5-17-89

Accession No. 407009-12

DATA EVALUATION RECORD

1. **CHEMICAL:** Ethyltrianol
Shaughnessey Number: 128997

2. **TEST MATERIAL:** HWG-1608 Technical; Lot No. 86R0082I; 96.28% active ingredient; an off-white powder

3. **STUDY TYPE:** Acute Toxicity Test for Freshwater Fish.
Species Tested: Lepomis macrochirus

4. **CITATION:** Surprenant, D. C. 1987. Acute Toxicity of HWG-1608 (Technical Grade) to Bluegill (Lepomis macrochirus) under Flow-Through Conditions: Bionomics Report #87-6-2409: Bionomics Study #274.1186.6127.105. Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. Submitted by Mobay Corporation, Stilwell, Kansas. Accession No. 407009-12.

5. **REVIEWED BY:**

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

Signature: Kimberly D. Rhodes
Date: April 5, 1989

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 4/5/89

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

Signature:
Date: 5/17/89
H.T. Craven 5/17/89

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for a 96-hour acute flow-through toxicity study for a warmwater fish species. The 96-hour LC50, based upon mean measured concentrations, of HWG-1608 to bluegill sunfish (Lepomis macrochirus) was 5.7 mg a.i./L. Therefore, HWG-1608 is considered moderately toxic to bluegill sunfish. The NOEC was < 1.4 mg/L. A more precise NOEC could not be determined due to the adverse effects observed at all test levels.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bluegill sunfish (Lepomis macrochirus) used in this test were obtained from a commercial fish supplier in Nebraska. The fish were held in a 500-L fiberglass tank containing well water for a minimum of 14 days at 20 - 21°C. The fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing and the exposure period. There was no mortality in the test fish population during the two days prior to testing. The bluegill sunfish used for this experiment had a mean wet weight of 0.434 gram (g) with a range of 0.17 to 0.75 g and a mean standard length of 32 millimeters (mm) with a range of 24 to 40 mm. The resulting test organism loading was 0.29 g of biomass per liter of test solution.

B. **Test System:** The test was conducted using an exposure system consisting of a continuous flow serial diluter (Benoit et al., 1982), a temperature controlled water bath, and a set of 14 test aquaria. The dilution water was from the same source as the water which flowed into the fish holding tank and was characterized as having a total hardness of 30 mg/L as $CaCO_3$, a total alkalinity of 34 mg/L as $CaCO_3$, a pH of 7.1, and a specific conductance range of 120 umhos/cm during the study period.

The test system was designed to provide five concentrations of test material with a 0.65 dilution factor, a dilution water control and solvent control. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a 19.5-cm high standpipe which maintained constant test water volume of 15 L. The diluter provided approximately 6.5 volume additions per aquarium every 24 hours. The aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test water temperature at 22 $\pm$ 1°C. A photoperiod of 16 hours of light and 8 hours of darkness was provided each day.

C. **Dosage:** 96-hour acute flow-through test.

D. **Design:** The test was initiated when 10 bluegill were impartially distributed to each aquarium. A control, solvent control, and five nominal HWG-1608 concentrations of 1.3, 2.1, 3.2, 4.9, and 7.5 mg a.i./L were tested. The solvent control solution contained the maximum amount of acetone present in any test concentration (324 uL/L). All concentrations were observed at 24, 48, 72, and 96 hours for mortality and abnormal effects. The water quality parameters (dissolved oxygen, pH, and temperature) were measured in both replicates of the controls and all test concentrations at 0, 24, 48, 72, and 96 hours of testing. Analytical determination of HWG-1608 was performed on all test solutions, control and solvent control at 0, 48, and 96 hours using high pressure liquid chromatography (HPLC).

E. **Statistics:** The concentration of test substance lethal to 50 percent of the test population (LC50) was determined by the computerized calculation program (Stephan et al., 1978). LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities.

12. **REPORTED RESULTS:** The mean measured test concentrations, the corresponding mortalities and the physical and biological observations made during the 96-hour test are presented in Table 2 (attached). The mean measured concentrations of HWG-1608 (Technical grade) in exposure solutions during the 96-hour definitive test were 1.4, 1.9, 2.9, 4.2, and 6.4 mg/L. The mean measured concentrations of HWG-1608 (Technical grade) ranged from 90 to 108% of the nominal concentrations. The 96-hour LC50 for bluegill exposed to HWG-1608 (Technical grade) was calculated by binomial probability to be 5.7 mg/L with a 95% confidence interval of 4.2 to 6.4 mg/L. Based on the results of this study, the no-observed-effect concentration for bluegill and HWG-1608 (Technical grade) was determined to be < 1.4 mg/L, the lowest concentration tested. The water quality parameters measured during this study remained within acceptable ranges for the survival of the bluegill and were unaffected by the concentrations of HWG-1608 (Technical grade) tested.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on the results of this study, the 24-, 48-, 72-, and 96-hour LC50 values for bluegill exposed to HWG-1608 (Technical grade) were calculated to be > 6.4, > 6.4, 6.1, and 5.7 mg a.i./L based on mean measured concentrations. The no-observed-effect concentration for bluegill and HWG-1608 (Technical grade) was determined to be < 1.4 mg/L, the lowest concentration tested.

A GLP compliance statement was included in the report and the study was audited by a QA unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards: Pesticide Programs (40 CFR 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 from the SEP as follows:

o The SEP states that the concentration of solvent is not to exceed 0.10 mL/L under flow-through conditions. During this test, a solvent concentration of 0.324 mL/L was used.

o The SEP states that individual fish should weigh between 0.5 and 5 grams. The fish used in this study weighed between 0.17 and 0.75 grams.

o The SEP states that use of a natural dilution water with a hardness of 40 to 48 mg/L as $CaCO_3$ can be used in lieu of reconstituted water. The dilution water used for the toxicity test was well water with a total hardness of 30 mg/L as $CaCO_3$.

B. **Statistical Analysis:** The concentration of test substance lethal to 50 percent of the test population (LC50) was determined by the EPA's Toxanal Computer program. The 96-hour LC50 value was estimated by the binomial method to be 5.7 mg a.i./L with a 95 percent confidence interval of 4.2 to 6.4 mg a.i./L.

C. **Discussion/Results:** The study results appear to be scientifically valid. The concentration of solvent used in this test (0.324 mL/L) exceeded the 0.1 mL/L limit for a flow-through test. However, the test is acceptable since no mortality was observed in the solvent control.

The 96-hour LC50 value, based upon mean measured concentrations, was estimated to be 5.7 mg a.i./L. Therefore, HWG-1608 is considered moderately toxic to bluegill sunfish (Lepomis macrochirus).

D. **Adequacy of the Study:**

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER:** Yes, 03-31-89

(HWG-1608)

Shaughnessey No. 128997 Chemical Name Ethyltrianol Chemical Class ____ Page ____ of ____

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validat Status
14-Day Single Dose Oral LD_{50} 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:		
14-Day Single Dose Oral LD_{50} 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 LC_{50} 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 LC_{50} 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 LC_{50} Species Lab ____ Acc.		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)= Slope= # Animals/Level= Temperature = 48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) Comments:		
96-Hour LC_{50} Species Lepomis macrochirus Lab Springborn Life Sciences 96.28% Acc. 407009-12		LC50 = 5.7 ppm (95% C.L. 4.2 - 6.4) (Binomial method) Con. Mor(%)= 0 Sol. Con. Mor.(%)= 0 Slope= N/A # Animals/Level= 20 Temp.= 22°C 96-Hour Dose Level ppm/(%Mortality) 1.4 (0), 1.9 (0), 2.9 (10), 4.2 (0), 6.4 (75) Comments: Based on mean measured concentrations	K.R 05/31/89 Core	
96-Hour LC_{50} Species Lab ____ Acc.		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)= Slope= # Animals/Level= Temp.= 96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) Comments:		

TEBUCONAZOLE

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KIMBERLY RHODES ETHYLTRIANOL LEPOMIS MACROCHIRUS 03-31-89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
6.4	20	15	75	2.069473
4.2	20	0	0	9.536742E-05
2.9	20	2	10	2.012253E-02
1.9	20	0	0	9.536742E-05
1.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 4.2 AND 6.4 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 5.714383

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.01652	3.523178	1.429653E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 6.904687

95 PERCENT CONFIDENCE LIMITS =-2.900261 AND 16.70963

LC50 = 5.610905

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3.673669

95 PERCENT CONFIDENCE LIMITS = 0 AND 5.967355

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KIMBERLY RHODES ETHYLTRIANOL SALMO GAIRDNERI 03-31-89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
6.1	20	16	80	.5908966
3.9	20	8	40	25.17223
2.5	20	1	5	2.002716E-03
1.5	20	0	0	9.536742E-05
1.1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.5 AND 6.1 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 4.340928

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1382494	4.35589	3.771038	5.212023

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1633509	1	.9809506

SLOPE = 6.371681
95 PERCENT CONFIDENCE LIMITS = 3.796459 AND 8.946903

LC50 = 4.404876
95 PERCENT CONFIDENCE LIMITS = 3.827715 AND 5.186866

LC10 = 2.783648
95 PERCENT CONFIDENCE LIMITS = 2.01665 AND 3.286574

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