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SHAUGHNESSEY NO.

5
REVIEW NO.

EEB BRANCH REVIEW

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FILE OR REG. NO. 264-GUE, 264 - GUR

PETITION OR EXP. PERMIT NO.

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TYPE PRODUCT(S): I, D, H, F, N, R, S Insecticide

DATA ACCESSION NO(S).

PRODUCT MANAGER NO. J. Ellenberger (12)

PRODUCT NAME(S) Larvin 500 : 264-GUR
Larvin 75WP: 264-GUE

COMPANY NAME

SUBMISSION PURPOSE Submission of further data in support of soybeans and cotton uses

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.
114501	Thiodicarb	

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DATA EVALUATION SHEET

1. CHEMICAL: Larvin

2. FORMULATION: Thiodicarb

   Shaughnessy Number: 114501

3. CITATION: Booth, et al. 1982. The Acute and Chronic Toxicities of Larvin to Daphnia magna Using a Static Renewal System. An unpublished report prepared by Brighan Young University for Union Carbide Corp. Acc# 247397

4. REVIEWER: Daniel Rieder
   Wildlife Biologist
   EEB/HED

5. REVIEW DATE: 6/24/82

6. TEST TYPE: Chronic/Reproduction Toxicity Test

   A. Species: Daphnia magna

   B. Material: Thiodicarb 97.3% a.i.

7. RESULTS: 48-hour LC50 is around 50 ppb.
   The maximum acceptable toxicant concentration (MATC) is between 9 and 18 ppb.

8. REVIEWERS CONCLUSION: The chronic toxicity study was scientifically sound and fulfills guideline requirements. The results are acceptable for use in hazard assessment preparation.

Methods

Organisms. Daphnia magna used in both the acute and chronic studies were obtained from a stock culture from Brigham Young University (BYU). Daphnids cloned from the same parthenogenetic female, which were at least 10-12 days old (those which have had at least two broods), were separated from the culture and put in a separate culture container prior to starting the test. Young daphnids $<$ 24 hours old were obtained from this culture to start the bioassays. The culture water was identical to that used in the toxicity tests.

Water. Filtered spring water (using Whatman No. 30 filter paper) from the fresh water laboratory at BYU was used in both bioassays. The water was vigorously aerated using air stones prior to the introduction of Larvin and the daphnids.

Chemicals. Both technical Larvin (97.3% Thiodicarb, 2.7% inert ingredients, Ref. No. 17343-40) and ^{14}C-Larvin (S.A. = 1.78 X 10^5 dpm/ug) were used in the chronic studies while nominal concentrations of technical Larvin were used in the acute bioassays. A stock solution of 20 ppm Larvin was used for all dilutions. The stock concentrations were checked for accuracy using U.V. spectrophotometry at 236 nm. The ^{14}C-Larvin was utilized in the chronic bioassays in order to verify and confirm the concentrations in each jar and because the parent compound cannot be chromatographed.

Test chambers and cleaning procedures. Glass squat jars (250 mL) were used in both the acute and chronic studies. The jars were washed with detergent and rinsed with water, 10% HCl, water, acetone, and twice with BYU distilled water prior to initial use and during each toxicant change.

Photoperiod and temperature. All studies were conducted in a walk-in growth chamber with a photoperiod of 16 hours of light and 8 hours of darkness and a temperature of 20°C. Light intensity on the shelves was 32-74 foot candles.

Acute toxicity experimentation. A 48-hour LC50 was determined prior to beginning the chronic and reproductive studies. Daphnid food was added initially to oxygenated test water to obtain a solution concentration of 30 mg/L. Two hundred milliliters of this water plus food solution was placed into each of 3 replicate jars for each test concentration. Appropriate amounts of a 20 ppm Larvin stock solution were added to each jar resulting in test concentrations of 0,1,3,5,7, 10,25,50, and 75 ppb.

Five first-instar daphnids, $\leq$ 26 hours old, were randomly placed into each jar and covered with loosely fitting lids. The location of the test containers was determined randomly and maintained at 20° $\pm$ 1°C with a photoperiod of 16 hours light and 8 hours dark. Mortality was determined at 49 hours. In addition, a predicted 48-hour LC50 evaluation was completed on the first two-days (48 hours) of the chronic and reproductive phases of the study.

A computerized probit analysis of the acute toxicity data was completed using an IBM 4341 computer system and a program by Barr et al. (1976). In order to perform the analysis, the 100% mortality figure was adjusted to 93.3% mortality. The raw data for the LC50 evaluations were provided.

Chronic and reproductive toxicity experimental design. The test concentrations used in both of these studies were 0, 0.018, 0.18, 1.8, 9.0, 18.0, 36.0, and 50.0 ppb. Concentrations at 0.018 and 0.18 ppb contained only ^{14}C-Larvin while all other concentrations contained mixtures of ^{14}C-Larvin and technical Larvin. The choices of concentrations were based on the data from the acute study the experimental design in general was consistent with guidelines proposed by the American Society for Testing and Materials (ASTM, 1981).

Basically, ten 250 mL squat jars were used for each toxicant concentration with the addition of two other control jars in the chronic study only. Seven of the jars at each concentration contained one daphnid each for collection of data on survival, growth and reproduction. Three of the jars (five for the chronic controls as noted above) contained five daphnids each for collection of data on survival as well as reproduction. The daphnids were added to the test solutions prior to the food which was added at a rate of 20 mg/L. Treatments and assignment of daphnids were randomized before the beginning of the bioassays. For discussion purposes, the experiment containing 5 daphnids per jar is referred to as the chronic-phase while the experiment containing 1 daphnid per jar is referred to as the reproductive-phase.

Three times each week the live and dead parents in all jars were counted and the live daphnids were transferred to jars containing the same Larvin concentrations as that from which they were removed. In the jars containing one parent daphnid, the offspring were counted and discarded. Jars were cleaned and refilled with 200 mL of the same concentration of fresh test solution. The concentrations were confirmed by using liquid scintillation techniques with Aquasol and an Isocap 300 Liquid Scintillation Spectrometer.

On day 21, the first generation (parent) daphnids were counted, individually measured to the nearest 0.01 mm from the apex of the helmet to the base of the spine with an ocular micrometer, and the number of young in each jar were counted.

RESULTS

Table 1.

Percent Mortality Data From Acute Study

	Concentrations (ppb)							
Replicates	Control	1	3	5	7	10	25	50	75
1	0	0	0	0	0	0	0	80	100
2	0	0	0	0	0	0	0	60	100
3	0	0	0	0	0	0	0	0	100

Percent Mortality

These data show that the 48-hour LC50 would be around 50 ppb.

Table 2. Results From Reproduction Study

	Concentrations							
At 21 days	control	0.018	0.18	1.8	9.0	18.0	36.0	50.0
Total surviving[a]	5	2	4	2	7	3	0	0
Total Young Produced	100	58	118	99	203	6	0	0

[a] out of 7; There were seven test containers each with one daphnid.

Table 3. Results From Chronic Study

	Concentration							
At 21 days	con trol	0.018	0.18	1.8	9.0	18.0	36.0	50.0
Percent[b] Surviving	76%	73	87	60	60	73	20	0
Total Young Produced	351	237	238	173	122	126	18	0

[b] Percent is used because there were 5 containers (5 daphnids each) in the control and only 3 containers (5 daphnids each) in the treatment.

The data in these tables show that chronic effects in daphnids may be expected at between 9 and 18 ppb. The weight/length information did not reflect any dose effected.

REVIEWERS EVALUATION

The protocol was acceptable and the results of this study are acceptable for use in hazard assessments.

Conclusion

Category: Core

DATA EVALUATION SHEET

1. CHEMICAL: Larvin

2. FORMULATION: Technical grade 97.3% Thiodicarb

   Shaughnessy Number: 114501

3. CITATION: Booth et al. 1982. The Effects of Larvin on the Early Life Stages (Embryo-Larvae) of Rainbow Trout (Salmo gairdneri) An unpublished report prepared by Brigham Young University For Union Carbide. Acc # 247396.

4. REVIEWER: Daniel Rieder
   Wildlife Biologist
   EEB/HED

5. REVIEW DATE: 6/18/82

6. TEST TYPE: Embryo Larvae

   A. Species: Rainbow Trout

   B. Material: Technical Larvin

7. RESULTS: The acute LC50 values were 1.06 and 2.39 ppm for the static and flow-through systems respectively. There was no observed dose/effect relationship at up to 1 ppm, so the MATC is assumed to be between 1 ppm and 1.06 ppm (the lowest LC50 value)

8. REVIEWERS CONCLUSION:

   A. Validation Category: Core

   B. Discussion: The study was scientifically sound and fulfilled guideline requirements for a fish embryo-larvae study. It showed that there were no dose related effects at up to 1 ppm concentration.

METHODS

Acute bioassays of Larvin to larvae. The static acute toxicity of Larvin to alevins was determined using jars containing 200 ml of spring water. The alevins averaged 0.0929 ± 0.0203 g and 2.32 ± 0.079 cm. Generally, a total of three larvae per jar was used which resulted in a total biomass of 1.3935 mg/L. The treatments were 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, and 10.0 ppm with 30 organ isms per treatment (10 replicates per treatment).

The flow-through acute toxicity of Larvin to the larvae was determined using the mini-diluter system described in section 2.3. A stock solution of 35 ppm of Larvin was dissolved in 1% dimethyl formamide (DMF) and pumped into the toxicant cell at a pump rate of 14.3 ml/min. The resulting treatments were 0, 0.32, 0.63, 1.25, 2.50, and 5.0 ppm with a total of 20 organisms per treatment (4 replicates per treatment). The larvae averaged 0.0964 ± 0.0161 g and 2.3 ± 0.094 cm.

Chronic Embryo-Larvae Study. Trout embryos (18 days old) were obtained from a local hatchery. The age of the embryos was selected so that they spent 7 days in the test solutions prior to hatching (ASTM, 1981). A total of 30 embryos were placed in each of 24 incubation cups (720 total embryos), and each unit was vertically oscillated using a 1 rpm motor. Dead embryos (visually opaque) were removed daily until hatch was completed.

Upon completion of the hatch, the total number of larvae in each replicate chamber were counted. Dead or deformed larvae were counted and subtracted from the total for a determination of normal larvae at hatch. Fifteen larvae from each cup were then placed into each of the replicate test chambers for the remainder of the study. The other live remaining larvae were weighed and measured from each replicate chamber.

The prolarvae were not fed for the first 16 days because they survive on the yolk-derived material from the sac. Also, the prolarvae were kept in subdued light through ca. one week posthatch. Afterwards, they were placed on the 14 hour light: 10 hour dark photoperiod. After the 16 day incubation period (or when the larvae began to "swim-up"), the rainbows were fed a diet of trout-starter at the rate of 4% of their body weight per day with 2-4 feedings per day. The unused food from the previous day was siphoned out at the beginning of each new working day.

Survival and abnormal behavior were determined on a daily basis for each replicate. Weekly length measurements were completed on every fish using a photographic method (Bushuev, 1969; Martin, 1967) that did not stress the fish.

The test was completed using a continuous flow mini-diluter exposure system similar to that developed by the EPA. The small system incorporates four replicate glass exposure chambers (18.7 x 7 x 9.2 cm high) at each of five concentrations plus a control. The mini-diluter delivers 15 ml of test water per minute to each

replicate 500 mL chamber. The test chambers were randomized in sets of four blocks. Water depth in each chamber was measured at 4.5 cm. All test chambers were carefully siphoned daily with an aspirator once the fish began feeding. Rainbow trout feeding began 16 days posthatch. The sac-fry (also called prolarvae) were kept in dim light until one week posthatch, after which the organisms were placed on a 14 hour light:10 hour dark photoperiod for the remainder of the study. Cool white fluorescent lamps were used as the main source of illumination. Light intensity at the water surface varied from 5-18 foot candles.

Unfiltered spring water from the fresh water laboratory at BYU was used throughout the studies. A complete chemical analysis of the water was completed and a record of the measured parameters kept. The temperature, pH, and dissolved oxygen were taken in each ELS treatment at least once per week. An additional test for the suitability of the water is whether the rainbow trout embryos would hatch and grow in the bioassay chambers under flow-through conditions. This was confirmed at BYU prior to the beginning of the embryo-larval test.

Technical Larvin (97.3% Thiodicarb, 2.7% inert ingredients, Ref. No. 17343-40) was used in the flow through bioassay. A stock solution of 20 ppm Larvin was made frest twice during a 24 hour period. The concentration was checked for accuracy using U.V. spectrophotometry at 236 nm. Stock solutions were delivered to the diluter by an FMI pump. The test concentrations were 1.0 ppm, 0.5 ppm, 0.25 ppm, 0.125 ppm, 0.063 ppm, and 0 ppm Larvin.

The test concentrations and proper performance of the system were confirmed empirically by using ^{14}C-Larvin (S.A. = 1.78×10^5 dpm/ug) once per week in the stock solution. This was done since the parent compound cannot be chromatographed.

At the termination of the study, the surviving fish were killed in ice water and growth was determined by weighing and measuring the length of each fish. Selected fish from each treatment were X-rayed in order to compare skeletal formation between treatments. These X-rays were taken using a Siemens Mammomat machine with direct exposure at 25 MaS and 28 Kv. The film was Eastman Kodak Min-R with Ultra Detail Min-R Screens.

Hatchability of embryos, normal larvae at hatch, weight and length of selected larvae at hatch, length of the larvae through time, and survival and mean weight of terminal fish in each replicate were subjected to statistical analysis.

RESULTS

Test conclusions were based on hatching success of the embryos and the survival and growth (weight & length) of the hatchlings.

Summary of Hatching Data

Treatment	Replicate	Cumulative # of embryos hatching	% hatch	$\bar{X}$ %
Control	1	30	100	
	2	30	100	100
	3	30	100	
	4	30	100	
0.063 ppm	1	28	93	
	2	28	93	
	3	29	97	95
	4	29	97	
0.125 ppm	1	30	100	
	2	30	100	
	3	29	97	99
	4	30	100	
0.250 ppm	1	28	93	
	2	29	97	98
	3	28	93	
	4	29	97	
0.5 ppm	1	30	100	
	2	30	100	98
	3	28	93	
	4	29	97	
1.0 ppm	1	29	97	
	2	30	100	98
	3	28	93	
	4	30	100	

At the end of the 53-day posthatch periods a total of 303 fish had survived out of a total beginning sample of 360. Eight Fish died in the bioassay chambers during the study and 49 either were damaged during cleaning or jumped out of the containers. No dose-response relationship was observed.

Weight Comparisons Between Control and 1 ppm Level at End of Study

		Weight (Replicate)			
		1	2	3	4
Control	$\bar{X}$	0.963 g	0.795 g	0.903 g	0.849 g
	S.D.	0.174	0.145	0.157	0.122
1.0 ppm	$\bar{X}$	0.811 g	0.800 g	0.940 g	0.787 g
	S.D.	0.126	0.179	0.151	0.155

According to the length information, there was no significant difference between the control and the highest concentration, although there was a trend observed in that the average lengths in the control were greater than those at 1 ppm.

	Length Comparisons			
	1	2	3	4
Control X	4.60 cm	4.40	4.56	4.42
S.D.	0.37	0.23	0.25	0.18
1.0 ppm X	4.45	4.32	4.49	4.19
S.D.	0.21	0.28	0.26	0.28

REVIEWERS EVALUATION

This study is considered acceptable. Between the embryo-larvae study and the static acute toxicity test, a Maximum Acceptable Toxicant Concentration (MATC) of between 1 and 1.06 ppm can be extracted.

The results of the two acute toxicity tests (static LC50= 1.06; flow-through LC50= 2.39 ppm) seem to show that Thiodicarb is more Toxic after 96 hours in static conditions. This suggests that a metabolite of thiodicarb could be more toxic than thiodicarb itself.

Conclusions

Category: Core

Larvin

100 Pesticide Label Information

Insecticide

103 Toxicological Properties

103.4.2 Embryo-larvae and Chronic Studies

The Union Carbide Corp. submitted two studies for validation, a fish embryo-larvae study with rainbow trout and an aquatic invertebrate chronic study with Daphnia magna.

Embryo-larvae (Rainbow trout)

This study was considered core and acceptable for use in hazard assessment preparation. It showed no dose related effects at a concentration of 1 ppm. Therefore the MATC is assumed to be between 1 ppm (the highest dose tested in the chronic study) and 1.06, the lowest LC50 value calculated.

Chronic study (Daphnia magna)

This study was considered core and acceptable for use in hazard assessment preparation. The MATC is between 9 and 18 ppb, the 48-hour LC50 is around 50 ppb.

Daniel Rieder

Daniel Rieder, Wildlife Biologist
Ecological Effects Branch

Norman Cook 6-29-82

Norman Cook, Head, Section #2
Ecological Effects Branch

6/29/82

Clayton Bushong, Branch Chief
Ecological Effects Branch