US EPA ARCHIVE DOCUMENT

UNDATED

This was a KBN study that was changed. The original study is followed by an explanat the first and last pages of the changed study.

DATA EVALUATION RECORD

1. **CHEMICAL:** Molinate.
Shaughnessey No. 041402.

2. **TEST MATERIAL:** 1. Non-radiolabelled Ordram Technical; Lot No. WRC 4921-8-9; 97.5 ingredient; an amber liquid;
2. ^{14}C-Ordram; Lot No. WRC 6334-46-5.

3. **STUDY TYPE:** Daphnid Flow-Through Life-Cycle Chronic Toxicity Test. Species Test Daphnia magna.

4. **CITATION:** Forbis, A.D. 1987. Chronic Toxicity of ^{14}C-Ordram to Daphnia magna U Flow-Through Test Conditions. Final Report No. 35222. Prepared by Analytical Laboratories, Inc., Columbia, MO. Submitted by Stauffer Chemical Company, Ric EPA MRID No. 406578-02.

5. **REVIEWED BY:**

Louis M. Rifici, M.S. **Signature:**
Associate Scientist
KBN Engineering and **Date:**
Applied Sciences, Inc.

6. **APPROVED BY:**

Pim Kosalwat, Ph.D. **Signature:**
Senior Scientist
KBN Engineering and **Date:**
Applied Sciences, Inc.

Henry T. Craven, M.S. **Signature:**
Supervisor, EEB/HED
USEPA **Date:**

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirem chronic, flow-through toxicity test for the freshwater invertebrate, Daphnia mag ^{14}C-Ordram, based on the most sensitive biological parameters, daphnid repro carapace length, was >0.38 ppm and <0.90 ppm mean measured concentrations (g mean = 0.59 ppm).

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Daphnia magna (<24 hours old) were obtained from in-house cu The primary culture was obtained from the Columbia National Fisheries Laboratory in Columbia, MO. The cultures were housed in a temperature con (20°±2°C) on a 16-hour daylight photoperiod with 30 minute dawn/dusk simul light intensity was maintained at 50-70 ft-candles.

Adult daphnids were fed a suspension of algae (Selenastrum capri supplemented with a Tetramin®, cereal leaves, and yeast suspension.

B. **Test System:** The proportional diluter delivered 2.9 mL/chamber/minute (or replacements per day). Flow splitting chambers were used to mix and divi solution. To minimize turbulence, the solutions were delivered to the te 14-gauge hypodermic needles. The diluter system was calibrated before use.

The test vessels were 1-L beakers with stainless steel screen (50 mesh) co drains. The test chambers were immersed in a temperature-controlled water 20°±2°C.

The characteristics of the aged well water are given in Table 1 (attached).

A sample of non-radiolabelled Ordram Technical was mixed with a 24 mg/mL st C-Ordram and diluted to volume in acetone. The radiopurity of the stock determined by liquid scintillation counting (LSC) to be 100%. The stoc delivered to the diluter using a syringe dispenser.

C. **Dosage:** Twenty-one-day, flow-through, life-cycle chronic toxicity test. B preliminary test, five nominal concentrations (0.072, 0.12, 0.25, 0.43, dilution water control and a solvent control (0.05 mL acetone/L) were se test.

D. **Design:** Four chambers were used for each concentration with ten randomly-p daphnids per chamber. Survival was recorded on Monday, Wednesday, and Fri neonates were first observed in the chambers; survival was then assessed d were counted every Monday, Wednesday, and Friday by removing the adult and the test solution through a 50-mesh stainless steel screen. The collect placed in shallow glass vessels, counted, and discarded. The test solutio and replaced, along with the adult daphnid, back into the chamber. The t were cleaned on each counting day. At test termination, the daphnids wer measured.

The daphnids were fed 20-30 mL of an algal suspension (Selenastrum capri three times daily and 2 mL of a Tetramin®-cereal leaves-yeast suspension on

The dissolved oxygen (D.O.) and pH were measured in the dilution water c middle, and high concentration on days 0, 4, 7, 14, and 21. The temperatu bath was measured daily with a mercury thermometer and continuously wi logger. The above parameters and conductivity, hardness, and alkalinity water were measured weekly.

^{14}C-Ordram Technical concentrations were measured by liquid scintillation c samples taken on days 0, 4, 7, 14, and 21.

E. **Statistics:** Daphnid survival, growth (length), and reproduction (young/adult/reproduction day) were analyzed using analysis of (ANOVA) and Dunnett's test. The proportional survival data were arc root transformed. The control and solvent control data were pooled

analysis.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.065, 0.11, 0.23, 0.38, and 0.90 µg/L and averaged 88-92% of nominal. Measured concentrat fairly consistent between sampling days (Table 2, attached).

The concentration of ^{14}C-Ordram had no significant effect on daphnid surviv 21-day test (Table 3, attached). Adult daphnid lengths at 0.11 and 0.9 measured concentration) were significantly lower than that of the pooled c the length difference was statistically different it was not considered significant since two higher test concentrations were not significantly controls."

No young were observed until 7 days into the study. The number of young reproductive day at "0.38 and 0.90 ppm" were significantly affected tha controls.

Based on the analysis of survival, growth, and reproduction, the maximum toxicant concentration (MATC) limits were estimated to be 0.38 and 0.90 measured concentration).

The pH of the test solutions ranged from 8.1 to 8.4. Dissolved oxygen ran 8.7 mg/L or 76 to 95% of saturation at 20°C. The temperature of the test 20°C during the study.

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

The author presented no conclusions other than those previously mentioned.

Quality Assurance and GLP Compliance Statements were included in the indicating adherence to USEPA GLP Regulations.

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

A. **Test Procedure:** The test procedures were generally in accordance wit protocols recommended by ASTM (1985), but deviated as follows:

The conductivity, hardness, and alkalinity of the dilution water w weekly. ASTM (1985) states that these parameters must be measured control, low, medium, and high concentration test solutions weekly.

Treatments must be randomly assigned to the test chambers. The rep not mention if the treatments were randomly assigned.

B. **Statistical Analysis:** The reviewer used one-way analysis of variance Version 3.3) to analyze the survival and reproduction (average numbe produced per adult per reproductive day) of daphnids after 21 days. data were arcsine square root transformed before the analysis. The effect concentration (NOEC) for survival and reproduction were 0.9 ppm, respectively (see attached printouts 1 and 2). Adult daphnid analyzed using Crunch Version 3, the raw length data (in µm), an analysis of variance. Daphnid length at 0.11 and 0.90 ppm was s lower than the control. However, as suggested by the author, it i daphnid length at 0.11 ppm was affected by the toxicant. Therefore was 0.38 ppm (mean measured concentration; see printout 3). The control and solvent control were pooled prior to all analyses.

C. **Discussion/Results:** In the Results section of the report (page 16), states that the mean young/adult/reproduction day at 0.38 and 0.90 significantly lower than the pooled controls. While the mean at 0.3 than the controls (6.443 vs. 8.116), the statistical analysis, repo reported MATC limits given in the report suggest that there was no difference at 0.38 ppm. The reviewer believes that the author made the only statistically significant effect on reproduction was at 0.90

This study is scientifically sound and meets the guideline requir chronic, flow-through toxicity test for the freshwater invertebrate, . The MATC, based on the most sensitive biological parameters, reproduction and length, was >0.38 ppm and <0.90 ppm mean meas concentration (geometric mean = 0.59 ppm).

D. **Adequacy of the Study:**

(1) **Classification:** ~~Core.~~Supplemental

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 06-17-91.

REFERENCES: ASTM. 1985. Proposed Standard Practice for Conducting Da magna Chronic Toxicity Tests in a Flow-Through System. Draft No. 4.

September 14, 1992

MEMORANDUM

Subject: Change of a Molinate chronic *Daphnia magna* "Supplemental." D182484, S425136, MRID 406578-02.

From:James J. Goodyear, Ph.D.
Biologist, Section 1
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

To: Files.

I have evaluated KGN's review of ICI's chronic *Daphnia magna* study re
Fobis, A.D. 1987. Chronic toxicty of ^{14}C-Ordram to *Daphnia ma*
under flow-through test conditions. Final report no. 35222.
Analytical BioChemistry Laboratories, Inc., Columbia, MO. Su
ICI Americas, Inc., Agricultural Products, Wilmington, Delawar

KBN rated the study "Core." However, since the study did not measure Molinate upon the dry weights of the Daphnids, it does not meet t requirements or the ASTM protocol, which they stated they were followin measurement that they did do (length) is less reliable than that of dry

EEB has downgraded the study to "Supplemental" for growth (LOEC = and NOEC = 0.90 ppm) with no possibility of repair. It is still "Cor effects (LOEC = 0.38 ppm and NOEC = 0.90 ppm).

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DATA EVALUATION RECORD
MOLINATE TEP

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5. **REVIEWED BY:**

James J. Goodyear Signature:
Biologist, Section 1
Ecological Effects Branchate:
Environmental Fate and Effects Division (H7507C)

6. **APPROVED BY:**
Leslie W. Touart Signature:
Head, Section 1
Ecological Effects Branchate:
Environmental Fate and Effects Division (H7507C)

7. **CONCLUSIONS:** This study is scientifically sound, but it does not meet the requir Guideline 72-4(b) Aquatic invertebrate life cycle. The reproduction and length p study are "Core." Their MATCs were both >0.38 ppm and <0.90 ppm mean measured concentrations (geometric mean = 0.59 ppm). The study did not measure the effect Ordram (TEP Molinate) upon dry weight, therefore EEB considers the study to be "Supplemental." The study must be repeated and the dry weight must be measured.

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

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MRID No. 406578-02

reproduction were 0.90 and 0.38 ppm, respectively (see attached printouts 1 and 2). Ad length was analyzed using Crunch Version 3, the raw length data (in pm), and two-way an variance. Daphnid length at and 0.90 ppm was significantly lower than the control. Ho suggested by the author, it is unlikely that daphnid length at 0.11 ppm was affected by Therefore, the NOEC was 0.38 ppm (mean measured concentration; see printout 3). The ne control and solvent control were pooled prior to all analyses.

C. **Discussion/Results:** In the results section of the report (page 16), the author s mean young/adult/reproduction day at 0.38 and 0.90 ppm were significantly lower than th controls. While the mean at 0.38 ppm is lower than the controls (6.443 vs. 8.116), the report summary, and reported MATC limits given in the report suggest that there was no difference at 0.38 ppm. The reviewer believes that the author made an error and the on significant effect on reproduction was at 0.90 ppm.

This study is scientifically sound but does not meet the guideline requirements for a c toxicity test for the freshwater invertebrate, Daphnia magna. The dry weights of the d taken. The MATC, based on daphnid reproduction and and length, was >0.38 ppm and <0.90 measured concentration (geometric mean = 0.59 ppm).

D. **Adequacy of the Study:**

(1) **Classification:** supplemental

(2) **Rationale:** The dry weights of the daphnids were not measured.

(3) **Repairability:** not repairable

15. **COMPLETION OF ONE-LINER FOR STUDY:** no

REFERENCE: ASTM. 1985. Proposed Standard Practice for Conducting Daphnia magna Chronic Toxicity Tests in a Flow-Through System. Draft No. 4.

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Pim Kosalwat, Ph.D. **Signature:**
Senior Scientist
KBN Engineering and **Date:**
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8. **RECOMMENDATIONS:** N/A.