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Data Evaluation Record

1. Chemical: Bolero

2. Formulation: Technical (SX-1127), 95.2-95.9%ai

3. Citation: Daphnia magna Chronic Study Testing, Bolero Technical, Union Carbide Corporation Environmental Services, Tarrytown, New York, November 8, 1979. Ref. 27 in Fish and Wildlife Safety Data to Support Registration of Bolero 10G Herbicide (acc #241483).

4. Reviewed by: Ann Rosenkranz
Aquatic Biologist
HED/EEB

5. Date Reviewed: July 23, 1980

6. Test Type: Aquatic invertebrate flow through chronic study, Daphnia magna

7. Reported Results: 21-day LC_{50}=0.009 (0.005-0.015)mg/l
21 day RI_{50}=0.002mg/l
MATC= ~~$\sqrt{0.001 < 0.002}$~~ 0.0017 mg/l F.M.M.

NOEL = 0.001 mg/l
LOEL = 0.003 mg/l

8. Reviewer's Conclusions: The study is scientifically sound and meets EPA guidelines for a chronic Daphnia magna study.

Test Procedure
Materials/Methods

	Parameter	Measurement, Setting or Condition
1.	Test material.	Bolero Technical (SX-1127), 95.2-95.9% purity; oily, pale amber liquid.
2.	Test type.	Daphnia magna flow-through chronic.
3.	Test dates.	4 to 25 October 1979, 21 days exposure.
4.	Physical test apparatus, toxicant injection system.	Proportional diluter of Mount and Brungs design. Dilution factor of 0.5 delivering approx. 4.0 ml/hr.
5.	Toxicant stock solution contentration.	0.038 mg/l ($\bar{X}$, n = 5). Acetone solvent.
6.	Mean measured test water concentration.	0.035, 0.016, 0.006, 0.003, and .0001 mg/l, plus control and solvent control (n = 7). Each test treatment and controls analyzed for Bolero concentrations on days 0, 3, 7, 11, 14, 18 and 21.
7.	Test vessels.	Twenty-eight glass battery jars 12.5 cm diam. 15.5 cm high, holding 1.7 liters, covered with #405 Nitex. Partially immersed in waterbath.
8.	Dilution water and volume.	UCES site well water, 8.8 min cycle time, 125 ml per test replicate per cycle.
9.	Mean dilution water characteristics (n = 4; temperature n = 22).	Total hardness 212 mg/l as $CaCO_3$, total alkalinity 141 mg/l as $CaCO_3$, total acidity 8 mg/l as $CaCO_3$, pH range 7.65-8.13, temperature 20.9 (range 18.9-24.3)°C, conductivity 600 umhos/cm, dissolved oxygen 9.2 mg/l. (hard quality)
10.	Photoperiod.	Continuous darkness due to photolability of test material, except 3 hours light each counting period and 30 mins twice weekly collecting water samples.
11.	Bioassy organism.	Daphnia magna Straus. Initial no. per test vessel; 15 first instars 20 hrs old. 4 replicates/concentration. 60 daphnids/ concentration.

12.	Feeding rate, food.	0.5 ml prepared suspension 4 times daily, 1.0 ml once daily on weekends (10 g trout mash, 3 g Cerophyl, in 500 ml well water).
13.	Mortality and productivity counts.	Counts made on days 1, 2, 3, 4, 6, 8, 11, 13, 15, 18 and 21. Dead individuals and instars produced removed on these days.

Statistical Analysis

Adult mortality and young production in replicate test vessels were analyzed by standard analysis of variance (ANOVA) according to Steel and Torrie (1960) and Duncan's (1955) multiple range test for differences among treatment means. All differences were considered statistically significant at $p < 0.01$.

Before calculating LC50's for 4, 8, 15 and 21 days exposure, cumulative mortalities were subjected to Abbott's formula (Amer. Publ. Health Assoc., 1975) to correct for control mortality, as that induced by handling, weak organisms, aging and chronic exposure to the solvent. In Abbott's formula, $P = (P' - C)/(1 - C)$, where P and P' are the corrected and observed proportions responding to the test material for a given treatment, and C is the proportion responding in the solvent control. Mean cumulative Bolero Technical analytical values were used to compute LC50's; e.g., data from test days 0, 3, 7, 11 and 14 were averaged for day 15 LC50 calculations. LC50 values and associated 95% confidence limits were calculated by the Spearman-Karber Estimator after Finney (1971).

MATC "effect-no effect" values were calculated from the highest concentration tested in which the number of young produced did not differ significantly from that in the controls, as determined by ANOVA and Duncan's test.

The RI50 (reproductive impairment) was calculated for 21 days exposure after Litchfield and Wilcoxon (1949), using total productivity values. The corresponding Bolero Technical analytical values were also cumulative means.

An estimated brood size per adult daphnid was calculated for each treatment, for each day on which young were produced. This was obtained by dividing total young produced by the number of viable adults present the day the count was made. This value reflects the effect on survival of the original 15 instars, as well as reproductive impairment, resulting from exposure to the test material.

Results/Discussion

A. Cumulative percent mortalities.

Conc, mg/l*	Test day 1	2	3	4	6	8	11	13	15	18	21
Control	0	0	3	3	5	7	8	8	8	8	10
Solvent Control	0	2	3	5	10	13	13	18	18	20	22
0.001	0	0	8	12	15	18	25	28	28	28	32
0.003	0	3	12	18	25	28	30	38	38	47	57
0.006	0	10	28	32	37	43	45	58	63	73	78
0.016	0	25	38	47	53	72	78	87	92	93	98
0.035	0	47	60	77	88	95	98	98	98	98	100

NOEL ?

*Mean (n = 7) measured concentrations.

LC50 values, mg/l.

		Day 4	Day 8	Day 15	Day 21
LC50		0.039	0.017	0.012	0.009
95% Confidence Limits	Lower	0.021	0.009	0.007	0.005
	Upper	0.072	0.031	0.021	0.015

Reproductive impairment as measured by a 21-day RI_{50} was 0.002 mg/l. This value was determined by comparing the total young produced at each concentration with that of the solvent control.

B. ANOVA Analyses

Since reduced productivity, most likely due to decreased illumination, was evident in both water and solvent control daphnids, significance in effect was evaluated by comparison to control performance.

Young Produced. The first day young were present was day 11 when one instar appeared in the control. Low numbers of young produced in the control and solvent control compared to no young produced in any test treatment resulted in statistical similarity of all exposures for days 11, 13 and 15. Daphnids is some replicates of the solvent control and two lowest treatments (0.001 and 0.003 mg/l; $\bar{X}$, n = 4) contained eggs in dorsal brood chambers on day 13. Young were present in both solvent and water controls on day 15; full brood chambers were also present in adults of all replicates of the two lowest treatments (0.001 and 0.003 mg/l; $\bar{X}$, n = 5) and some replicates of the median and second highest treatments (0.006 and 0.015 mg/l; $\bar{X}$, n = 5). On day 18 the solvent control and two lowest treatments contained statistically equivalent numbers of young. A significantly greater number of young were present in the water control at day 18. All replicates of the median treatment and some replicates of the second highest treatment (respectively 0.007 and 0.017 mg/l; $\bar{X}$, n = 6) contained adults with full brood chambers.

On day 21, significantly greater numbers of instars were present in the water control than in the solvent control and all test treatments, all of the latter being statistically equal. Throughout the test, young were never released in the three highest treatments tested and brood chambers were never observed in daphnids at the highest Bolero concentration of 0.035 mg/l.

An ANOVA of cumulative productivity for the exposure period indicates significantly greater young were produced by the water control daphnids than in any other exposure. Statistically equivalent numbers of daphnids were produced by the animals in the solvent control and two lowest test treatments (0.001 and 0.003 mg/l; $\bar{X}$, n = 7).

TOTAL YOUNG PRODUCED

Conc. (Mg/l	Test Day 11	13	15	18	21
Control	1	10*	16*	106	254
Solvent Control	0	0*	6*	40	60
0.001	0	0*	0*	33	70
0.003	0	0*	0*	19	1*
0.006	0	0	0*	0*	0*
0.016	0	0	0*	0*	0*
0.035	0	0	0	0	0

*Eggs present in brood chambers of some daphnids.

Adult mortality. No mortalities occurred in any test concentration or control groups by day 1. Using Duncan's test and comparing treatment means, mortalities in the water and solvent controls and lowest treatment were statistically the same through day 8. After day 8, relatively constant control mortalities together with slowly increasing mortalities in the solvent control and lowest treatment (0.001 Mg/l) maintained statistical equivalency between both controls, but mortalities in the lowest treatment remained equivalent only to those in the solvent control. Mortalities in all other treatment groups were significantly higher.

D. MATC

The estimated 21-day MATC, based on productivity and further defined by the 21-day RI50, is $\geq$0.001$\leq$0.002 mg/l.

E. Mean Brood Size

Mean broad sizes on each counting day from day 11, when the first young were produced, were determined as the number of instars produced during each designated test interval (days 0-11, 11-13, 13-15, 15-18, 18-21)/the number of surviving adults. Brood size was low in all exposures throughout the study, which is most likely due to the greatly reduced illumination. Water control daphnids produced the greatest number of instars.

Daily Mean Brood Size

Conc. mg/l	Test Interval, Days 0-11+	11-13	13-15	15-18	18-21
Control	0	0	0	2	5
Solvent Control	0	0	0	1	1
0.001 [#5]	0	0	0	1	2
0.003 [#4]	0	0	0	1	0
0.006 [#3]	0	0	0	0	0
0.016 [#2]	0	0	0	0	0
0.035 [#1]	0	0	0	0	0

Reviewer's Evaluation
Test Procedure
Materials/Methods

The reported procedure was compared to Tentative Procedure for Daphnia Magna Chronic Tests in a Flowing System in the Federal Register, June 25, 1975.

This study used a faster flow rate than recommended (12 volumes/24 hr. instead of 2-4 volumes/24 hr), larger number of organisms to water volume (15 daphnids/ 1.7 l instead of 5 daphnids/2 l) , different photoperiod and temperature (basically 24h dark vs. 16L-8D and 20.9°C vs 18°C), and different feeding regimen (4 times daily instead of continuously).

The lack of illumination was responsible for the small brood sizes. Although there were more daphnids per chamber than recommended, the procedure can be accepted since D.O. levels remained high and control mortality was within recommended levels (<30%).

Statistical Analysis

The LC_{50} values were re-calculated using Stephan's program with Abbott's formula. Two-way ANOVA's (SAS) were used to analyze young production, comulative percent mortalities (using Arcsin transformations of the percentages), and daily brood mean size. Duncan's multiple range test was used to determine differences among treatment means and replicate means. Differences were considered to be statistically significant at an alpha level of 0.05.

Results/Discussion

	LC_{50} Values, Mg/l			
	Day 4	Day 8	Day 15	Day 21
LC_{50}	0.0163	0.0094	0.0063	0.0035
95% C.I.	0.0126-0.0224	0.0079-0.0114	0.0053-0.0074	0.0030-0.0042

LC_{50} values derived with the probit method of Stephan's program are slightly higher than the values calculated by the Spearman-Karber Method. However, since they all are within the very highly toxic range of 0.1 mg/l, the values for the same time periods can be considered to be equivalent.

The 21-day reproductive impairment value of 0.002 mg/l. is valid.

Young Produced

The 2-way ANOVA indicated that there were significant differences among the treatments and among the sampling times ($P \leq 0.0001$). Additionally there was a significant interaction between the two factors ($P \leq 0.0001$).

The Duncan test indicated that production basically increased with time and with decreasing Bolero concentrations. The numbers of young produced during the first 3 sampling times from days 11 through 15, regardless of the Bolero concentration, were not significantly different. However the numbers of young produced on days 18 and 21 were significantly different from each other and from the production on the earlier days. Day 21 had the greatest production. The total number of young produced by the Daphnia exposed to Bolero concentrations less than or equal to 0.003 mg/l were statistically equivalent. Likewise, production was statistically equivalent in the solvent control and the group exposed to 0.001 mg Bolero/l. Daphnia in the water control group were the most productive of any treatment group. The interaction between the two factors is statistically significant mainly because no young were produced at concentrations greater than or equal to 0.006 Mg Bolero/liter.

Adult Mortality

The 2-way ANOVA and Duncan's test basically verify the reported results. Adult mortality basically increased with time and with Bolero concentration. Results for several of the treatment groups were statistically equivalent-- control and solvent control, solvent control and 0.001 Mg Bolero/l., and 0.001 and 0.003 Mg Bolero/l. Likewise results for several of the sampling times were statistically equivalent-- days 3 through 6, 4 through 8, 6 through 11, 8 through 18, and 13 through 21.

Mean Brood Size

The 2-way ANOVA and Duncan's test indicate that any significant differences among treatment groups or among sampling times regarding mean brood size are mainly due to larger production from days 18 to 21 in the control group.

Conclusions

1. Category: Core
2. Rationale: The study is scientifically sound and meets the requirements of the EPA guidelines of 1978.