

12-24-90 *Mr. Rhodes*
128850

MRID No. 413961-08

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Glufosinate.
Shaughnessey No. 128850.
- 2. **TEST MATERIAL:** HOE 039866 200 g/L Soluble Concentrate; Code #HOE 039866 OH SL18 A507; 18.5% active ingredient; a liquid.
- 3. **STUDY TYPE:** Estuarine Fish Toxicity Test.
Species Tested: Sheepshead minnow (Cyprinodon variegatus).
- 4. **CITATION:** Ward, G.S. 1986. Acute Toxicity of HOE 039866 200 g/L Soluble Concentrate (Code: HOE 039866 OH SL18 A507) to the Sheepshead minnow (Cyprinodon variegatus). Prepared by Hunter/ESE, Gainesville, Florida. ESE Project No. 86-341. Report No. A34014. Submitted by Hoechst Celanese Corporation, Somerville, New Jersey. MRID No. 413961-08.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Kimberly D. Rhodes*
Date: *June 1, 1990*

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *6/1/90*

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

Signature: *H.T. Craven*
Date: *12/20/90*
M. Rhodes 12/24/90

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a 96-hour static acute toxicity test for estuarine fish. The 96-hour LC50 value for Cyprinodon variegatus exposed to HOE 039866 200 g/L soluble concentrate was 13.1 mg/L, based on nominal concentrations of whole test material. Therefore, HOE 039866 200 g/L soluble concentrate is classified as slightly toxic to sheepshead minnows. The NOEC was determined to be 7.0 mg/L nominal concentration of whole test material after 96 hours of exposure.

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 hatched and reared at the testing facility. Adult fish from which eggs were obtained were collected from the Gulf of Mexico near Pensacola, Florida. During the 96-hour period before the test, the salinity of the water was 20 parts per thousand (ppt) and the temperature was 22-23°C. Mortality was 5 percent during the 48-hour holding period immediate before the test and was 0 percent during the 24-hour period preceding the test. No diseases were observed and no disease treatments were performed during holding. During holding, fish were fed live brine shrimp nauplii daily. Fish were approximately 2 to 3 weeks old at test initiation. Test fish were 11 ± 1 millimeters (mm) standard length and 28.0 ± 4.8 milligrams (mg) wet weight. Loading density was 0.28 g/L.

B. Test System: The static test was conducted in 1.6-L glass test containers, each of which received a final volume of 1.0 L of test solution or control seawater at a depth of approximately 4 centimeters (cm). All test concentrations and the control were duplicated. The test was conducted at 21°C under fluorescent lighting on a 14-hour light and 10-hour dark photoperiod. No aeration was provided during the test.

The saltwater used for culture and testing of the sheepshead minnows was filtered (5- μ m) natural seawater. The dilution water was collected at Marineland, Florida, and diluted to a salinity of 20 ppt with well water. Prior to addition to the test containers, seawater was sterilized by ultraviolet light. At test initiation, the dilution water control was characterized as having a salinity of 20 ppt, a dissolved oxygen concentration of 7.8 mg/L, and a pH of 8.1.

C. Dosage: 96-hour static acute test.

D. Design: Based on the results of a 96-hour range-finding test, a control, and five nominal HOE 039866 200 g/L soluble concentrate concentrations of 7.00, 11.7, 19.4, 32.4, and 54.0 mg/L (as whole material)

were selected for the definitive test. Ten sheepshead minnow were added to each test concentration and control within 30 minutes following addition of test material. All concentrations were observed once every 24 hours for mortality and abnormal effects.

The dissolved oxygen concentration and pH were measured and recorded at each 24-hour interval in all concentrations and the control throughout the exposure. The salinity was measured in the seawater control test container at test initiation. The temperature was measured and recorded in the seawater control at each 24-hour interval during the exposure period.

E. **Statistics:** The concentration of test substance lethal to 50 percent of the test population (LC50) was determined by the computer program developed by Stephan (1982).

12. **REPORTED RESULTS:** The nominal test concentrations with the corresponding mortality rates during each 24-hour interval are shown in Table 3-2 (attached). HOE 039866 200 g/L soluble concentrate was acutely toxic to sheepshead minnows at test concentrations ≥ 11.7 mg/L. After 96 hours of exposure, mortality ranged from 0 percent in 7.0 mg/L to 100 percent in test concentrations ≥ 19.4 mg/L. There was no control mortality. The 96-hour LC50 was 13.5 mg/L with 95 percent confidence limits of 7.0 to 19.4 mg/L. The no-observed-effect concentration (NOEC) was 7.0 mg/L.

Water quality parameters remained within acceptable limits throughout the test. Dissolved oxygen concentrations remained ≥ 6.0 mg/L (>79% of saturation) and pH ranged from 8.0 to 8.3 in all test containers throughout the test. Test salinity was 20 ppt and temperature was 21°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards.

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 from the SEP as follows:

o The SEP states that the fish must be randomly assigned to the test containers. This toxicity report did not state whether the fish were randomly distributed among the test containers. However, the ESE test protocol attached as Appendix C to the report states that the fish would be randomly assigned to the test containers.

o The SEP states that individual fish should weigh between 0.5 and 5 grams. The fish used in this study weighed between 0.019 and 0.032 grams.

o The SEP states that organisms must not be used if more than 3% die during the 48 hours immediately prior to testing. During this toxicity test, mortality was 5% during the 48-hour holding period immediately before the test.

o The SEP states that natural or reconstituted seawater of 10 to 17 ppt salinity should be used when testing estuarine (euryhaline) fish species. The natural seawater used during the toxicity study had a salinity of 20 ppt.

o The SEP states that temperature should be recorded every six hours in at least one test vessel during the entire study period if the temperature is controlled by a water bath. During the study, the test temperature was measured and recorded every 24 hours.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The photoperiod during this toxicity test was 14 hours of light and 10 hours of darkness. The report did not state whether 15- to 30-minute transition periods between light and dark were maintained.

o There is a discrepancy in the report involving the percent saturation of the dissolved oxygen concentration. The author determined the percent saturation for the lowest dissolved oxygen concentration (6.0 mg/L) to be 79 percent. However, the reviewer determined the percent saturation for the lowest dissolved oxygen concentration to be 67 percent at 20 ppt and 21°C.

B. Statistical Analysis: The reviewer used the EEB's Toxanal computer program to calculate the 96-hour LC50 value and 95 percent confidence interval. These

calculations are attached. The binomial test provided a 96-hour LC50 value of 13.1 mg/L nominal concentration with a 95 percent confidence interval of 7.0 to 19.4 mg/L which is similar as that reported by the author (i.e., 13.5 mg/L with a 95% confidence interval of 7.0 to 19.4 mg/L). The NOEC was determined to be 7.0 mg/L nominal concentration as whole material.

- C. **Discussion/Results:** This study appears to be scientifically valid. Although the mortality of the culture fish population 48-hours prior to test initiation exceeded the 3 percent criterion, there was no mortality during the 24-hour period prior to test initiation and the mortality of all remaining fish from this population was 0 percent during the 6 days following test initiation. These data, along with the 100 percent control survival in the test, confirm that the culture fish population were in good health and this deviation did not affect the test results. The 96-hour LC50 value based upon nominal concentrations of HOE 039866 200 g/L soluble concentrate was determined to be 13.1 mg/L as whole material. Therefore, HOE 039866 200 g/L soluble concentrate as whole material is classified as slightly toxic to sheepshead minnows (Cyprinodon variegatus). The NOEC as whole material was determined to be 7.0 mg/L nominal concentration after 96 hours of exposure.

D. **Adequacy of the Study:**

- (1) **Classification:** Core.
- (2) **Rationale:** Although the test procedures deviated from the guidelines, the deviations probably did not significantly affect the toxicity results.
- (3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 05-18-90.

16. **REFERENCES:**

Stephan, C.E. 1982. Methods for Calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota.

Shaughnessy No. 128850
Study/Species/Lab/
Accession _____

Chemical Name Glyphosate Chemical class _____ Page _____ of _____
(HOE-039866a 200 g/L Soluble Concentrate)

14-Day Single Dose Oral LD50
Species _____
Lab _____
Acc. _____

Results
LD50 = . mg/kg (95% C.L.) Contr. Mort. (%) = _____
Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____
14-Day Dose Level mg/kg/(% Mortality)
(, , , ,)
Comments: _____

14-Day Single Dose Oral LD50
Species _____
Lab _____
Acc. _____

LD50 = mg/kg. (95% C.L.) Contr. Mort. (%) = _____
Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____
14-Day Dose Level mg/kg/(% Mortality)
(, , , ,)
Comments: _____

8-Day Dietary LC50
Species _____
Lab _____
Acc. _____

LC50 = ppm (95% C.L.) Contr. Mort. (%) = _____
Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____
8-Day Dose Level ppm/(% Mortality)
(, , , ,)
Comments: _____

8-Day Dietary LC50
Species _____
Lab _____
Acc. _____

LC50 = ppm (95% C.L.) Contr. Mort. (%) = _____
Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____
8-Day Dose Level ppm/(% Mortality)
(, , , ,)
Comments: _____

48-Hour LC50
Species _____
Lab _____
Acc. _____

LC50 = pp (95% C.L.) Contr. Mort. (%) = _____
Slope = # Animals/Level = _____ Sol. Contr. Mort. (%) = _____
Temperature = _____
48-Hour Dose Level pp/(% Mortality)
(, , , ,)
Comments: _____

96-Hour LC50
Species Cyprinodon variegatus
Lab Hunter/ESE
Acc. 413961-08

LC50 = 13.1 PP100 (95% C.L.)
Slope = N/A # Animals/Level = 10
Con. Mort. (%) = 0
Sol. Con. Mort. (%) = N/A
Temp. = 21°C
96-Hour Dose Level ppm/(% Mortality)
7.00 (0), 11.7 (30), 19.4 (100), 32.4 (725), 54.0 (100)
Comments: Based on nominal concentrations of the whole material.

96-Hour LC50
Species _____
Lab _____
Acc. _____

LC50 = PP (95% C.L.)
Slope = # Animals/Level = _____
Con. Mort. (%) = _____
Sol. Con. Mort. (%) = _____
Temp. = _____
96-Hour Dose Level pp/(% Mortality)
(, , , ,)
Comments: _____

K.R.
5/18/90 Core

KIMBERLY RHODES HOE 039866 200 G/L SOLUBLE CONCENTRATE
CYPRINODON VARIEGATUS 05-17-90

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
54	10	10	100	9.765625E-02
32.4	10	10	100	9.765625E-02
19.4	10	10	100	9.765625E-02
11.7	10	3	30	17.1875
7	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT (7 AND 19.4) 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 13.13163

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

Table 3-2. Mortality of Sheepshead Minnows (Cyprinodon variegatus)
Exposed to HOE 039866 200 g/L Soluble Concentrate

Nominal Concentration (mg/L; ppm)	Cumulative Mortality (%)			
	24 hr	48 hr	72 hr	96 hr
Control	0	0	0	0
7.00	0	0	0	0
11.7	30	30	30	30
19.4	70	100	100	100
32.4	100	100	100	100
54.0	100	100	100	100

Source: ESE, 1986.