

US EPA ARCHIVE DOCUMENT

1/31/00

MRID No. 406578-02

This was a KBN study that was changed. The original study is followed by an explanatory letter and by 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% active ingredient; an amber liquid;  
2.  $^{14}\text{C}$ -Ordram; Lot No. WRC 6334-46-5.
3. **STUDY TYPE:** Daphnid Flow-Through Life-Cycle Chronic Toxicity Test. Species Tested: Daphnia magna.
4. **CITATION:** Forbis, A.D. 1987. Chronic Toxicity of  $^{14}\text{C}$ -Ordram to Daphnia magna Under Flow-Through Test Conditions. Final Report No. 35222. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Stauffer Chemical Company, Richmond, CA. EPA MRID No. 406578-02.
5. **REVIEWED BY:**  

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

Pim Kosalwat, Ph.D. Senior Scientist KBN Engineering and Applied Sciences, Inc.	Signature:  Date:
Henry T. Craven, M.S. Supervisor, EEB/HED USEPA	Signature:  Date:
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a chronic, flow-through toxicity test for the freshwater invertebrate, Daphnia magna. The MATC for  $^{14}\text{C}$ -Ordram, based on the most sensitive biological parameters, daphnid reproduction and carapace length, was  $>0.38$  ppm and  $<0.90$  ppm mean measured concentrations (geometric 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 cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in a temperature controlled area ( $20^{\circ}\pm 2^{\circ}\text{C}$ ) on a 16-hour daylight photoperiod with 30 minute dawn/dusk simulations. The light intensity was maintained at 50-70 ft-candles.

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

B. Test System: The proportional diluter delivered 2.9 mL/chamber/minute (or 4.2 volume replacements per day). Flow splitting chambers were used to mix and divide each test solution. To minimize turbulence, the solutions were delivered to the test vessels using 14-gauge hypodermic needles. The diluter system was calibrated before use.

The test vessels were 1-L beakers with stainless steel screen (50 mesh) covered notched drains. The test chambers were immersed in a temperature-controlled water bath set at  $20^{\circ}\pm 2^{\circ}\text{C}$ .

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 stock of  $^{14}\text{C}$ -Ordram and diluted to volume in acetone. The radiopurity of the stock solution was determined by liquid scintillation counting (LSC) to be 100%. The stock solution was delivered to the diluter using a syringe dispenser.

C. Dosage: Twenty-one-day, flow-through, life-cycle chronic toxicity test. Based on a preliminary test, five nominal concentrations (0.072, 0.12, 0.25, 0.43, and 1.0 ppm), a dilution water control and a solvent control (0.05 mL acetone/L) were selected for the test.

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

The daphnids were fed 20-30 mL of an algal suspension (*Selenastrum capricornutum*) three times daily and 2 mL of a Tetramin®-cereal leaves-yeast suspension once daily.

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

<sup>14</sup>C-Ordram Technical concentrations were measured by liquid scintillation counting from 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 variance (ANOVA) and Dunnett's test. The proportional survival data were arcsine square root transformed. The control and solvent control data were pooled before data 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 concentrations were fairly consistent between sampling days (Table 2, attached).

The concentration of <sup>14</sup>C-Ordram had no significant effect on daphnid survival during the 21-day test (Table 3, attached). Adult daphnid lengths at 0.11 and 0.90 ppm (mean measured concentration) were significantly lower than that of the pooled controls. "While the length difference was statistically different it was not considered to be

biologically significant since two higher test concentrations were not significantly different from the controls."

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

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

The pH of the test solutions ranged from 8.1 to 8.4. Dissolved oxygen ranged from 7.0 to 8.7 mg/L or 76 to 95% of saturation at 20°C. The temperature of the test solutions were 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 report 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 with protocols recommended by ASTM (1985), but deviated as follows:

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

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

**B. Statistical Analysis:** The reviewer used one-way analysis of variance (Toxstat Version 3.3) to analyze the survival and reproduction (average number of young produced per adult per reproductive day) of daphnids after 21 days. The survival data were arcsine square root transformed before the analysis. The no-observed



effect concentration (NOEC) for survival and reproduction were 0.90 and 0.38 ppm, respectively (see attached printouts 1 and 2). Adult daphnid length was analyzed using Crunch Version 3, the raw length data (in  $\mu\text{m}$ ), and two-way analysis of variance. Daphnid length at 0.11 and 0.90 ppm was significantly lower than the control. However, as suggested by the author, it is unlikely that daphnid length at 0.11 ppm was affected by the toxicant. Therefore, the NOEC was 0.38 ppm (mean measured concentration; see printout 3). The negative control and solvent control were pooled prior to all analyses.

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

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

D. **Adequacy of the Study:**

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

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

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

Upgraded back to core  
Nick Mastrotot  
1/31/00

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

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

1. CHEMICAL: Molinate.  
Shaughnessey No. 041402.

2. TEST MATERIAL: 1. Non-radiolabelled Ordram Technical; Lot No. WRC 4921-8-9; 97.5% active ingredient; an amber liquid; 2. <sup>14</sup>C-Ordram; Lot No. WRC 6334-46-5.

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

4. CITATION: Forbis, A.D. 1987. Chronic Toxicity of <sup>14</sup>C-Ordram to *Daphnia magna* Under Flow-Through Test Conditions. Final Report No. 35222. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Stauffer Chemical Company, Richmond, CA. MRID No. 406578-02, D182484, S425136.

5. REVIEWED BY:

James J. Goodyear                      Signature:  
  
\_\_\_\_\_  
Biologist, Section 1  
Ecological Effects Branch          Date:  
  
\_\_\_\_\_  
Environmental Fate and Effects Division (H7507C)

6. APPROVED BY:

Leslie W. Touart                      Signature: \_\_  
Head, Section 1  
Ecological Effects Branch          Date:  
  
\_\_\_\_\_  
Environmental Fate and Effects Division (H7507C)

7. CONCLUSIONS: This study is scientifically sound, but it does not meet the requirements for Guideline 72-4(b) Aquatic invertebrate life cycle. The reproduction and length portions of the 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 of <sup>14</sup>C 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.

upgraded  
M.P.M.

8

Whole study upgraded to 'core.'  
Nick Mastrototaro 1/31/00

MRID No. 406578-02

September 14, 1992

MEMORANDUM

Subject: Change of a Molinate chronic *Daphnia magna* to  
"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 report:

Fobis, A.D. 1987. Chronic toxicity of <sup>14</sup>C-Ordram to *Daphnia magna* under flow-through test conditions. Final report no. 35222. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by ICI Americas, Inc., Agricultural Products, Wilmington, Delaware 19897.

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

EEB has downgraded the study to "Supplemental" for growth (LOEC = 0.38 ppm and NOEC = 0.90 ppm) with no possibility of



repair. It is still "Core" for reproductive effects (LOEC = 0.38 ppm and NOEC = 0.90 ppm).

The Reject Rate Analysis concluded that chronic freshwater invertebrate studies shall not be rejected based on lack of dry weight measurements. (pp. 131-132)

L. J. M.

TABLE 1

Chemical Characteristics of Well Water Used by  
ABC's Aquatic Toxicology Division.

Parameter	Concentration
Temperature	15-20°C <sup>b</sup>
Dissolved Oxygen <sup>a</sup>	9.2-10.1 ppm <sup>b</sup>
pH	7.8-8.3 <sup>b</sup>
Hardness (CaCO <sub>3</sub> )	225-275 ppm <sup>b</sup>
Alkalinity (CaCO <sub>3</sub> )	325-375 ppm <sup>b</sup>
Conductivity	700 µmhos/cm
NO <sub>3</sub> -N	0.58 ppm
NO <sub>3</sub> -NO <sub>2</sub> -N	0.69 ppm
PO <sub>4</sub> -P	<0.50 ppm
Aluminum	<20 ppb
Cadmium	<3 ppb
Chromium	<5 ppb
Cobalt	<5 ppb
Copper	<5 ppb
Iron	<5 ppb
Lead	<20 ppb
Nickel	<10 ppb
Silver	<5 ppb
Zinc	<1 ppb
TCC	1.6 ppm <sup>c</sup>
Suspended Solids	0.60 ppm <sup>c</sup>
Measured organophosphorus pesticides	<0.10 ppb <sup>c</sup>
Measured organochlorine pesticides plus PCB's	<0.50 ppb

<sup>a</sup>After aeration.<sup>b</sup>Represents seasonal variation, with the monthly range not exceeding 10%.<sup>c</sup>Water used in chronic studies.

Sample dates: Trace elements = May 12, 1986, Organophosphate and organochlorine analyses = May 12, 1986.

TABLE 2  
Measured Concentrations of  $^{14}\text{C}$ -Ordram During The 21-Day  
Chronic Life Cycle Toxicity Study with Daphnia magna

	Measured Concentrations (ppm)					
	Day 0	Day 4	Mean Day 7	Day 14	Day 21	( $\pm$ S.D.)
Level #1 (0.072 ppm) <sup>a</sup>	0.069	0.060	0.065	0.066	0.064	0.065 ( $\pm$ 0.0033)
Level #2 (0.12 ppm) <sup>a</sup>	0.12	0.10	0.11	0.11	0.11	0.11 ( $\pm$ 0.007)
Level #3 (0.25 ppm) <sup>a</sup>	0.24	0.21	0.22	0.24	0.22	0.23 ( $\pm$ 0.013)
Level #4 (0.43 ppm) <sup>a</sup>	0.42	0.36	0.37	0.40	0.37	0.38 ( $\pm$ 0.025)
Level #5 (1.0 ppm) <sup>a</sup>	1.0	0.83	0.89	0.91	0.88	0.90 ( $\pm$ 0.062)
Stock (10,564 ppm) <sup>a</sup>	12,062	12,046	13,061	14,769	13,986	13,185 ( $\pm$ 1196.3)

<sup>a</sup> Nominal concentrations.

TABLE 3

Percent Survival, Adult Length and Young/Adult/Reproduction Day of Daphnia magna  
Continuously Exposed to <sup>14</sup>C-Ordram During a 21-Day Life Cycle Study

Chamber I.D. (nominal concentrations)	Mean Measured Concentration (mg/l)	Mean Percent Survival	Adult Mean Length mm	Mean Young/Adult/ Reproduction Day
Control	---	100	4.0	8.1
Solvent Control	---	98	4.0	8.1
Controls Pooled <sup>a</sup>	---	99	4.0	8.1
Level #1 (0.072 mg/l)	0.065	98	4.0	8.4
Level #2 (0.12 mg/l)	0.11	100	3.8**	7.1
Level #3 (0.25 mg/l)	0.23	100	3.9	8.5
Level #4 (0.43 mg/l)	0.38	100	4.0	6.4
Level #5 (1.0 mg/l)	0.90	92	3.6*	4.9*

\*Denotes values significantly different ( $\alpha=0.05$ ) from the pooled controls using one-way analysis of variance (ANOVA) and Dunnett's Multiple means test.

\*\*Denotes value statistically different but not biologically significant.

<sup>a</sup>Represents pooled control and solvent control values. The controls were combined since statistical analysis showed no significant difference between the measured parameters.

# Printout 1

406578-02, Ordram, Daphnia Proportional Survival

File: a:40657802.dml

Transform: ARC SINE(SQUARE ROOT(Y))

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.4120	CALCULATED t VALUE =	1.0000
GRP2 (BLANK CRTL) MEAN =	1.3713	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0407		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Shapiro Wilks test for normality

D = 0.108

W = 0.783

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Hartley test for homogeneity of variance

Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.

Additional transformations are useless.

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT -

Ho:Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	GRPS 1&2 POOLED	1.392				
2	0.65	1.371	24.00	12.00	4	
3	0.11	1.412	28.00	12.00	4	
4	0.23	1.412	28.00	12.00	4	
5	0.38	1.412	28.00	12.00	4	
6	0.90	1.295	19.50	12.00	4	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

LC  
6/17/91



# Printout 2

406578-02, Ordram, Daphnia Young/Reproductive Day  
 File: A:\40657802.DM2 Transform: NO TRANSFORMATION

## t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	8.1375	CALCULATED t VALUE =	0.0583
GRP2 (BLANK CTRL) MEAN =	8.0950	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0425		

TABLE t VALUE (0.05 (2), 6) =	2.447	NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) =	3.707	NO significant difference at alpha=0.01

## Shapiro Wilks test for normality

D = 49.499  
 W = 0.938  
 Critical W (P = 0.05) (n = 28) = 0.924  
 Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

## Bartlett's test for homogeneity of variance

Calculated B statistic = 18.71  
 Table Chi-square value = 15.09 (alpha = 0.01)  
 Table Chi-square value = 11.07 (alpha = 0.05)  
 Average df used in calculation ==> df (avg n - 1) = 3.67  
 Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

## WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	GRPS 1&2 POOLED	8.116				
2	0.065	8.920	35.00	12.00	4	
3	0.11	7.147	24.00	12.00	4	
4	0.23	8.480	31.00	12.00	4	
5	0.38	6.443	21.00	12.00	4	
6	0.90	4.880	10.00	12.00	4	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Fmax for testing homogeneity of between subjects variances: 49.47  
Number of variances= 24 df per variance= 10.

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	274	4237.5859			
C (CONC)	5	1128.1810	225.6362	22.478	0.0000
R (REP)	3	148.2104	49.4035	4.922	0.0024
CR	15	441.6087	29.4406	2.933	0.0003
Subj w Groups	251	2519.5857	10.0382		

Analysis of Variance

File: ordram

Date: 06-14-1991

FILTER: None

Post-hoc tests for factor C (CONC)

Printout 3

Level	Mean	Level	Mean
1	59.911	6	53.865
2	59.974		
3	57.075		
4	58.725		
5	59.025		

units =  $\mu\text{m}$

Comparison	Bon- ferroni
1 < 2	
1 > 3	0.0000 *
1 > 4	
1 > 5	
1 > 6	0.0000 *
2 > 3	0.0011
2 > 4	
2 > 5	
2 > 6	0.0000
3 < 4	
3 < 5	0.0952
3 > 6	0.0003
4 < 5	
4 > 6	0.0000
5 > 6	0.0000

1 = pooled controls

2-6 = mean measured concentrations

Post-hoc tests for factor R (REP)

Level	Mean
1	58.731
2	59.429
3	57.676
4	57.729

Comparison	Bon- ferroni
1 < 2	
1 > 3	
1 > 4	
2 > 3	0.0080
2 > 4	0.0102
3 < 4	

Shaughnessey # 041402 Chemical Name Molinate Chemical Class \_\_\_\_\_ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Chronic Fish		Concentrations Tested (ppm) - _____		
Species:		MATC - > _____ < _____ ppm.		
Lab:		Effected Parameters - _____		
MRID #		Control Mortality (%) - _____ Solvent Control Mortality (%) - _____		
		Comments:		

Chronic Invertebrate		Concentrations Tested (ppm) - <u>0.065, 0.11, 0.23, 0.38, 0.90</u>		
Species: <u>Daphnia magna</u>		MATC - > <u>0.38</u> < <u>0.90</u> ppm.	<u>LR</u>	<u>Coe</u>
Lab: <u>Analytical Biochemistry Laboratories</u>		Effected Parameters - <u>Daphnid Carapace length, young/adult Rep- Reproductive Day</u>	<u>6/17/91</u>	
MRID # <u>406578-02</u>		Control Mortality (%) - <u>100</u> Solvent Control Mortality (%) - <u>98</u>		
		Comments: <u>* mean measured concentrations</u>		