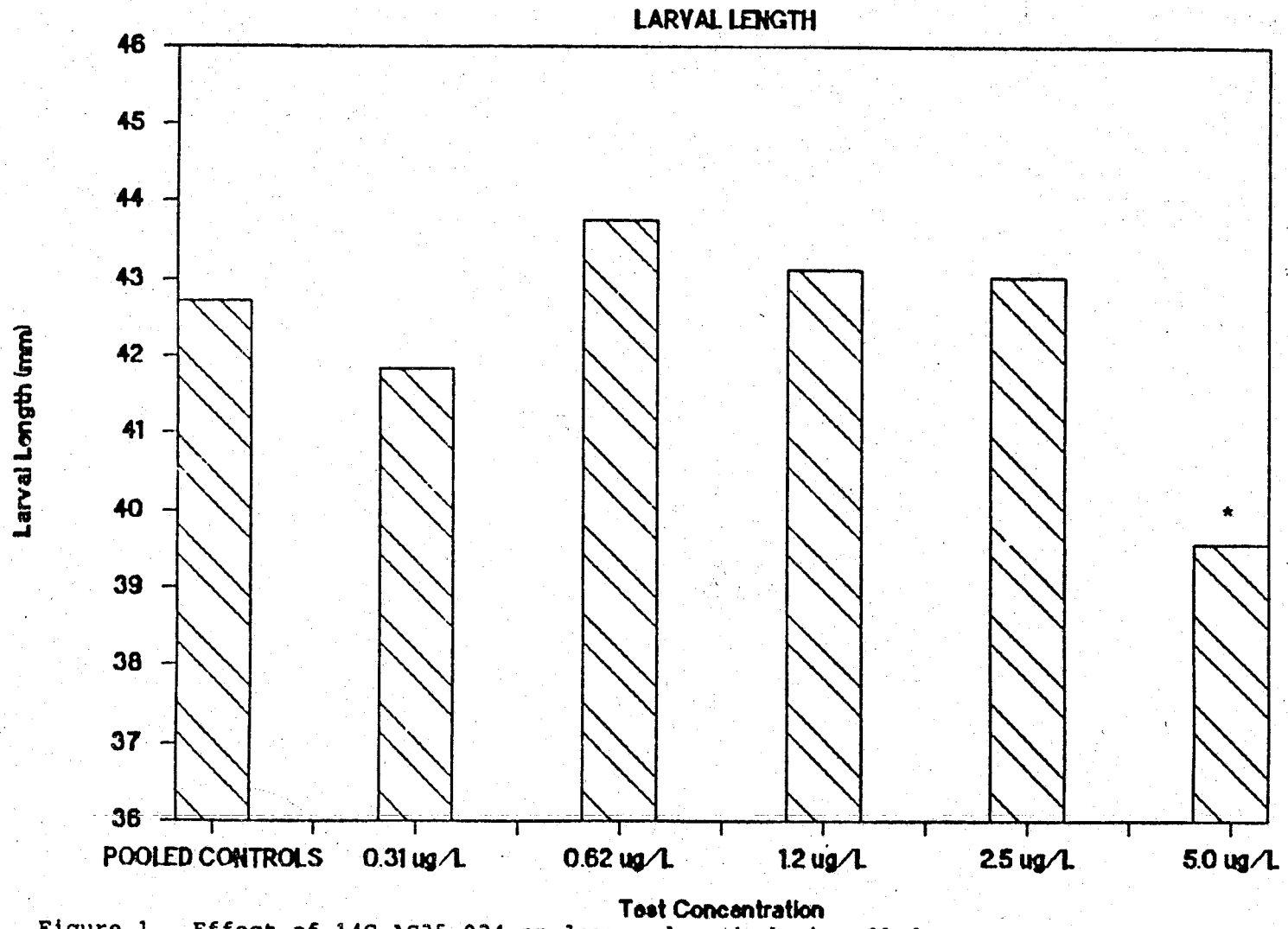

Figure 1. Effect of 14C-AC35,024 on larvae length during 60 day post-hatch exposure. Concentration reported are based on nominal treatment levels.

* ... different from controls

Figure 2. Measurement of egg survival(hatchability), larval survival and larval weight of Rainbow trout exposed to 14C-AC35,024 in 60 day post-hatch study. No significant differances detected.