

US EPA ARCHIVE DOCUMENT

10/2/91

INVALIDATED

MRID No. 418852-04

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Chlorpyrifos (Dursban ME 20).  
Shaughnessey No. 051701.
- 2. **TEST MATERIAL:** Dursban® 20 MEC (XRM-5160); Identification:  
AGR 286398; Dow Registry No. DR-0320-1647; 25.6% active  
ingredient chlorpyrifos; a milk-colored liquid.
- 3. **STUDY TYPE:** Freshwater Fish Static Acute Toxicity Test.  
Species Tested: Rainbow Trout (Oncorhynchus mykiss).
- 4. **CITATION:** Mayes, M.A., S.J. Gorzinski, C.H. Richardson,  
J.T. Weinberg, and R.B. Potter. 1991. XRM 5160  
(Microencapsulated Insecticide): Acute Toxicity to the  
Rainbow Trout, Oncorhynchus mykiss Walbaum. Laboratory  
Project Study ID. ES-DR-0320-1647-5. Prepared by  
Environmental Toxicology & Chemistry Research Laboratory,  
The Dow Chemical Company, Midland, MI. Submitted by  
DowElanco, Indianapolis, IN. EPA MRID No. 418852-04.

5. **REVIEWED BY:**

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

Signature: *Louis M. Rifici*  
Date: 7/19/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.  
Senior Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *P. Kosalwat*  
Date: 7/19/91

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

Signature: *Henry T. Craven*  
Date: 10/2/91  
*H. T. Craven*  
9-30-91

- 7. **CONCLUSIONS:** This study is not scientifically sound. The  
analysis for chlorpyrifos was highly variable and suggests  
that the actual concentrations of chlorpyrifos the fish were  
exposed to are unknown. Under the conditions of the test,  
the 96-hour LC<sub>50</sub> of XRM-5160 for rainbow trout was 8.7 mg/L  
mean measured concentration of formulated product.  
Therefore, XRM-5160 is classified as moderately toxic to  
rainbow trout. Sublethal effects were noted at all

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concentrations; therefore an NOEC was not generated in this test.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Rainbow trout (*Oncorhynchus mykiss*) were obtained as eyed embryos from a commercial supplier in Red Bluff, CA, and were hatched and reared in the laboratory and they were fed a commercially available fish food daily. The fish were held on a 16-hour light/8-hour dark photoperiod for at least 14 days prior to testing. The fish were acclimated to the test temperature for at least 48 hours prior to testing and feeding was discontinued 72 hours prior to testing. Mortality of the test fish was less than 3% in the 7 days before testing.

The mean standard length and mean weight of the control fish at the end of the test were 35.8 (31-42) mm and 0.68 g, respectively.

B. **Test System:** The test was conducted in 12-L glass beakers containing 10-L of test solution. The beakers were held in a water trough set to maintain  $12.0 \pm 1^\circ\text{C}$  and fitted with glass covers. A 16-hour light/8-hour dark photoperiod was provided using cool-white fluorescent tubes.

The dilution water was obtained from the upper Saginaw Bay of Lake Huron. It was limed and flocculated with ferric chloride by the City of Midland Water Treatment Plant. The water was sand-filtered, pH-adjusted with  $\text{CO}_2$ , carbon-filtered, and UV-irradiated in the laboratory before use. The water had a pH of 7.5-7.7, an alkalinity of 46-47 mg/L as  $\text{CaCO}_3$ , a hardness of 70-73 mg/L as  $\text{CaCO}_3$ , and a conductivity of 170-185  $\mu\text{mhos/cm}$ .

C. **Dosage:** Ninety-six-hour static test. Based on a preliminary test, six nominal concentrations (3.8, 6.5, 11, 18, 30, and 50 mg/L) and a dilution water control were used. The concentrations made were based on the total product (tested as a formulation).

- D. **Design:** Five rainbow trout were impartially distributed to each beaker; two beakers per concentration. The biomass loading rate in the control was 0.34 g/L. The test solutions were prepared by combining an appropriate amount of the test material with test dilution water to a final volume of 2 L. After thorough mixing, the solution was added to the test beakers containing 8 L of dilution water and the fish. The test solutions were lightly aerated during the test.

Observations of mortality and sublethal responses were made every 24 hours. Dead fish were removed. The temperature, dissolved oxygen (D.O.), and pH were measured in all test beakers containing live fish daily. The temperature was also measured continuously in one representative vessel.

The concentrations of chlorpyrifos in the solutions after 0 and 96 hours were measured using gas chromatography .

- E. **Statistics:** The 96-hour median lethal concentration ( $LC_{50}$ ) and associated 95% confidence interval (C.I.) was calculated using a computer program developed by Stephan et al.

12. **REPORTED RESULTS:** "The results of the analysis of chlorpyrifos can be found in Table 5 (attached). The slightly lower than expected analyzed concentrations (compared to nominal XRM-5160 concentrations) found at hour 0 indicate that the microencapsulated material may have coated out on the aquaria (or that some of the chlorpyrifos that leached from the capsules may have coated out on the glass) due to insolubility. Results of the 96 hour analysis show that the analyzed concentrations of chlorpyrifos greatly decreased from the hour 0 sampling." Measured concentrations were adjusted for the percent recovery (78.3%) of chlorpyrifos from the laboratory water.

The responses of rainbow trout are given in Table 7 (attached). The 96-hour  $LC_{50}$  value (based on nominal concentrations) was 13.4 mg/L (95% C.I. = 10.8-16.6 mg/L). Sublethal effects were noted in all concentrations. The fish in the 3.8 and 6.5 mg/L concentrations recovered by the end of the test. The no-observed-effect concentration (NOEC) was given as 6.5 mg/L.

Dissolved oxygen ranged from 7.5 to 10.0 mg/L or 69 to 93% of saturation. The pH values ranged from 7.0 to 7.7. The temperature was reported as 12.1°-12.7°C throughout the test.

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

The authors stated that XRM-5160 can be characterized as slightly toxic to rainbow trout.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

**A. Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated as follows:

*Not recommended*

The test was conducted using a formulated product. The technical grade is required for testing.

An inert or carrier ingredient control was not included in the test design.

The test solutions were aerated during the test. However, it is acceptable since the test concentrations were measured during the test.

The length of the acclimation period to the temperature was given as at least 48 hours. An acclimation period of at least two weeks is recommended.

The report did not state whether the recommended 15-30 minute transition period between light and dark was used.

The alkalinity, hardness and conductivity of the test solutions were not measured during the test.

The results of the continuous temperature monitoring were not given in the test.

The age of the test fish was not given in the report.

**B. Statistical Analysis:** The reviewer used EPA's Toxanal program and mean measured concentrations of the

formulated product to calculate the LC<sub>50</sub> values and obtained a 96-h LC<sub>50</sub> of 8.7 (95% C.I. = 6.8-10.1); see attached printout. The slope of the probit line was 14.1.

- C. **Discussion/Results:** The test material is a microencapsulation which apparently slowly leaches the active ingredient, chlorpyrifos, which may then adhere to the glass test vessels. A static system is probably not adequate for testing this formulation. According to the authors, "microcapsules settled to the bottom of the test vessels" and the concentration of chlorpyrifos sampled at 96 hours "greatly decreased from the hour 0 sampling." Using a flow-through system may improve the consistency of the analytical results (i.e. more constant concentrations) but may still underestimate the toxicity of the test material because of the slow leach rate of the toxic component, chlorpyrifos.

This study is not scientifically sound. The physical properties of the formulation (microencapsulation) caused the analysis for chlorpyrifos to be highly variable and suggests that the actual concentrations of chlorpyrifos the fish were exposed to are unknown. Under the conditions of the test, the 96-hour LC<sub>50</sub> of XRM-5160 for rainbow trout was 8.7 mg/L mean measured concentration of formulated product. Sublethal effects were noted at all concentrations, therefore an NOEC was not generated in this test.

- D. **Adequacy of the Study:**

- (1) **Classification:** Invalid.
- (2) **Rationale:** The actual concentrations of chlorpyrifos the fish were exposed to are unknown.
- (3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 07-19-91.

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Page \_\_\_\_\_ is not included in this copy.

Pages 6 through 7 are not included.

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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.

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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.

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LOU RIFICI CHLORPYRIFOS ONCORHYNCHUS MYKISS 7-19-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
35	10	10	100	9.765625E-02
16	10	10	100	9.765625E-02
10	10	8	80	5.46875
8	10	3	30	17.1875
3	10	0	0	9.765625E-02
1	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 3 AND 16 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.730762

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.7449965	7.669243	5.230829	9.779569

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
12	.7446208	1	.9999998

SLOPE = 14.13449  
 95 PERCENT CONFIDENCE LIMITS = 1.937639 AND 26.33134

LC50 = 8.715402  
 95 PERCENT CONFIDENCE LIMITS = 6.758851 AND 10.13496 *2.2 ppm ai* (1.73φ - 2.59φ)

LC10 = 7.086573  
 95 PERCENT CONFIDENCE LIMITS = 1.682131 AND 8.056878

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Shaughnessey # 041701

Chemical Name Chlorpyrifos

Chemical Class \_\_\_\_\_

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Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
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48-Hour EC<sub>50</sub>

EC<sub>50</sub> - pp 95% C.L. ( ) Control Mortality (%) -  
 Solvent Control Mortality (%) -

Species:

Slope - # Animals/Level - Temperature -

Lab:

48-Hour Dose Level pp / (% Effect)  
 ( ), ( ), ( ), ( ), ( )

MRID #

Comments:

96-Hour LC<sub>50</sub>

25.6 LC<sub>50</sub> - 8.7 \* 95% C.L. ppm ( 6.8 - 10.1 ) Control Mortality (%) - 0  
 Solvent Control Mortality (%) - N/A

Species:

Oncorhynchus mykiss Slope - 7.1 <sup>LR 7/11/91</sup> # Animals/Level - 10 Temperature - 12°C  
 14.1

Lab: Environ. tox. + Chem.  
Dow Chemical Co.

96-Hour Dose Level pp<sup>\*</sup> / (% Mortality)  
 1 (6), 3 (6), 8 (30), 10 (80), 16 (100)

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Comments:

35 (100)

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\* based on measured concentrations of the formulated product