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Data Evaluation Record

1. Chemical: Bolero (108401)

2. Formulation: Bolero 8EC, 84.7% active ingredient.

3. Citation: Effects of Bolero 8EC on Survival, Growth, and Development of the Grass Shrimp, Palaemonetes pugio., EG&G Bionomics, Marine Research Laboratory, Pensacola, Florida, January, 1977. Ref. 26 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 14, 1980

6. Test Type: Aquatic subchronic toxicity test-estuarine species, Grass shrimp-Palaemonetes pugio.

7. Reported Results: Survival significantly decreased at $\geq$ 21 ug/l. Growth significantly decreased through day 21 at $\geq$ 36 ug/l. Noticeable delay in metamorphosis to postlarval stage by day 14 at $\geq$ 36 ug/l.

Recalculated Results: 56-day LC_{50}=33 ug/l. Growth significantly decreased through day 14 at $\geq$ 36 ug/l and through day 21 at $\geq$52 ug/l.

8. Reviewer's Conclusions: The study is scientifically sound but does not meet the requirements for an aquatic toxicity study with estuarine invertebrates.

Test Procedure
Materials/Methods

The test system was a flow-through system with a 500-ml proportional diluter (Mount and Brungs, 1967), constructed to provide 50% dilution. The flow rate of 2.5 l/hr resulted in a 99% replacement of water in test containers every 5 hr. Bolero 8EC was tested at nominal concentrations of 30, 60, 120, 250, and 500 ppb. The compound was dissolved in glass-distilled water prior to delivery to the test system.

Test containers were 3-l uncovered glass battery jars, the mouths of which were encircled by a 2-4 cm. band of Nitex with a mesh opening of 274 um. The water depth in the jars was maintained at 20 cm. There were 4 replicate jars of each of the 5 test concentrations plus 4 replicate control jars. The jars were placed randomly in water baths in which the temperature was maintained at 25°C. The photoperiod was maintained on a 16-L, 18-D cycle.

Fifty 4-day old grass shrimp zoea were placed into each jar, for 200 shrimp per treatment. Shrimp were fed 10 ml of newly hatched brine shrimp nauplii daily. Water temperature, salinity, DO, and pH were recorded daily. Chemical analyses were determined weekly. Three shrimp from each replicate container (12 shrimp/treatment) were sampled each week for growth measurements and preserved in 20% formalin.

The test was terminated after 56 days with shrimp at the adult stage of development. To reach the adult stage, shrimp complete approximately 10 zoeal stages, and a postlarval and a juvenile stage.

Statistical Analysis

At the termination of the test, percentage survival was calculated and differences among the control and the treatments were determined by ANOVA. Mean size of shrimp was calculated for each sampling period and differences were also determined by ANOVA. Statistical comparison between the control and each treatment was made by Newman-Keuls tests of the means. Differences were considered significant at the 95% confidence level.

Results/Discussions

Salinity ranged from 20 to 30 ppt with a mean value of 27 ppt. Temperature ranged from 25 to 30°C with a mean value of 25.9°C. D.O. varied from 3.2 to 7.1 ppm (47-100% of saturation). The pH ranged from 7.9 to 8.5 with no apparent effect due to the presence of Bolero.

Chemical analyses of the water samples indicated that mean measured concentrations were 42 to 70% of the nominal concentrations.

Concentration (ug/l; ppb)

Nominal	Measured	%nominal	Range
0	22±(11)	-	47-44
30	21±(11)	70	47-71
60	36±(14)	60	16-91
120	52±(22)	43	18-130
250	105±(35)	42	41-230
500	218±(57)	44	160-360

A probable reason for the difference between nominal and measured concentrations was that stock solutions were intended to be nearly saturated but were not. There was, apparently, some contamination of control containers. This contamination occurred sporadically and in no particular replicate container and was attributed to human errors during sampling and/or analysis.

Survival of shrimp exposed to mean measured concentrations of Bolero $\geq$ 21ppb was significantly less than survival of control shrimp throughout the 56-day study period.

Concentration (ug/l;ppb)		Survival (%)	
Nominal	Measured	Observed	Relative to the control
Control	22	62	100
30	21	28[a]	45
60	36	33[a]	54
120	52	20[a]	32
250	105	14[a]	23
500	218	0[a]	0

[a]Significantly (P 0.05) less than control survival.
[b]Shrimp sampled for growth measurements were disregarded in survival calculations.

Ninety-seven percent of the grass shrimp zoea exposed to 218 ug/l were dead after 7 days. All observed mortalities except for one in a control tank occurred within the first 21 days of exposure.

Mean survival of control shrimp, disregarding those sampled for growth measurements, was 62%. This survival rate is in agreement with Sandifer (op. cit.) who found 60% control survival, Tyler-Schroeder (1975) who found 65% control survival, and EG&G, Bionomics (1975a) who found control survival ranging from 61 to 71% in three 35-day studies. (In the studies cited, no shrimp were sampled or those which were sampled were disregarded when calculating control survival.)

There are two reasons for the accountable shrimp at the end of the study not totaling 200 in all concentrations and the control.

Concentration (ug/l;ppb)		Number Sampled	Number observed dead	Number of live shrimp (day 56)	Number accountable (of 200)
Nominal	Measured				
Control	22	96	6	65	167
30	21	96	2	29	127
60	36	95	3	35	133
120	52	96	3	21	120
250	105	94	6	15	115
500	218	6	49	0	55

First, during the first week of development when the zoea were very small, a dead larva could easily go undetected or disintegrate rapidly. The second reason for the unaccountability of shrimp is that after the shrimp metamorphosed to postlarvae (approximately day 14) they had the ability to jump. Evidence of this was seen when two control shrimp jumped out of the water and were found dead on the screen collar of a test container.

Through day 21 of the exposure, shrimp larvae in several test concentrations (> 36ppb) were significantly smaller than control shrimp, but this effect was not apparent on day 28. However, on day 35, shrimp exposed to 105 ppb of Bolero were significantly smaller than control shrimp. For shrimp exposed to 21 ppb, there was no significant effect on growth until day 28, at which time the shrimp were significantly larger than control shrimp. Shrimp exposed to 21 ppb were also significantly larger than control shrimp on day 56, as were shrimp exposed to 105 ppb. The reason for the growth enhancement in the lowest concentration (21 ppb) of Bolero is not understood, but the growth enhancement in the 105 ppb concentration may have resulted from a decrease in the number of animals competing for a constant food supply.

At the start of the test, all larvae (4 days old) were in either the second or third zoeal stage. All larvae were between the fourth and eighth zoeal stage at day 7. No noticeable delay in development was seen until day 14, when the difference between those shrimp in ninth or tenth zoeal stages and those which had already metamorphosed to postlarvae was apparent. Based on the shrimp sampled on day 14, the following ratios of zoea:postlarvae were observed: control, 3:9; 21 ppb, 0:12; 36 ppb, 6:6; 52 ppb, 8:4; 105 ppb, 7:5; and 218 ppb, 2:0. Thus, development was apparently related to growth. Shrimp exposed to 21 ppb grew and developed more rapidly than did control shrimp, while growth and development of shrimp in all other concentrations was slower than that of control shrimp.

The presence of one ovigerous female at 52 ppb on day 56 indicates that the shrimp had reached the adult stage of development.

Reviewer's Evaluation

Test Procedures

The test procedure was evaluated according to the "Entire Life-Cycle Toxicity Using Grass Shrimp (Palaemonetes pugio Holthuis)" by Dana Beth Tyler-Schroeder in Bioassay Procedures for the Ocean Disposal Permit Program, EPA-600/9-78-010, March, 1978. This test procedure basically complies with the EPA protocol except that they used zoeal instead of postlarval and juvenile shrimp and an exposure period of 56 days instead of 35 days. However the earlier life stage and longer exposure period are acceptable since both factors allow for greater sensitivity.

Statistical Analysis

An ANOVA could not be conducted on any of the data since the results were not listed in terms of the individual replicates.

Results/Discussion

The explanation that the sporadic contamination in some of the control replicates was caused by human error during sampling and/or analysis is plausible and does not necessarily invalidate the study. Neither does the fact that there were large variations between nominal and measured concentrations. However, both facts indicate that greater care should have been taken during the research.

Prior to calculation of the percent survival the percent of larvae that were recovered on day 56 was calculated for each concentration.

Conc.(ug/l,ppb)		Recovered (dead & alive/#start - #sampled)	
Nominal	Measured	Number	Percent
0	22	71/104	68.3
30	21	31/104	29.8
60	36	38/105	36.2
120	52	24/104	23.1
250	105	21/106	19.8
500	208	49/194	25.3

Based upon the actual number of larvae that were alive at 56 days and the number of larvae exposed for 56 days but minus the number sampled for growth during the study the following mortality values are obtained.

Conc. (ug/l; ppb)	%Alive	%Dead	%Dead using Abbott's formula
0	62	38	0
21	28	72	54.8
36	33	67	46.8
52	20	80	67.7
105	14	86	77.4
218	0	100	100

Using the mortality values which were corrected for control mortality an LC_{50} value was derived by the method of Litchfield and Wilcoxon. For the 56-day exposure period, the LC_{50} value is 33 mg/l (ppb).

The researcher is justified in counting the missing larvae as dead since they cannot be disregarded. Based on personal experience it is known that early stage larvae are so fragile that they disintegrate after dying; therefore, counting the missing ones as dead is a common technique. However, Dana Beth Tyler of EPA's laboratory at Gulf Breeze told me that the recovery rate for the controls was lower than the rates she normally obtained in bioassays with this species (~80% recovery). It is possible that some live and or/dead larvae were lost through overflow. Another possiblity is contamination of the controls, but this phenomenon only occurred sporadically. The raw data in terms of live animals/replicate/day are needed to know what is responsible for the low recoveries.

Without the raw data it is difficult to account for some of the peculiar observations in the growth data. The data for growth in the control shrimp indicate that, as expected, growth increased from day 0 to day 35 (3.7 to 14.0mm), but decreased from day 35 to day 42 (14.0 to 13.2mm) and day 49 to day 56 (17.0 to 15.9mm). Therefore what appears to be greater growth on day 56 in the shrimp exposed to 21 and 105 ug/l (18.6 and 18.2 mm, respectively) as compared to the controls may actually be reflecting an inaccuracy in the control measurements. The increased growth on day 28 in the shrimp exposed to 21 ug/l as compared to the controls may only be an anomaly since the growth measurements for this group are statistically equivalent to those of the control group at the other sampling times. Therefore, it appears that exposure to Bolero causes a negative effect on growth only through the first 21 days at concentrations greater than or equal to 52 ug/l and through the first 14 days at concentrations greater than or equal to 36 ug/l.

The more rapid development on day 14 in shrimp exposed to 21 ug/l as compared to the controls may be due to the fact that low concentrations of pesticide inhibit fungal and bacterial diseases that can adversely affect development.

Conclusions:

1. Category: Supplemental
2. Rationale: Use of formulated product instead of technical product. Lack of raw data that are needed for statistical analyses.
3. Repairability: Submission of raw data will help verify results, but use of formulated product will not upgrade study to core.