VULCAN 4-8-91

DATA EVALUATION RECORD

1. **CHEMICAL:** Pentachlorophenol
Shaughnessy #063001

2. **TEST MATERIAL:** Pentachlorophenol 88%

3. **STUDY TYPE:** Fish Early Life-Stage

4. **STUDY IDENTIFICATION:** Spehar, R.L., Nelson, H.P., Swanson, M.H., and Renoos, J.W., Pentachlorophenol Toxicity to Amphipods and Fathead Minnows at Different Test pH Values, U.S. Environmental Protection Agency Environmental Research Laboratory, 6201 Congdon Blvd., Duluth, Minnesota, 55804, submitted by Penta Task Force/Scientific Research Associates, Inc., 1220 19th Street, N.W., Suite 202, Washington, D.C., 20036.
MRID # 409241-15

5. **REVIEW BY:** Harry A. Winnik
Biologist
EFED/EEB
Signature:
Date: 3-12-91

6. **APPROVED BY:** Henry Craven
Supervisory Biologist
EFED/EEB
Signature:
Date: 4/8/91

7. **CONCLUSIONS:** The study appeared scientifically sound but could not be fully analyzed due to the lack of raw data. The study is considered supplemental but may be upgraded upon submission and analysis of raw data.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** The study was submitted by the Penta Task Force to support the reregistration of Pentachlorophenol and the Sodium Salt of Pentachlorophenol.

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. Test Animals: (excerpted from the submission)

Fathead minnows (Pimephales promelas) cultured at the U.S. EPA's Environmental Research Laboratory in Duluth, Minnesota. Information regarding species history and health is maintained at the laboratory.

B. Test System: (excerpted from the submission)

Lake Superior water was filtered through sand and heated to 25 ± 2°C for 32-d early life stage tests with fathead minnows. Chemical characteristics of the dilution water for tests conducted at four pH values were determined according to methods described by the American Public Health Association. The ranges for water hardness, alkalinity and acidity were 42 to 47, 10 to 52 and 0 to 9 mg/L (as $CaCO_3$) for all tests respectively.

Tests were conducted with continuous-flow minidiluter exposure systems that delivered five PCP concentrations and a control to four replicate chambers per treatment. Duplicate exposure chambers per treatment were used in the acute toxicity tests due to the smaller number of animals available. Glass test chambers measured 7 cm wide x 19 cm long x 9 cm high, with a water depth of 4.5 cm. The flow rate to each chamber was 15 ± 1 ml/min. Sylvania Cool White fluorescent bulbs provided a light intensity of 100 to 300 lux at the water surface during a 16-h photoperiod.

C. Dosage: (excerpted from the submission)

Technical-grade PCP (88% active ingredient: Dowicide EC7) was supplied by Dow Chemical (Midland, Michigan) and used as the toxicant source. Superstocks were made by dissolving PCP with NaOH in distilled water. Diluter stocks were also prepared with distilled in 19-liter glass bottles and were delivered to the diluter systems via fluid-metering pumps to produce the desired test concentrations (pH 6.5-control,3.1, 5.1, 8.9, 16.5, 34.6 µg/L; pH 7.5 - control, 5.6, 12.7, 27.6, 58.2, 124.0 µg/L; pH 8.0 - control, 12.7, 32.0, 75.0, 161.0, 327.0 µg/L; pH 8.5 - 29.3, 63.7, 125.0, 237.0, 451.0 µg/L). The desired pH was obtained and maintained by pumping dilute solutions of either HNO_3 or NaOH into aerated dilution water located in head tanks throughout the tests.

D. Design: (excerpted from the submission)

Early life stage tests with fathead minnows were begun by randomly distributing 25 <24-h-old embryos from (the) culture unit into 120-ml glass jars with 40-mesh Nytex screen bottoms. The jars were oscillated in the exposure chambers with a 2 rpm motor to determine hatchability of the embryos. After 2 d of incubation, the number of developed embryos was randomly thinned to 15 in each replicate chamber (60 embryos per treatment) to eliminate embryo mortality due to fungus. Subsequently, these embryos were incubated until they hatched (~4-5 d). After all embryos were hatched, the larvae were transferred to the exposure chambers by gently inverting the egg cups in the dilution water until all larvae were released. All animals were fed ad libitum amounts of brine shrimp nauplii three times per day, except on weekends when they were fed once daily. After a total of 32 d of exposure, the juvenile fish were killed in ice water, blotted dry and each fish was weighed to the nearest milligram.

Fathead minnows that survived the 32-d tests were analyzed on a whole body basis (wet weight) for PCP. Fish from each replicate chamber were composited and analyzed as one sample. The fish were placed in 125-ml glass-stoppered bottles containing 50 ml of concentrated H_2SO_4 and were digested in an oven at 90° C for approximately 2 h. After cooling and the addition of 50 ml hexane, the samples were extracted, derivatized and analyzed in the same manner as the water samples. The limit of detection for this procedure was <0.1µg/g. The mean percentage recovery and SD for five spiked samples were 96 ± 3.7.

E. Statistics: (excerpted from the submission)

For early life stage tests with fathead minnows, survival and embryo hatchability data were transformed to arc sin % for variance stabilization. Individual weights from fish in replicate chambers were pooled (after no significant differences were found between replicates) before the data were subjected to a one-way analysis of variance (p=0.05) and Dunnet's one-sided comparison of treatment means to control means (p=0.05).

Linear regression analysis was used to describe the relationship between PCP toxicity and accumulation versus pH. Analysis of covariance was used to determine slope comparability.

12. Reported Results: (excerpted from the submission)

Survival and growth of fathead minnows exposed to different concentrations of PCP for 32 d and to four pH values are shown in Table 2 (attached). Embryo hatchability was consistently the least sensitive measure of effect in all tests. Although fish hatched in all concentrations,

morphological and behavioral abnormalities such as scoliosis, pericardial edema and convulsive swimming patterns were observed in larvae exposed to the highest concentrations. Survival of the larvae was the most sensitive measure of PCP effect at pH 6.5 and 8.5, whereas growth, as measured by wet weight, was the most sensitive parameter measured in tests at pH 7.5 and 8.0. Chronic values defined as the geometric mean of the upper chronic limit (the lowest test concentration that caused significant decreases from the control) and the lower chronic limit (the highest test concentration that did not cause significant decreases from the control) based on the above results were 24, 40, 49 and 89 µg/L at the respective increasing pH values.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

Egg hatchability was not significantly reduced in any exposure level when compared to the controls. Growth and survival were significantly reduced at the highest exposure levels at each pH level when compared to the controls.

Survival of the larvae was the most sensitive measure of PCP effect at pH 6.5 and 8.5, whereas growth, as measured by wet weight, was the most sensitive parameter measured in tests at pH 7.5 and 8.0. Chronic values defined as the geometric mean of the upper chronic limit (the lowest test concentration that caused significant decreases from the control) and the lower chronic limit (the highest test concentration that did not cause significant decreases from the control) based on the above results were 24, 40, 49 and 89 µg/L at the respective increasing pH values.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. Test Procedures: This study appears to be scientifically sound but it was not possible to verify that the study meets the Guideline requirements for a Fish Early Life-Stage study since the submission lacked the following information:

- All raw data concerning the acclimation period
- All raw data concerning the actual study

B. Statistical Analysis: Survival, hatchability, length and weight data could not be reanalyzed by the reviewer since no raw data was submitted.

C. Discussion of Results: Due to the lack of data, the results of this study could not be analyzed.

D. Adequacy of the Study:

(1) Classification: Supplemental

(2) Rationale: The study appeared scientifically sound but could not be fully analyzed due to the lack of raw data.

(3) Repairability: The study may be upgraded upon submission and analysis of raw data.

15. **COMPLETION OF ONE-LINER FOR STUDY:** N/A

Attachments

Table 1

Table 2. Survival and growth of fathead minnows exposed to various concentrations of PCP for 32 d at pH values of 6.5, 7.5, 8.0 and 8.5

Measured water concentration (μg/L)	Embryo hatchability[a] (%)	Normal larvae at hatch (%)[a]	Survival[a] (%)	Mean weight[b] (mg)
pH = 6.5				
<0.3[c] (control)	100 ± 0.0	100 ± 0.0	91 ± 6.7	81 ± 20 (54)
3.1 ± 0.3[d]	98 ± 3.5	98 ± 3.5	96 ± 4.0	74 ± 20 (58)
5.1 ± 0.4	100 ± 0.0	100 ± 0.0	91 ± 10.1	80 ± 20 (43)
8.9 ± 0.9	100 ± 0.0	100 ± 0.0	95 ± 6.7	74 ± 20 (58)
16.5 ± 1.8	100 ± 0.0	100 ± 0.0	95 ± 6.7	74 ± 17 (56)
34.6 ± 2.4	100 ± 0.0	0[e]	23 ± 8.8[e]	88 ± 37 (14)
pH = 7.5				
<0.3[c] (control)	100 ± 0.0	95 ± 6.7	90 ± 4.0	98 ± 28 (41)
5.6 ± 0.6[d]	100 ± 0.0	95 ± 6.7	91 ± 10.1	103 ± 28 (39)
12.7 ± 1.4	100 ± 0.0	93 ± 9.4	88 ± 11.5	102 ± 24 (52)
27.6 ± 4.2	100 ± 0.0	100 ± 0.0	98 ± 3.5	90 ± 26 (59)
58.2 ± 7.6	100 ± 0.0	88 ± 6.3	84 ± 6.1	81 ± 30 (50)[e]
124.0 ± 6.4	82 ± 6.7[e]	0[e]	0[e]	—
pH = 8.0				
<0.3[c] (control)	100 ± 0.0	97 ± 4.0	88 ± 10.0	100 ± 24 (53)
12.7 ± 2.6[d]	98 ± 3.5	97 ± 7.0	88 ± 10.0	108 ± 24 (53)
32.0 ± 4.3	100 ± 0.0	95 ± 6.7	91 ± 3.6	109 ± 32 (54)
75.0 ± 5.8	95 ± 6.3	93 ± 5.7	92 ± 10.1	90 ± 26 (54)
161.0 ± 6.1	95 ± 3.5	0[e]	29 ± 19.3[e]	88 ± 41 (18)
327.0 ± 13.4	54 ± 22.3[e]	0[e]	0[e]	—
pH = 8.5				
<0.3[c] (control)	100 ± 0.0	97 ± 4.0	93 ± 5.1	108 ± 31 (56)
29.3 ± 2.3[d]	100 ± 0.0	100 ± 0.0	93 ± 5.7	104 ± 35 (55)
63.7 ± 4.4	98 ± 3.5	97 ± 4.0	92 ± 10.1	107 ± 28 (52)
125.0 ± 6.1	100 ± 0.0	100 ± 0.0	75 ± 22.8[e]	104 ± 30 (44)
237.0 ± 16.0	100 ± 0.0	0[e]	75 ± 14.7[e]	88 ± 41 (45)
451.0 ± 33.8	82 ± 16.7[e]	0[e]	13 ± 0.0[e]	7 ± 0.0 (1)

[a]Mean of 15 organisms per replicate.
[b]Number of fish weighed in parentheses.
[c]Detection limit of analyses.
[d]Mean ± SD of 8 to 10 samples.
[e]Values significantly less than controls ($p = 0.05$).