5-17-89

Accession No. 407009-15

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** HWG-1608 Technical; 96.28% active ingredient; an off-white powder

3. **STUDY TYPE:** Freshwater invertebrate chronic test.
   Species Tested: Cladoceran (Daphnia magna)

4. **CITATION:** Burgess, D. 1988. Chronic Toxicity of HWG-1608 Technical to Daphnia magna under Flow-Through Test Conditions. Prepared by Analytical Biochemistry Laboratories, Inc., Columbia, Missouri. Submitted by Mobay Corporation, Stilwell, Kansas. Accession Number 407009-15.

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
   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: 5/17/89
   Date: Henry T. Craven 5/17/89

7. **CONCLUSIONS:** The data submitted appears scientifically sound ~~but~~ does ~~not~~ fulfill the Guideline requirements for a freshwater invertebrate chronic test. The MATC for Daphnia magna exposed to HWG-1608 Technical was > 0.12 mg/L < 0.23 mg/L mean measured concentration. The NOEC was 0.12 mg/L mean measured concentration.

   Core 12/28/89

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** All daphnids were cultured and tested in a temperature controlled area at 20 ± 2°C. The lighting was 40-80 footcandles on a 16-hour daylight photoperiod, 8-hour darkness and 30-minute transition periods. During the holding period, the daphnids were fed a suspension of algae (Selenastrum capricornutum) supplemented with a Tetramin/cereal leaves/yeast suspension. Only first-instar daphnids (<24 hours old) were selected for testing.

B. **Test System:** The test was conducted in a half-liter proportional diluter system described by Mount and Brungs (1967), utilizing a Hamilton Micro Lab 420 syringe dispenser. A dilution factor of 50 percent was used. The diluter delivered five concentrations of HWG-1608, a dilution water control and a solvent control to four replicate one-liter test aquaria. The diluter provided for approximately 5.8 volume replacements per 24-hour period. A photoperiod of 16 hours of light and 8 hours of darkness with a 30-minute transition period was provided. Test temperature was maintained at 20 ± 2°C by a temperature controlled water bath.

The water parameters of the well water used as dilution water were, total hardness: 206-275 mg/L as $CaCO_3$; total alkalinity: 224-336 mg/L as $CaCO_3$; pH: 7.6 to 8.4; dissolved oxygen: 7.4 - 9.1 mg/L; and conductivity: 500-650 umhos/cm.

C. **Dosage:** 21-day flow-through life-cycle test.

D. **Design:** Forty D. magna (≤ 24 hours old) were impartially distributed to each test concentration (10 per replicate) to initiate the test. A control and nominal HWG-1608 Technical concentrations of 0.024, 0.048, 0.10, 0.20, and 0.40 mg a.i./L were tested. The test daphnids were uniformly fed an equal volume per test chamber of an algal suspension (Selenastrum capricornutum) three times daily providing approximately 2-4 x 10^8 cells total to each replicate on each day of the study. Daphnids were also supplemented once daily with 0.2 mL per test chamber of Tetramin, cereal leaves, vitamins and yeast mixture. Survival and abnormal

effects of first brood of the organisms were recorded daily throughout the study. Reproduction success was measured by counting and discarding the offspring produced in each concentration every Monday, Wednesday, and Friday for the duration of the study.

Exposure concentrations of HWG-1608 Technical were analytically measured on days -6, 0, 4, 7, 14, and 21 through the use of HPLC. The mean measured test concentrations reported were 0.042, 0.069, 0.12, 0.23 and 0.51 mg a.i./L. Water quality parameter of dissolved oxygen and pH were measured on days 0, 4, 7, 14, and 21 in alternating, duplicate replicates of the control, solvent control, low, middle, and high test concentrations. Temperature measurements of the water bath were made daily with a mercury thermometer and were recorded continuously with a computerized data logger.

E. **Statistics**: The selected parameters of survival, adult length (pooled) and total young/adult/reproduction day were analyzed using a one-way analysis of variance. When treatment effects were indicated following a significant F-test of the mean square ratios a multiple means comparison test, Dunnett's Test, was used to determine which exposure levels differed from the control value.

Percent survival data were transformed for analysis. All differences were considered significant at the 95% confidence level. Total young/adult/reproduction day for each replicate was calculated by dividing the total number of young produced by the total number of adult reproduction days. The number of reproduction days (normally 13-15) were counted from the day instars were first observed, which for this study ranged from Day 9 to Day 12. Adult reproduction days were calculated for each change in survival in order to be corrected for mortality. Total number of adult reproduction days for each replicate was the sum of each adult reproduction day for each change in survival. The 21-day EC50 was calculated by employing a computerized EC50 program developed by Stephan et al (1978).

12. **REPORTED RESULTS**: Statistical analysis of survival for Daphnia magna after a 21-day exposure to HWG-1608 Technical indicated that daphnid survival rates in the mean measured test concentration of 0.51 mg/L were significantly different ($P \leq 0.05$) from the pooled controls (Table 4, attached). A

21-day EC50, based on adverse effects was calculated to be 0.33 mg/L.

The mean young/adult/reproduction day after 21 days were significantly affected in the mean measured exposure levels of 0.23 and 0.51 mg/L of HWG-1608 Technical (Table 4, attached).

A one-way analysis and Dunnett's multiple means test indicated that the daphnid lengths in the HWG-1608 Technical mean measured concentrations of 0.23 and 0.51 mg/L were significantly different from the pooled controls (Table 4, attached).

Based on the statistical analyses of adult mean length, survival and young/adult/reproduction day from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be the HWG-1608 Technical mean measured concentrations of >0.12 and <0.23 mg/L.

The mean measured concentrations of HWG-1608 Technical during the 21-day exposure were 0.042, 0.069, 0.12, 0.23, and 0.51 mg a.i./L. The mean measured concentrations ranged from 115% to 175% of expected nominal concentrations. The stock solution was 119% of the expected nominal concentration. The dissolved oxygen concentration ranged from 8.1 to 9.0 mg/L, representing 93 and 103% saturation at 20°C. The water quality parameters were considered adequate for testing.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Based on the statistical analysis of adult mean length, survival and young/adult/reproduction day from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be the HWG-1608 Technical mean measured concentrations of >0.12 and <0.23 mg/L. A 21-day EC50, based on adverse effects was calculated to be 0.33 mg/L.

A GLP compliance statement was included in the report by the author 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:** At the present time, there is no SEP for Daphnia magna flow-through chronic test. Therefore, the ASTM Guidelines were used for this data validation. The report deviated from the ASTM as follows:

o The ASTM states that hardness, alkalinity, and conductivity in the control, low, medium and high concentration test solutions must be measured weekly during the test. This test did not measure these water quality parameters.

o The ASTM states that measured concentration of test material in any chamber should be no more than 30% higher or lower than the nominal concentration. If the concentration of the test chamber is too high, the stock solution of test solution may have been prepared incorrectly. All measured concentrations on day 21 were consistently high, ranging from 141% to 317% of the nominal concentrations (Table 2, attached), which might indicate an incorrect stock solution.

The toxicity report did not provide the following information required by the ASTM:

o A report of the results of a test must include data on the survival of first generation daphnids, production of young by first generation daphnids at various times for each treatment, and the lengths of first generation daphnids (i.e., individual measurements) at the end of the test. The above raw data were not presented by the author.

B. **Statistical Analysis:** Validation of the survival, reproduction, and growth data could not be conducted because of the lack of raw data.

C. **Discussion/Results:** The data submitted does not fulfill the Guideline requirements for a Daphnia magna flow-through chronic toxicity test since the lack of data on the survival, reproduction, and growth of the first generation daphnids at test termination prevents the validation of statistical analyses. In addition, it is not clear whether the duplicate water samples collected from each treatment were collected from the splitter boxes or from individual test aquaria. The sample collection procedure should be clarified. Based on the

author's statistical analyses, the MATC of HWG-1608 Technical for Daphnia magna was > 0.12 mg/L < 0.23 mg/L mean measured concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Supplemental~~ core 12/30/89

(2) **Rationale:** Lack of ~~raw~~ data on survival, reproduction, and ~~growth~~ of first generation daphnids ~~prevent~~ validation of statistical analysis. was submitted

(3) **Repairability:** Yes, ~~submit~~ the above raw data.

15. **COMPLETION OF ONE-LINER:** Yes, 03-30-89.

Page ____ is not included in this copy.

Pages 7 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.

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(HWG-1608 Technical)

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

Study/Species/Lab/ Succession	Chemical % Active	Results					Reviewer/ Date	Valid: Stati
Avian Reproduction, Species:		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	1Ch Inh.		
		Control						
Lab:		Treatment I						
		Treatment II						
Acc		Treatment III						
		Study Duration:						
		Comments:						

Field Study(Simulated/Actual) Species:		Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
		Control						
Lab:		Treatment I						
		Treatment II						
Acc.		Treatment III						
		Crop/Site:		Study Duration:				
		Comments:						

Chronic fish, Species		Concentrations Tested (ppm)=			
Lab:		MATC = >____ <____ pp__.	Effected Parameter =		
Acc.		Contr. Mort.(%)=	Sol. Contr. Mort.(%)=		
		Comments:			

Chronic invertebrate		Concentrations Tested (ppm)= 0.042, 0.069, 0.12, 0.23, 0.51			
Species Daphnia magna 96.28%		MATC =>0.12 < 0.23 ppm.	Effected Parameter(s) Survival, Reproduction + Length	K.R. 3/30/89	Supvl.
Lab Analytical Biochemistry Laboratories		Contr. Mort.(%)= 0	Sol. Contr. Mort.(%)= 0		
Acc. 407009-15		Comments: Based on mean measured concentrations.			

12/20/89