7-21-88

(15)

Accession Number 405508-02

DATA EVALUATION RECORD

1. CHEMICAL: Iprodione Technical.

2. TEST MATERIAL: Iprodione Technical, 100% purity, a white-colored powder.

3. STUDY TYPE: Estuarine Invertebrate Life-Cycle Test.
Species Tested: Mysidopsis bahia.

4. CITATION: Surprenant, D. C. 1988. Chronic Toxicity of Iprodione Technical to Mysid Shrimp (Mysidopsis bahia). Prepared by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Rhone-Poulenc AG Company, Research Triangle Park, North Carolina. Report No. 88-2-2640. Accession No. 405508-02.

5. REVIEWED BY:

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Date: 7/11/88

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Date: 7/21/88

Cindy Brassard 7/21/88

7. CONCLUSIONS: This study is scientifically sound and can be used in ecological risk assessment of Iprodione technical. However, it does not meet the guideline requirements for an invertebrate life-cycle test because the MATC could not be accurately determined. Based on the most sensitive indicator (i.e., reproduction), the MATC of Iprodione technical for Mysidopsis bahia was estimated to be less than 0.015 mg/L mean measured concentration.

see Addendum attached

8. RECOMMENDATIONS: N/A.

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9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile (≤ 24 hours old) mysids were obtained from cultures maintained at Springborn Life Sciences, Inc. (SLS). The test organisms were cultured and held in natural seawater at conditions compatible with those in the test, i.e., a salinity of approximately 30 grams per liter (g/L), and a temperature of 25 degrees Celsius (^{o}C), prior to distribution into the test aquaria.

The mysid culture area received a regulated photoperiod of 16 hours of light and 8 hours darkness. Light at an intensity of 70-110 footcandles at the culture surface was provided. Mysids were fed live brine shrimp nauplii supplemented with Selco twice daily and Hatch Fry Encapsulon, a high protein supplement, three times weekly. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 ± 1^{o}C.

B. Test System: The life-cycle test was conducted using an exposure system consisting of a modified-flow proportional diluter (Mount and Brungs, 1967), temperature-controlled water bath, and a set of 14 test aquaria. The test system was designed to provide five concentrations of the test material, a dilution water control and a solvent control. The solvent control was maintained at 88 uL of acetone per liter of solution which was equal to the solvent concentration in the highest treatment level. Filtered natural seawater was used as dilution water.

The diluter was constructed to deliver 0.5 L of exposure solution per cycle to each replicate test aquarium at a dilution ratio of 50%. During the study, the diluter provided the exposure solutions at a rate of approximately 7 volume additions per aquarium per day. Test aquaria were impartially positioned in a temperature-controlled water bath.

The mysid retention chambers were glass Petri dishes, 10 cm in diameter, 2 cm deep, to which a 15 cm high nylon screen collar was attached. The retention chambers were partially submerged in the replicate exposure aquaria and were used to maintain the mysids during the initial phase (15 days) of the chronic exposure. At the time of sexual maturity (day 15) individual pairs (male/female) of mysids were transferred into cylindrical glass isolation jars (5.1 cm diameter, 10 cm high) containing two 1.9-cm holes covered with nylon screen. A photoperiod of 16 hours light and 8 hours dark with a light intensity of 15-110 footcandles at the test solution surface was maintained throughout the test period.

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C. **Dosage**: 28-day life-cycle flow-through test.

D. **Design**: Based on a preliminary range-finding test, conducted at SLS under flow-through conditions, the nominal concentrations of Iprodione Technical selected for the definitive life-cycle exposure were 0.031, 0.063, 0.13, 0.25, and 0.50 mg/L.

A total of 420 test organisms, $\leq$ 24 hours old, were removed from the SLS culture units and placed into a 1-L glass beaker containing culture water (25 $\pm$ 1°C). The organisms were impartially selected and distributed to the 28 retention chambers by adding three organisms to each chamber and then repeating the procedure four additional times, totaling 15 organisms per retention chamber, yielding 30 mysids per replicate, and 60 organisms per concentration or control.

When mysids had reached sexual maturity (day 15), they were redistributed within the test aquaria. Mature male/female pairs within each exposure aquaria were transferred from the retention chambers to ten glass isolation jars. The remaining mysids (after isolation of male-female pairs) were pooled and placed in a clean retention chamber within each aquarium where they were maintained for the duration of the chronic test. Male mysids from this pool were used to replace dead males from the paired (male/female) isolation jars. Females which died in the isolation jars were not replaced. If development of brood pouches distinguishing female organisms from males was delayed due to toxicant exposure, all test organisms were maintained in clean retention chambers until maturity was observed or until test termination.

Throughout the test, mysids were fed live, approximately 24-hour old (post-hatch) brine shrimp nauplii supplemented with a commercial food at a minimum of twice daily. During the first 14 days of the test, the number of dead organisms and any unusual appearance or behavior were recorded. Mortality was determined by lack of movement after gentle prodding with a glass pipet tip. After males and females had been paired (day 15), the number of dead males and females, the number of offspring produced by each individual female, and the appearance and abnormal behavior, if observed, of the adult mysids were recorded. Observations were made daily throughout the study. Dead parental mysids and juveniles released were removed, recorded, and discarded when observed during the test.

At test termination, all mysids were sacrificed by immersion in cold deionized water for approximately 15 minutes and blotted dry on absorbent paper. Mysids were separated into male and female groups for each replicated exposure system. At this time, brine shrimp nauplii were removed from the female brood sacs when

observed, but eggs and juveniles were not removed. Male and female groups were transferred to aluminum pans and dried for approximately 24 hours at 60°C and then cooled in a desiccator. Individual body weight to the nearest 0.01 mg was determined. Individual weights of all surviving males and females were recorded separately for each replicate of each concentration and the controls.

The reproductive success, i.e., the number of offspring per female per reproductive day in each treatment and control, was calculated for each mysid pair as the ratio of total number of offspring produced by an individual female during the test and the number of female reproductive days. The number of female reproductive days was determined as the number of days that an individual female was alive, counting from the day that offspring was first observed in any treatment or control (day 17).

Salinity and temperature were measured daily in the dilution water control. Dissolved oxygen concentrations and pH were measured every day in one replicate of each treatment level and the controls throughout the exposure. Solution temperature was continuously monitored throughout the study in one replicate of the solvent control. Samples were removed from all replicate test solutions and the controls on test days 0, 7, 14, 21, 23, and 28, and analyzed for Iprodione Technical.

E. Statistics: At the termination of the chronic test, data obtained from the paired (male/female) and unpaired organisms were statistically analyzed. The toxic endpoints used for determination of significant adverse effect included survival, and growth (total dry weight) of males and females. Reproductive success was determined for only the paired organisms. Growth and survival data points were entered as mean values by replicate in the statistical analyses. Reproductive success data were entered by individual replicates.

One-way, single classification ANOVAs were conducted to compare results of the measured or calculated toxic endpoint between control and solvent control replicates. Since no statistically significant differences ($P \leq 0.05$) were found between control and solvent control, data were pooled and this pooled data set was subsequently used to detect treatment effects.

Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. Statistical comparison between results of pooled control and various levels of Iprodione Technical was based on Williams' method.

The theoretical threshold concentration expected to produce no

deleterious effects at the 95% level of certainty was estimated as the Maximum Acceptable Toxicant Concentration (MATC).

12. REPORTED RESULTS: Table 1 (attached) presents the results of the measurements for dissolved oxygen concentration and pH in the controls and the treatment level solutions during the 28-day life cycle test. Daily measurements of temperature and salinity in the dilution water control ranged from 23-26°C and 30-32 parts per thousand, respectively.

The mean measured concentrations of Iprodione established in the test solutions were typically between 40 and 60% of nominal treatment levels. The diluting system operated consistently and prepared and delivered the test solutions properly throughout the exposure period.

A summary of the mean percentage survival of mysids after 28 days of the exposure to Iprodione Technical is presented in Table 3 (attached). Mysid survival in the two highest measured test concentrations, 0.25 and 0.10 mg/L Iprodione (42 and 65%, respectively) was significantly lower than the pooled control organism survival. Based on the survival data, the established NOEC and LOEC concentrations were 0.063 and 0.10 mg/L, respectively, with a geometric mean (MATC) of 0.079 mg/L Iprodione.

Measurements of growth, as dry body weight, were made at test termination on all surviving mysids, and are summarized in Table 4 (attached). Statistical comparison of this data established that no significant reduction in growth occurred for male and female mysids exposed to Iprodione concentrations of 0.063, 0.031, and 0.015 mg/L when compared to the pooled control data. Statistical analyses of mysid dry weights for organisms exposed to 0.25 and 0.10 mg/L were not performed since an adverse survival effect was observed at concentrations lower than the levels which adversely affected survival of the test organisms.

An adverse effect on reproduction was the most sensitive indicator of the toxicity Iprodione technical to mysid shrimp. At test termination, reproduction among mysid shrimp exposed to all tested concentrations of Iprodione technical was adversely affected as compared to the reproduction of the control organisms (Table 3). As a statistically significant difference was found between the reproductive success of the controls and that of the lowest Iprodione concentration tested, an estimate of the No Observed Effect Concentration (NOEC) was obtained by extrapolation of the concentration vs. biological effect relationship. The NOEC obtained was 0.003 mg/L Iprodione.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The chronic toxicity of Iprodione to mysid shrimp in the concentration range tested was expressed as a survival effect, geometric mean MATC was 0.079 mg/L; and an effect on reproduction, geometric mean MATC was < 0.015 mg/L (the lowest tested concentration). Based on a strongly apparent concentration-effect relationship, the NOEC for the reproductive effect observed was estimated to be 0.003 mg/L Iprodione. Effects of Iprodione on mysid growth were not observed at concentrations ≤ 0.063 mg/L.

The raw data and the final report for this study were inspected by the Springborn Life Sciences' Quality Assurance Unit to assured compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS: Since there is no SEP developed for mysid life-cycle test, this study was reviewed based on the "Proposed New Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids" by ASTM (June, 1986).

A. Test Procedure: The test procedure generally follows the ASTM Proposed Guideline, except for the following differences:

o The test was conducted at 25°C. The ASTM recommends 27°C for Mysidopsis bahia test.

o There was no 15- to 30-minute transition period between light and dark photoperiod. This transition period is important for mysids because they tend to be stressed by instantaneous changes in light intensity.

o The ASTM recommends the dissolved oxygen concentration be maintained between 60 and 100% of saturation. In this test, the lower ranges of dissolved oxygen concentrations were generally low (Table 1) and at one time the d.o. fell as low as 4.0 mg/L or 44% of saturation (100% of saturation at 31 ppt and 25°C is 9.1 mg/L).

o The test probably should have included lower concentrations of Iprodione technical than the lowest concentration tested since all selected concentrations affected reproduction of mysids (see results). Therefore, a more accurate MATC cannot be estimated.

B. Statistical Analysis: The statistical analyses conducted by the author were appropriate. However, reproduction and growth data of all treatment levels should have been included in the statistical analyses. The reviewer reanalyzed the data and obtained similar results (attached).

C. Discussion/Results: The drop in dissolved oxygen concentrations probably did not adversely affect the test organisms since the data showed high percent survival in both controls and most treatment levels. According to the raw data, the percent survival of Replicate B in the dilution water control should be 87%, instead of 83% as reported in Table 3.

Adult weight was not a good indicator of Iprodione toxicity to mysids. The most sensitive indicator was reproductive success measured by counting the number of offspring a female produced per reproductive day. All Iprodione concentrations tested significantly reduced the reproductive success of Mysidopsis bahia. Therefore, the MATC based on reproduction could be reported only as less than 0.015 mg/L of mean measured concentration of Iprodione technical which is the lowest concentration tested.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: Since the selected Iprodione concentrations did not include the no-observed-effect level, an accurate MATC value could not be determined.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, July 8, 1988.

IPRODIONE

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MYSID SHRIMP LIFE-CYCLE TEST

Analysis of Survival Data Using Dunnett's Test

Arcsin square root transformation applied before data analysis.
For this set of data, the minimum significant difference is 0.061.
This represents a 7.19% reduction in Survival.
T= 2.66 p=0.05 (one-tailed test)

Survival Data

Group	1	2	3	4	Mean	S
1 CONTROL	80	87	87	87	85	
2 0.015 ppm	87	87	0	0	87	
3 0.031 ppm	80	60	0	0	70	
4 0.063 ppm	83	77	0	0	80	
5 0.10 ppm	53	77	0	0	66	
6 0.25 ppm	53	30	0	0	41	*

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	0.395	0.079	6.701	3.69
Within	8	0.094	0.012		
Total	13	0.490			

MYSID SHRIMP LIFE-CYCLE TEST

Analysis of Re Success Data Using Dunnett's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 0.149.
This represents a 18.52% reduction in Re Success.
T= 2.66 p=0.05 (one-tailed test)

Re Success Data

Group	1	2	3	4	Mean	S
1 CONTROL	0.82	0.90	0.73	0.77	0.81	
2 .015 ppm	0.49	0.45	0.00	0.00	0.47	*
3 .031 ppm	0.29	0.31	0.00	0.00	0.30	*
4 .063 ppm	0.26	0.41	0.00	0.00	0.33	*
5 .10 ppm	0.14	0.04	0.00	0.00	0.09	*
6 .25 ppm	0.06	0.03	0.00	0.00	0.04	*

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	1.138	0.228	54.327	3.69
Within	8	0.034	0.004		
Total	13	1.171			

Estimation of NOEC by extrapolation of concentration and reproductive effect relationship

Regression analysis

Conc. (mg/L)	Log conc.	# offspring/female/reprod. day
0.015	-1.824	0.49
		0.45
0.031	-1.509	0.29
		0.31
0.063	-1.201	0.26
		0.41
0.100	-1.000	0.14
		0.041
0.25	-0.602	0.056
		0.033

Linear regression equation: $Y = a + b(X)$

Where: Y = # of offspring/female/reproductive day
X = Log concentration
a = Intercept = -0.182
b = slope = -0.35

$Y = -0.182 + (-0.35)(X)$ $\qquad r^2 = 0.80$

Assuming the average reproductive success (# of offspring per female per reprod. day) in the pooled control (0.80) is the normal reproductive success without any adverse effects, the no-observed-effect concentration of Iprodione could be estimated as follows:

$$\text{Log Concentration} = \frac{0.80 - (-0.182)}{-0.35}$$

$$= -2.806$$

Therefore, the NOEC = 0.002 mg/L.

MYSID SHRIMP LIFE-CYCLE TEST

Analysis of Male Wt Data Using Dunnett's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 0.162.
This represents a 21.56% reduction in Male Wt .
T= 2.66 p=0.05 (one-tailed test)

Male Wt Data

Group	1	2	3	4	Mean	S
1 CONTROL	0.76	0.81	0.61	0.83	0.75	
-2 .015 ppm	0.72	0.78	0.00	0.00	0.75	
3 .031 ppm	0.73	0.79	0.00	0.00	0.76	
4 .063 ppm	0.74	0.66	0.00	0.00	0.70	
5 .10 ppm	0.57	0.65	0.00	0.00	0.61	
6 .25 ppm	0.66	0.66	0.00	0.00	0.66	

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	0.041	0.008	1.636	3.69
Within	8	0.040	0.005		
Total	13	0.080			

MYSID SHRIMP LIFE-CYCLE TEST

Analysis of Female Wt Data Using Dunnett's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 0.140.
This represents a 15.11% reduction in Female Wt .
T= 2.66 p=0.05 (one-tailed test)

Female Wt Data

Group	1	2	3	4	Mean	S
1 CONTROL	0.96	0.97	0.90	0.87	0.92	
2 .015 ppm	0.86	0.87	0.00	0.00	0.87	
3 .031 ppm	0.85	0.87	0.00	0.00	0.86	
4 .063	0.77	0.86	0.00	0.00	0.82	
5 .10 ppm	0.79	0.77	0.00	0.00	0.78	*
6 .25 ppm	0.82	0.63	0.00	0.00	0.73	*

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	0.066	0.013	3.574	3.69
Within	8	0.029	0.004		
Total	13	0.095			

Msy No. _____ Chemical Name Iprodione Technical Chemical Class _____ Page 2 of _____

Study/Species/Lab/ Succession	Chemical % Active	Results				Reviewer/ Date	Validat Status
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Che Inh.	
Species:		Control					
		Treatment I					
Lab:		Treatment II					
		Treatment III					
Acc*;		Study Duration:					
		Comments:					

Field Study(Simulated/Actual)		Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
Species:		Control						
		Treatment I						
Lab:		Treatment II						
Acc.*;		Treatment III						
		Crop/Site:		Study Duration:				
		Comments:						

Chronic fish,		Concentrations Tested (pp__)=					
Species		MATC = >____ <____ pp__.	Effected Parameter =				
Lab:		Contr. Mort.(%)=	Sol. Contr. Mort.(%)=				
Acc.*;		Comments:					

Chronic invertebrate		Concentrations Tested (ppm*)= 0.015, 0.031, 0.063, 0.10, 0.25			
Species Mysidopsis bahia	100	MATC =>____ < 0.015 ppm.*	Effected Parameter(s) Reproduction		Sup
Lab Springborn Life Sciences, Inc.		Contr. Mort.(%)= 16	Sol. Contr. Mort.(%)= 13	PK 7/8/88	
Acc.* 405508-02		Comments: * mean measured concentrations.			

Addendum to Mysid Life Cycle Study
Iprodione

In addition to the discrepancies noted in section 14, the following concerns were also identified.:

As mentioned earlier in the review, a reproductive success NOEL was not determined. The lowest concentration tested was significantly different from the control. To estimate the NOEL by extrapolating the concentration and reproductive effect relationship is unwise especially, since the proposed use patterns exceed the lowest dose tested.

THe solvent control data and the control data should not be pooled when conducting statistical analysis. Even with using just the solvent control data, a NOEL was not determined for reproductive success using the ANOVA.

In the future, the study design should include length as well as weight for a measurement of growth.

The study author did not indicate if the mysids were randomly distributed within the test aquaria when pairing the organisms on day 15.

The no. of young per treatment level are reported in Table A.

The reported measured concentrations were higher than 30 % than the time-weighted average measured concentration for more than 5% of the duration of the test. See Table 2. Specifically, the 0.015 (mean measured)treatment level showed concentrations as high as 0.021 mg/l. The 0.031 concentration level showed levels as high as 0.044 mg/l, and the highest dose tested showed levels as high as 0.37 mg/l. On the average, the measured concentrations were less than 50 % of the nominal concentrations. The same testing facility demonstrated in a fish early life stage study that the measured concentrations were 84 -89 % of the nominal at approximately the same concentrations levels tested. The study author should account for this discrepancy.

The mysid weight should have been recorded to the nearest ug, not 0.01 mg.

Conclusions:

The study is scientifically sound, but does not fulfill guideline requirement no. 72-4, for Marine Invertebrate Life Cycle Study. A NOEL was not determined for reproductive success. There was an effect at the lowest dose tested, 0.015 mg/l mean measured concentration. Before the study is repeated, the discrepancies noted in section 14 and in the addendum should be reviewed by the testing laboratory.

TABLE A
No. F1 Generation Per Treatment Group

mean measured concentrations mg/l		no. young per treatment group
Control	A	91
	B	107
Solvent Control	A	78
	B	92
0.015	A	57
	B	54
0.031	A	34
	B	33
0.063	A	28
	B	26
0.10	A	12
	B	2
0.25	A	6
	B	1

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