

DATA EVALUATION RECORD

1. CHEMICAL: Methomyl
2. FORMULATION: Technical (>99%)
3. CITATION: Brittelli, M.R. 1982. Chronic toxicity of methomyl to Daphnia magna. Unpublished Haskell Lab. Report No. 46-82; submitted by E.I. Dupont de Nemours & Co., Inc., Newark, Delaware; in support of Reg. Nos. 352-342 and 352-370; Acc. No. 071268.
4. REVIEWED BY: John J. Bascietto
Wildlife Biologist
EEB/HED
5. DATE REVIEWED: 2-3-83
6. TEST TYPE: Aquatic Invertebrate - ("Life-cycle") Chronic toxicity with reproduction.

A.) Test Species: Daphnia magna

7. REPORTED RESULTS: "The 48-hour LC_{50} for Daphnia magna exposed to nominal methomyl concentrations was estimated to be 28.7 ug/l. At the concentrations tested during the 21-day chronic test, methomyl did not affect daphnid survival and growth; however, a statistically significant effect was observed on reproduction. Based upon these reproductive effects, the Maximum Acceptable Toxicant Concentration (MATC) for Daphnia magna exposed for 21 days to methomyl is estimated to be $>1.6 <3.1$ ug/l, nominal concentration ($>1.6 <3.5$ ug/l, mean measured concentraton).
8. REVIEWER'S CONCLUSIONS: The study is scientifically sound. Based on the results the MATC for Daphnia magna exposed to methomyl is $>1.6 <3.1$ ug/l. Daphnid survival and growth were not affected at ≤ 13.8 ug/l measured. Reproduction was significantly decreased at ≥ 3.1 ug/l. The first day of reproduction was significantly delayed at ≥ 0.8 ug/l (measured). The study would fulfill the requirement for an aquatic invertebrate life cycle study if the raw data is submitted.



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9. Materials/Methods

A. Test Procedures -

Stock adult daphnid cultures were kept under same as experimental conditions (dilution water, food type, density, water renewal frequency and feeding schedule) for at least 21 days prior to initiation of test. All tests used 1st instar daphnids (<24 hrs. old).

Dilution water was from a deep well, aerated and filtered prior to use. Test temperature was 20°C. 16 hr. light/8 hr. darkness photoperiod (fluorescent).

Food was 4:1 trout chow and active dry yeast + dilution water. Daphnids were cultured in same lab for several years under the above conditions.

For the chronic test, a stock methomyl solution (1 mg/ml) was used to prepare renewal dilutions. Prior to the chronic test a 48-hr LC₅₀ was estimated using two series of test solutions; one with food, one, without food (to determine effects of food on toxicity). Fed group got 30 mg/L food

Six nominal test concentrations were used for the chronic test (0.4, 0.8, 1.6, 3.1, 6.2 and 12.5 ug/l) and a dilution water control. Exposure was in 200 ml/250 ml glass beakers. Each test chamber, including controls, tested one (1) daphnid with ten (10) replicate chambers per concentration. For survival and growth measurements, three (3) replicates contained five (5) daphnids per beaker; for reproductive measurements, seven (7) replicates contained one (1) daphnid per beaker.

To begin the test fresh solutions were added to the appropriate chambers along with food (30 mg./L). 1st instars were added. Test solutions were renewed each Monday, Wednesday and Friday. Each test beaker had one (1) first-generation daphnid; examined daily and the 1st day of reproduction noted.

At each renewal, 1st generation daphnids were counted and transferred to new test chambers with food. Offspring were discarded except for those beakers used for the reproduction counts, where offspring were counted before being discarded. The test ended on Day 21. Surviving first-generation daphnids were counted. Lengths of surviving adults in the 5-daphnid beakers were measured (apex of helmet to base of terminal spine) with a Wild M5A Stereomicroscope calibrated with stage micrometer.

Chemical-Physical procedure - EDTA hardness, total alkalinity, and conductivity of the control were measured X1 per week; D.O. and pH were measured at beginning and at each renewal on one old and one new test solution in control, high, medium, and low toxicant concentrations. Temperature was measured each 4 hours on work days.

Analytical Chemistry - samples of 200 ml of stock and test solutions were taken 0 and 24, 48 or 72 hours. Each concentration of test solutions were pooled prior to analysis. Samples were frozen and analyzed by Biochemistry Dept. by H-P liquid chromatography.

B. Statistical Analysis

For the acute test, probit analysis was employed. In the 21-day study, results were analyzed by ANOVA after percentages were arc sin transformed. If significant ($P < 0.05$) differences were noted, LSD and Dunnett's procedure were used to determine which treatments varied from controls. These results were used to estimate MATC.

10. Results

- A. slight decrease in acute toxicity was observed for daphnids fed 30 mg/L food.

$$\begin{array}{lcl} 48\text{-hr LC}_{50} & = & 28.7 \text{ ug/l (unfed)} \\ \text{" " " } & = & 38.1 \text{ ug/L (fed)} \end{array}$$

Table I - 48 hr acute Toxicity Results

Nominal Conc. ug/L (ppb)	Observed % Mortality ^(a)			
	24 Hrs		48 Hrs	
	Fed	Unfed	Fed	Unfed
0 (Control)	0	0	5	0
10	0	0	10	0
18	0	0	0	0
24	0	0	5	0
32	11	10	100	0
42	75	74	90	76
56	72	100	100	100
75	100	100	100	100
100	95	100	100	100
LC ₅₀ ug/L	42.7	38.3	28.7	38.1
95% c.i.		(35.7-41.2)		(35.6-40.8)

(a) - average of duplicate chambers containing a nominal total of 10 daphnids each

Table II

Physical and Chemical Parameters - Acute Study

Nominal Concentrations (ug/l)	1000	42	10	Control
<u>D.O. (ppm)</u>				
0 HR	7.8	7.8	7.8	7.8
48 HR	7.8	7.8	7.7	7.9
<u>pH</u>				
0 HR	8.1	8.1	8.0	8.0
48 HR	7.6	7.4	7.1	6.7
<u>Total Alkalinity (mg Ca CO₃)</u>				
0 HR	-	-	-	121
<u>EDTA Hardness (mg CaCO₃)</u>				
	-	-	-	101
<u>Conductivity (umhos)</u>				
0 HR	-	-	-	220

Table III

Summary of
21-Day Mean Reproductive Data^(a)

<u>ug/l</u> <u>Nominal</u>	<u>ug/l</u> <u>Mean Measured</u>	<u>#Young</u> ^(b)	<u>Young/Adult</u> <u>Reproductive Day</u> ^(b)	<u>1st Day</u> <u>of Repro.</u> ^(b)
Control	<0.2 ^(c)	149 (22)	10.3 (1.7)	7.4 (0.8)
0.4	0.7	159 (30)	10.4 (2.1)	7.6 (0.5)
0.8	1.0	143 (39)	10.6 (3.0)	8.5 (0.5) ^(d)
1.6	1.6	162 (18)	12.0 (1.0)	8.5 (0.8) ^(d)
3.1	3.5	105 (24) ^(e)	8.2 (1.8)	8.4 (0.8) ^(d)
6.3	7.5	95 (38) ^(e)	7.8 (2.2) ^(d)	9.6 (0.8) ^(e)
12.5	13.8	114 (22) ^(d)	8.6 (0.6)	8.7 (0.8) ^(e)

a - one daphnid/beaker; ten replicates

b - mean (+ standard deviation)

c - detection limit

d - significantly different ($p < 0.05$) from control by LSD

e - Significantly different ($p < 0.05$) from control by Dunnett and LSD tests.

Table IV

Summary of 21-Day Mean Survival and Growth Data^(a)

<u>Nominal conc.</u> <u>ug/l (ppb)</u>	<u>Mean Mesured</u> <u>Conc. ug/l (ppb)</u>	<u>Daphnid</u> <u>Survival (%)</u>	<u>Daphnid</u> <u>Length (mm)^(b)</u>
Control	< 0.2 ^(c)	100	3.7 (0.1)
0.4	0.7	100	3.8 (0.1)
0.8	1.0	93.3	3.8 (0.1)
1.6	1.6	93.3	3.8 (0.1)
3.1	3.5	93.3	3.8 (0.1)
6.3	7.5	93.3	3.8 (0.1)
12.5	13.8	93.3	3.8 (0.1)

(a) N = 15 (5 daphnids/beakers; 3 beakers/conc.)

(b) Mean (± standard deviation)

(c) Detection limits of HPLC

Table V

Physical and Chemical Parameters - 21-Day Chronic Study

Nominal Conc. (ug/l)	12.5	1.6	0.4	Control (o)
<u>D.O (ppm)^a</u>				
Fresh test solution	7.4 (0.3)	7.4 (0.3)	7.4 (0.3)	7.4 (0.4)
old " "	4.6 (0.8)	4.5 (1.0)	4.2 (0.6)	5.4 (0.5)
<u>pH^b</u>				
Fresh test solution	7.5 - 8.6	7.5-8.6	7.5-8.6	8.1-8.6
old " "	6.3 - 7.7	6.9-7.5	7.1-7.7	6.2-7.6
<u>Total Alkalinity^{a,c}</u>	-	-	-	11.8 (5.3)
EDTA Hardness ^{a,c}	-	-	-	92.8 (1.7)
<u>Conductivity (umhos)^a</u>	-	-	-	216 (4.7)

a mean (+ standard deviation)

b Range

c mg/l as CaCO_3

11. Reviewer's Evaluation

- A. Procedures: these were acceptable as they were performed in accordance with "Proposed Standard Practice for Conducting Daphnia magna Renewal Chronic Toxicity Tests" currently under development by Committee E-47 of the American Society for Testing and Materials (Draft No. 8; 2/81).

Procedures for the acute phase were not detailed.

B. Statistical Analysis:

the experimental results were not statistically validated for this review because the raw data on reproduction and growth were not submitted.

C. Results -

The acute 48-hr LC₅₀ appears to be a valid acute result, although not necessary as these data were already known.

The summary chronic results indicate that for Daphnia magna the methomyl MATC >1.6 >3.1 ug/l. This exposure results in a significant decrease in observed reproduction, but not in survival and growth.

D. Conclusions

1. Category: Supplemental for Chronic study.
Supplemental for acute study.
2. Rationale: no raw data submitted for the chronic study. The acute study was a "range-finder" for which procedural details were not submitted.

(to core)

3. Repair: For the chronic study - submit the raw data on survival, growth, and reproduction.

(to core)

For the acute study - provide details of the procedure.