

9/1/94

MRID No. 421449-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorpyrifos.
Shaughnessey No. ~~038011~~.
2. **TEST MATERIAL:** Chlorpyrifos Technical; Lot No. 489205; 95% active ingredient; a tan crystalline solid.
3. **STUDY TYPE:** Mollusc 96-Hour Flow-Through Shell Deposition Study. Species Tested: eastern oyster (*Crassostrea virginica*).
4. **CITATION:** Surprenant, D.C. 1989. Acute Toxicity of Chlorpyrifos To Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions. Project No. 89-2-2931. Prepared by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Makhteshim-Agan (America) Inc., New York, NY. EPA MRID No. 421449-05.
5. **REVIEWED BY:**

Darlene R. Lintott
Aquatic Toxicologist
Toxikon Environmental
Sciences

Signature: *D Lintott*
Date: *3/30/92*

William S. Robert
8/4/94
Signature: *P. Kosalwat*
for L.M. Rifici
Date: *4/16/92*

Signature: *Boodyean*
Date: *9 1 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*

Signature: *Boodyean*
Date: *9 1 94*

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a flow-through mollusc shell deposition study. Control mean shell deposition in 96 hours (1.8 and 1.6 mm) was lower than recommended (2 mm). The 96-hour EC₅₀ value was 84 µg a.i./l mean measured concentration. Therefore, Chlorpyrifos Technical is classified as very highly toxic to eastern oysters. The NOEC was 41 µg 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: Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Dennis, Massachusetts where they were reared in natural flowing seawater from approximately the same source (Massachusetts Bay) as that used as dilution water during the toxicity test. The oysters were held under test conditions 48 hours prior to test initiation. They were supplied with unfiltered natural seawater supplemented with algae (*Isochrysis galbana* Parke, and *Tetraselmis maculata*) during the holding period and throughout the test period. During the last two days of holding, the temperature was 20-21°C, the salinity was 32-34 parts per thousand (ppt), the pH was 7.5-7.9, and the dissolved oxygen concentration (DO) was 79-97% of saturation.

Oysters had an average length of 42 mm ($\pm$ 5 mm standard deviation). No mortality was observed during the holding period. Twenty-four hours prior to testing, 3-5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge using a grinding wheel. Immediately prior to test initiation, the outer shell edge of each oyster was buffed with an emery board.

- B. Test System: A continuous-flow, serial diluter was used. The test chemical was dissolved in acetone and delivered to the test system by a syringe pump. The test chambers were 60 x 30 x 30 cm glass aquaria equipped with a 10 cm side drain which maintained a test solution volume of approximately 18 liters. The contents of each aquarium was continuously circulated. The flow of test solution to each aquarium was 75 ml/minute which provided approximately 9.8 volume replacements every 24 hours. A nylon impeller pump located in each aquarium recirculated the test solution in the aquarium. The flow rate of the recirculating test solution was 1.75 liters per minute or about 7 l per oyster per hour. The aquaria were randomly positioned in a temperature-controlled water bath designed to maintain 20 $\pm$ 2°C. The laboratory environment was maintained on a 16-hour light photoperiod.

Unfiltered natural seawater was collected at Cape Cod Canal, Bourne, Massachusetts for use as dilution water. The salinity of the dilution water was 32-33 ppt and the pH was 7.9-8.0 (based on measurement of control solutions).

- C. **Dosage:** Ninety-six-hour acute flow-through toxicity test. Based on preliminary test results, five concentrations (50, 83, 140, 230 and 380 $\mu\text{g a.i./l}$), a solvent control (0.17 ml acetone/l) and a dilution water control were chosen for the definitive test.
- D. **Design:** Twenty oysters were impartially selected and distributed to each aquarium, two replicates per concentration, for a total of 40 oysters per concentration. Three times daily, the oysters received supplemental feedings of algae resulting in an algal density of 10^5 cells/ml in each aquarium.

Observations of mortality were made daily. At the end of the test, the length of the longest finger of new shell growth on each oyster was measured to the nearest 0.1 mm using a calibrated micrometer. Shell growth inhibition in each treatment group was expressed as a percentage of the mean growth in the controls. The DO, pH, temperature and salinity were measured in each test chamber daily. The temperature of one aquarium was recorded continuously.

Water samples from each test chamber were collected at the beginning and end of the test from the mid-point of the aquaria using a glass volumetric pipet. The concentration of Chlorpyrifos Technical was determined using gas chromatography.

- E. **Statistics:** The 96-hour median effect concentration (EC_{50}) and associated 95% confidence intervals (C.I.) were calculated using a method of inverse prediction (Sokal and Rohlf, 1969). The NOEC was estimated by analysis of variance and Williams' Test.

12. **REPORTED RESULTS:** A precipitate of chlorpyrifos was observed and removed daily from the end of stock syringe tube. No undissolved material was observed in any of the test solutions. The mean measured concentrations were 17, 41, 85, 150 and 180 $\mu\text{g a.i.}$ (Table 2, attached). These values represent 34-65% of nominal concentrations. Measured concentrations of Chlorpyrifos in the exposure solutions were generally consistent between sampling intervals.

There were no mortalities during the test. Oysters in both replicates of the 180 $\mu\text{g}/\text{l}$ concentration and in replicate A of the 110 $\mu\text{g}/\text{l}$ concentration exhibited reduced fecal and pseudofecal production as compared to the control oysters. Growth between the control and solvent control oysters was not significantly different, therefore, treatment responses were compared to the pooled control response. The length measurements indicated significant shell growth inhibition ranging from 53% in the 85 $\mu\text{g}/\text{l}$ group to 94% in the 180 $\mu\text{g}/\text{l}$ group (Table 3, attached). The 96-hour EC_{50} and 95% confidence interval calculated by linear regression were 84 (71-99) $\mu\text{g a.i.}/\text{l}$ Chlorpyrifos Technical. The no-observed-effect-concentration (NOEC) was determined to be 41 $\mu\text{g}/\text{l}$.

The DO and pH values are presented in Table 1 (attached). The results of continuous temperature monitoring in addition to the individual measurements during the test established the test temperature range as 18 to 22°C.

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

Based on the criteria established by the EPA, chlorpyrifos technical would be classified as very highly toxic to eastern oysters.

Good Laboratory Practice Compliance and Quality Assurance Statements were included in the report indicating compliance to with EPA Good Laboratory Practice Standards under the Federal Insecticide, Fungicide, and Rodenticide Act. 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 and Butler and Lowe (1978), except for the following:

According to an amendment to the SEP, control oysters must produce a minimum of 2mm of new shell growth in 96 hours. The dilution water control and solvent control oysters produced, on average 1.8 and 1.6 mm, respectively.

Oysters were not held at the testing site for 10 days prior to test initiation. This is considered acceptable since culturing conditions provided by the commercial supplier for 14 days prior to test initiation were nearly identical to test conditions.

The flow rate of unfiltered seawater to each aquarium was 75 ml/min. This is equivalent to a flow of 0.255 l of "once through" water per oyster per hour. Butler and Lowe (1978) recommend a flow rate of 5 l/oyster/hour. However, supplemental additions of algae were made three times daily.

A 15- to 30-minute dawn and dusk simulation is recommended. The report does not state whether a dawn and dusk simulation was used.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC50 value and obtained similar results (see attached printout). The slope of the concentration-response curve was 4.8. The raw shell deposition data were not included in the report so the NOEC could not be verified by the reviewer.
- C. **Discussion/Results:** Control shell deposition in 96 hours (1.6 and 1.8 mm mean shell deposition) was less than the required 2 mm. The flow of "once through" unfiltered seawater (0.225 l/oyster/hour) was lower than recommended. However, a supplemental diet of marine algae was added which should have provided adequate food supply. The test is scientifically sound but the results should be interpreted with caution. The 96-hour EC₅₀ value was 84 µg a.i./l (based on mean measured concentrations) with 95% confidence limits of 77 and 91 µg/l. Therefore, Chlorpyrifos Technical is classified as very highly toxic to eastern oysters. The NOEC was 41 µg/l mean measured concentration. The slope of the concentration-response curve was 4.8.
- D. **Adequacy of the Study:**
- (1) **Classification:** Supplemental.
 - (2) **Rationale:** Control shell deposition was lower than recommended.
 - (3) **Repairability:** N/A.

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

References:

Butler, P.A. and J.I. Lowe. 1978. Flowing Sea Water Toxicity Test Using Oysters (*Crassostrea virginica*). EPA-600/9-78-010.

Page _____ is not included in this copy.

Pages 6 through 9 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
- ☐ The product confidential statement of formula.
- ☐ Information about a pending registration action.
- ☒ FIFRA registration data.
- ☐ The document is a duplicate of page(s) _____.
- ☐ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Scott Ward Chlorpyrifos Crassostrea virginica 03-26-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
180	100	94	94	0
150	100	88	88	0
85	100	53	53	0
41	100	6	6	0
17	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 81.76695

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	1.012427E-02	77.30959	71.5578 83.41826

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.266369E-02	1	.9473881

SLOPE = 4.805977
95 PERCENT CONFIDENCE LIMITS = 4.082463 AND 5.529491

LC50 = 84.16972
95 PERCENT CONFIDENCE LIMITS = 77.48645 AND 90.87126

LC10 = 45.80379
95 PERCENT CONFIDENCE LIMITS = 39.23455 AND 51.66773
