9/1/94

MRID No. 421449-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorpyrifos. 059101
Shaughnessey No. 038011.

2. **TEST MATERIAL:** Chlorpyrifos Technical; Lot No. 489205; 95% active ingredient; a tan colored solid.

3. **STUDY TYPE:** Estuarine Fish Flow-Through Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Surprenant, D.C. 1989. Acute Toxicity of Chlorpyrifos to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions. Project No. 89-1-2909. Prepared by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Makhteshim-Agan (America) Inc., New York, NY. EPA MRID No. 421449-04.

5. **REVIEWED BY:**

Darlene R. Lintott
Aquatic Toxicologist
Toxikon Environmental Sciences

Signature: D Lintott
Date: 3/30/92

William S. Rabert
8/3/94

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat for L.M. Rifici
Date: 4/16/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: H Craven
Date: 9 1 94

7. **CONCLUSIONS:** This study is scientifically sound, but does not meet the guideline requirements for an acute estuarine fish toxicity study. The tests concentrations were less than 100 mg/l and had no effect on the test organisms. The 96-hour LC_{50} value was >0.076 mg a.i./l mean measured concentration. The NOEC was 0.076 mg a.i./l mean measured concentration.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile sheepshead minnows (*Cyprinodon variegatus*) were obtained from a commercial supplier in New York. The fish were maintained in the laboratory (500-l holding tank) in a closed-loop recirculating system for a minimum of 14 days. The natural seawater used was characterized as having a salinity range of 31-35°/oo, a pH of 7.5 and a dissolved oxygen concentration of 92-96% of saturation. The photoperiod during holding was 16-hours light/8-hours dark. The temperature in the holding tank was 20-21°C during the 14-day acclimation period. Fish were fed a commercially available pelleted fish food *ad libitum* daily. No mortality was observed during the 48 hours prior to testing.

Mean wet (blotted) weight and total length of a representative sample (N=30) of the test population were 0.24 (0.08-0.55) g and 23 (17-28) mm, respectively, resulting in a loading of 0.03 g/l/day.

B. **Test System:** The test system consisted of a continuous flow serial diluter (Benoit *et al.*, 1982). The test chambers were glass aquaria (39 x 20 x 25 cm) filled with 11 l of test solution. The test solution depth was approximately 14.5 cm. The test aquaria were impartially positioned in a waterbath set to maintain 22 ±1°C. A 16-hour daylight photoperiod was maintained with a mean light intensity of 46 footcandles (range of 30-60).

The dilution water was natural filtered (5 µm) seawater collected from the Cape Cod Canal, Bourne, Massachusetts.

A stock solution (5.73 mg a.i./l) was prepared by diluting the test material (1.5075 g) to a total volume of 250 ml in acetone. A pump was used to deliver the stock solution at a rate of 0.0276 ml/minute. The diluter system mixing chamber also received 316 ml/minute of dilution water. This procedure provided a high treatment concentration of 0.50 mg a.i./l which was serially diluted (65% dilution factor) to provide the desired concentration range.

The flow rate provided 7 volume replacements per day to each aquarium.

C. **Dosage:** Ninety-six-hour flow-through test. Based on solubility and toxicity data developed through preliminary testing and consultation with the study sponsor, five nominal concentrations (0.089, 0.14, 0.21, 0.33 and 0.50 mg a.i./l), a dilution water control, and a solvent control were used.

D. **Design:** Ten sheepshead minnows were impartially selected and equally distributed to each aquarium, two aquaria per concentration. The fish were not fed during the test.

Observations of mortality and sublethal responses were made at test initiation and every 24 hours thereafter. The dissolved oxygen concentration (DO), pH, salinity, and temperature were recorded in each test chamber daily. The temperature of a solvent control aquarium was recorded continuously.

Water samples from each test vessel were collected at test initiation and termination. The concentration of Chlorpyrifos in the samples was determined using gas chromatography.

E. **Statistics:** No statistics were required for this data set.

12. **REPORTED RESULTS:** No insoluble material was observed at any concentration. The mean measured concentrations were 0.019, 0.023, 0.038, 0.055 and 0.076 mg/l (Table 2, attached). The concentration of test material in the exposure solutions increased by approximately 35% between day 0 and 4.

The concentration of the test material had no effect on the sheepshead minnows. The 96-hour LC_{50} value was greater than 0.076 mg/l mean measured concentration. The no observed effect concentration (NOEC) was 0.076 mg/l.

The temperature, pH, DO and salinity during the test are presented in Table 1 (attached). The results of continuous temperature measurement in a solvent control replicate indicated that the test temperature was 21-23°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author stated that "Chlorpyrifos Technical was not acutely toxic to sheepshead minnow at the material's limit

of solubility in seawater (0.076 mg A.I./L under the maintained test conditions)." The variability observed between the measured concentrations during the study (increase of 35% from day 0 to 4) was due to the limited solubility of Chlorpyrifos Technical in seawater and the behavior of the test material in the exposure system.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory Practice Regulations set forth in 40 CFR Part 160. The dates of quality assurance inspections were also reported.

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

A. Test Procedure: The test procedures were generally in accordance with the SEP except for the following:

The fish population was fed during the 48 hours prior to test initiation. The SEP states that fish must not be fed during the final 48 hours before the test.

The SEP states that fish should be between the range of 0.1 to 5 g wet weight. Some fish weighed less than 0.1 g.

No information was provided regarding a transition period between the light and dark photoperiod. The SEP states that a 15- to 30-minute transition period between light and dark should be used.

Solvent control concentration was not given. The SEP states that solvent control concentration must not exceed 0.5 ml/l.

B. Statistical Analysis: No statistics were required for this data set.

C. Discussion/Results: This study is scientifically sound but does not meet the guideline requirements for an acute estuarine fish toxicity study. The maximum solubility of Chlorpyrifos technical cannot be determined from the report. Therefore, the requirement of testing compounds with water solubility less than 100 mg/L at the maximum solubility may not have been achieved. The increase in test concentrations (57 to 162%) from test initiation to termination suggests that maximum solubility may not have been reached and at minimum, that stable test concentrations were not maintained. In addition, the lack of any observed

undissolved test substance also indicates that solubility of the test substance in dilution water was not achieved. Under the conditions of the test, the 96-hour LC_{50} value was >0.076 mg a.i./l (based on mean measured concentrations). The NOEC was 0.076 mg a.i./l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Lack of information on solubility of Chlorpyrifos Technical under test conditions. The test concentrations were less than 100 mg/l and there was no effect on the test fish.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 03-24-92.

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