7/20/88

Accession Number 405508-01

DATA EVALUATION RECORD

1. CHEMICAL: Iprodione Technical.

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

3. STUDY TYPE: Freshwater Fish Early Life Stage Test.
   Species Tested: Pimephales promelas.

4. CITATION: Surprenant, D. C. 1988. The Toxicity of Iprodione Technical to Fathead Minnow (Pimephales promelas) Embryos and Larvae. Prepared by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Rhone-Poulenc AG Company, North Carolina. Report No. 88-2-2639. Accession No. 405508-01.

5. REVIEWED BY:

   Prapimpan Kosalwat, Ph.D.
   Staff Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: Prapimpan Kosalwat
   Date: 7/11/88

6. APPROVED BY:

   Isabel C. Johnson, M.S.
   Principal Scientist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: Isabel C. Johnson
   Date: 7-11-88

   Henry T. Craven
   Supervisor, EEB/HED
   USEPA

   Signature: Henry T. Craven 7/20/88
   Date: Cindy Brassard 7/20/88

7. CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for a fish early life stage test. Based on significant adverse effects on larval survival, the MATC of Iprodione Technical for Pimephales promelas was estimated to be > 0.26 mg/L and < 0.55 mg/L (geometric mean MATC = 0.38 mg/L).

8. RECOMMENDATIONS:

8. RECOMMENDATIONS: The testing laboratory should be informed that the early life stage toxicity study should include four replicate embryo cups per test concentration. Since this study has been conducted since the issuance of the SEP Guidelines, it should have been conducted according to the recommended protocols in the 1986 SEP Guidelines for fish early life stage toxicity testing. Other discrepancies noted in section 14 should be reviewed by the testing facility.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Fertilized eggs of fathead minnow (Pimephales promelas) were obtained from the fathead minnow culture unit maintained at Springborn Life Sciences, Inc. (SLS).

B. Test System: A modified proportional diluter, similar to that described by Mount and Brungs (1967) with a 0.50 dilution factor, was used to prepare and deliver the selected test concentration range of Iprodione Technical to the aquaria during the 34-day exposure. The dilution and control water was well water which was pumped into an epoxy-coated concrete reservoir where it was supplemented with Town of Wareham untreated well water and aerated. Weekly characterization of the well water established the total hardness and alkalinity ranges of 24-32 and 26-32 mg/L as $CaCO_3$, respectively; the pH range was 7.2-7.3; and the specific conductivity range was 90-140 umhos/cm during the study period.

The diluter was calibrated to deliver five nominal concentrations of Iprodione Technical, a dilution water control, and a solvent (79 uL/L of acetone) control to duplicate test aquaria. The solvent control solutions contained a concentration of acetone which equaled the solvent level in the highest Iprodione Technical treatment level.

Each glass test aquarium measured 39 x 20 x 25 cm with a 19.5-cm high side drain that maintained a constant exposure solution volume of 15 L. The diluter delivered 0.5 L of solution to each aquarium at an average rate of 195 times per day. This delivery rate was equivalent to a flow rate of approximately 6.5 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 9 hours. Sixteen hours of light at an intensity of 26-140 footcandles at the water surface were provided each day. The aquaria were impartially positioned in a water bath containing circulating water designed to maintain the test solution temperatures at 25 $\pm$ 1°C.

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C. Dosage: The study included the following five concentrations: 2.5, 1.2, 0.62 0.31, and 0.15 mg/l (nominal concentrations), and a solvent control and control.

D. Design: Based on a preliminary range-finding test conducted at Springborn Life Sciences, Inc., under flow-through conditions, sixty (<24 hour old) embryos were impartially selected and distributed to each of 14 embryo incubation cups, one of which was then suspended in each duplicate test aquarium per exposure concentration and the controls. Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex screen bottoms. A rocker arm apparatus was used to gently oscillate the incubation cups in the test solutions. Dead embryos were counted daily until hatching was complete. Hatching was deemed complete (exposure day 4) when no more than 5 unhatched viable embryos remained in any egg incubation cup. Calculations of percentage survival of organisms at hatch were based on the number of live larvae and embryos per incubation cup after hatching was complete compared to the number of embryos per cup on test day 0.

To initiate the 30-day post-hatch larval exposure, 40 live larvae were impartially selected from the surviving larvae in each incubation cup on test day 4 and placed into their respective exposure aquaria. Larvae were fed live brine shrimp (Artemia salina) nauplii three times daily on weekdays and twice daily on weekends and holidays. Aquaria were brushed and siphoned when necessary to remove excess food and fecal matter.

Behavior and appearance of larvae were observed and recorded daily, and larval survival was estimated twice weekly. At 30 days post-hatch exposure (test termination), the percentage larval survival was determined. The larvae were measured and weighed individually to calculate the mean and standard deviation of total length and wet weight.

Dissolved oxygen concentration, pH and temperature were measured in each replicate aquarium daily. Total hardness as $CaCO_3$ was measured on day 0 and weekly thereafter in alternating replicates of the high and low test concentrations, the dilution water control, and the solvent control. Samples were removed from all replicate test solutions and the controls on test days 0, 4, 11, 18, 25, 32, and 34, and analyzed for Iprodione Technical.

E. Statistics: Statistical analyses were performed using the mean organism response of each replicate aquarium. One-way, single classification analyses of variance were conducted on each measured or calculated endpoint to compare with the control and solvent control data. Since no statistically significant ($P \leq 0.05$) differences were found between control and solvent control

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data for any of the measured toxic endpoints, the 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 dose levels of Iprodione Technical was based on Williams' test.

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: At the concentrations of Iprodione Technical tested, mean dissolved oxygen, pH, and total hardness varied minimally and were not affected by the established concentration gradient of Iprodione Technical (Table 1, attached). During the test, the diluter system consistently prepared and delivered the appropriate concentration of Iprodione Technical to the exposure aquaria. The mean measured concentrations of Iprodione established in the test solutions during the exposure period were typically between 84 and 92% of the nominal treatment levels.

A summary of the biological results of the continuous 34-day exposure of fathead minnow embryos and larvae to measured concentrations of Iprodione is presented in Table 5 (attached). Fathead minnow survival at completion of the hatching period (day 4) in the highest treatment level (2.2 mg/L Iprodione Technical) was reduced as compared to the survival of the pooled control organisms. After 30 days post-hatch exposure, larval survival in the 2.2-, 1.1-, and 0.55-mg/L treatment levels was significantly less than the survival of the pooled control larvae.

Since larval survival was significantly reduced at the three highest test concentrations, growth data for these treatment levels were not statistically compared to the growth of control organisms. Mean total length and mean wet weight of larvae in the two lowest treatments were not statistically different from those of the pooled control larvae.

Based on the significantly ($P \leq 0.05$) reduced larval survival at concentrations $\geq$ 0.55 mg/L Iprodione, the MATC for this material and fathead minnow was estimated to be > 0.26 and < 0.55 mg/L (geometric mean MATC = 0.38 mg/L).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: Based on significant adverse effects on larval survival, the MATC of Iprodione Technical for fathead minnow was estimated to be > 0.26 mg/L and < 0.55 mg/L (geometric mean MATC = 0.38 mg/L).

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

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure is generally in accordance with the SEP guidelines and the study appears to be well conducted. However, there were some deviations from the protocols as follows:

o There was no information on how fertilized eggs were obtained from the culture.

o The total hardness of dilution water used in the test was slightly lower than the recommended hardness of 40-48 mg/L as $CaCO_3$. Also, there was no report whether the water had been analyzed for pesticides, heavy metals, and other possible contaminants.

o There was a contradiction in the report concerning the turnover rate of solution in each aquarium. The author stated that "the delivery rate was equivalent to a flow rate of approximately 6.5 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 9 hours." A 90% replacement time of 9 hours would mean the flow rate of much less than 6.5 aquarium volumes per 24-hour period.

o Only two replicate incubation cups with 60 embryos in each cup were used per treatment level and control. The SEP recommends a minimum of 20 embryos per replicate cup with four replicates per concentration (80 embryos total).

o Time to swim-up was not noted in the study report or in the raw data.

o The counting should have been on days 11, 18, 25 and 32 of the study. See Table A for the no. live fry and observations on days 9, 16, 23 and 30.

o The study author should not have pooled the solvent control and the control.

o The relative standard deviation (RSD) of weights of the fish that were alive at the test termination in the control chamber must

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not be greater than 40 %. This study indicated that the RSD was as great as 38% in the control, replicate B.

o The study author should have indicated if the eggs used for the study were obtained from at least three females and fertilized from the sperm of at least three males.

o See Table B for time to hatch. Hatching time ranged from 3 to 5 days, depending on the concentration level.

o The light intensity varied considerably, from 26 to 140 footcandles.

B. Statistical Analysis: The statistical analyses conducted by the author were appropriate. The reviewer reanalyzed the data and obtained similar results (attached).

C. Discussion/Results: Larval survival was the most sensitive indicator of Iprodione toxicity among the toxic endpoints used in this study. Based on statistical analysis ($P \leq 0.05$), larval survival was reduced at concentrations $\geq$ 0.55 mg/L Iprodione. Therefore, the MATC of Iprodione Technical for fathead minnow was determined to be > 0.26 mg/L and < 0.55 mg/L.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: Although the test procedure slightly deviated from the SEP guidelines, the reviewer does not believe it affected the toxicity results.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, July 6, 1988.

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% Survival at hatch.

GROWTH AND SURVIVAL 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 0.009.
This represents a 1.12% reduction in Survival.
T= 2.65 p=0.05 (one-tailed test)

Survival Data

Group	1	2	3	4	Mean	S
1 CONTROL	75	82	77	82	79	
2 0.13 ppm	75	83	0	0	79	
3 0.26 ppm	83	82	0	0	83	
4 0.55 ppm	75	77	0	0	76	
5 1.1 ppm	78	80	0	0	79	
6 2.2 ppm	73	67	0	0	70	*

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	0.025	0.005	3.044	3.69
Within	8	0.013	0.002		
Total	13	0.039			

% Larval Survival

GROWTH AND SURVIVAL 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 0.015.
This represents a 1.64% reduction in Survival.
T= 2.66 p=0.05 (one-tailed test)

Survival Data

Group	1	2	3	4	Mean	S
1 CONTROL	90	90	90	88	90	
2 0.13 ppm	85	88	0	0	87	
3 0.26 ppm	78	90	0	0	84	
4 0.55 ppm	83	78	0	0	81	*
5 1.1 ppm	55	48	0	0	52	*
6 2.2 ppm	5	2	0	0	4	*

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	1.774	0.355	127.443	3.69
Within	8	0.022	0.003		
Total	13	1.796			

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GROWTH AND SURVIVAL TEST

Analysis of Length Data Using Dunnett's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 1.953.
This represents a 6.46% reduction in Length .
T= 2.66 p=0.05 (one-tailed test)

Length Data

Group	1	2	3	4	Mean	S	
1 CONTROL	31.00	29.00	31.00	30.00	30.25		
2 0.13 ppm	31.00	31.00	0.00	0.00	31.00		
3 0.26 ppm	30.00	29.00	0.00	0.00	29.50		
4 0.55 ppm	29.00	28.00	0.00	0.00	28.50		
5 1.1 ppm	27.00	27.00	0.00	0.00	27.00	*	
6 2.2 ppm	20.00	22.00	0.00	0.00	21.00	*	

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	142.607	28.521	39.682	3.69
Within	8	5.750	0.719		
Total	13	148.357			

STUDENT'S T-TEST (two-tailed)

Enter the name of the DATAFILE you wish to analyze: WETWT.DAT
(Press RETURN if you wish to skip directly to T evaluation)

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

	1 'solcon'	2 'con'
Means =	267	259
Variances =	18	146.0001

Are these INDEPENDENT or PAIRED samples? (I or P) I

T = .8834522 df = 2

p = .4701871

The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples can be calculated as:

8 +/- T(2) * 1

Do you want another T-TEST using this datafile?

GROWTH AND SURVIVAL TEST

Analysis of Wet Weight Data Using Dunnett's Test

No transformation applied before data analysis.
For this set of data, the minimum significant difference is 26.517.
This represents a 10.08% reduction in Wet Weight.
T= 2.66 p=0.05 (one-tailed test)

Wet Weight Data

Group	1	2	3	4	Mean	S
1 CONTROL	268.00	250.00	270.00	264.00	263.00	
2 0.13 ppm	270.00	273.00	0.00	0.00	271.50	
3 0.26 ppm	261.00	234.00	0.00	0.00	247.50	
4 0.55 ppm	240.00	233.00	0.00	0.00	236.50	
5 1.1 ppm	186.00	205.00	0.00	0.00	195.50	*
6 2.2 ppm	68.00	90.00	0.00	0.00	79.00	*

Asterisk (*) indicates significant difference from controls.

Analysis of Variance

Source	DF	Sum of Sq.	Mean Sq.	Calc F	F(0.05)
Among	5	55646.880	11129.380	83.995	3.69
Within	8	1060.000	132.500		
Total	13	56706.880			

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Study No. _____ Chemical Name Iprodione Technical Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Succession _____ Chemical % Active _____ Results _____ Reviewer/ Date _____ Valid Stat

Avian Reproduction,

Species: _____

Lab: _____

Acc*; _____

Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Chg Inh.
Control				
Treatment I				
Treatment II				
Treatment III				

Study Duration:

Comments:

Field Study(Simulated/Actual)

Species: _____

Lab: _____

Acc.*; _____

Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)
Control				
Treatment I				
Treatment II				
Treatment III				

Crop/Site: Study Duration:

Comments:

Chronic fish,

Species Pimephales Promelas 100

Lab: Springborn Life Sciences, Inc.

Acc.*; 405508-01

Concentrations Tested (ppm*)= 0.13, 0.26, 0.55, 1.1, 2.2

MATC = >0.26<0.55 ppm* Effected Parameter = Larval Survival

Conctr. Mort.(%)= 10 Sol. Contr. Mort.(%)= 11

Comments: * mean measured concentrations.

PK 7/6/88

Chronic invertebrate

Species _____

Lab _____

Acc.* _____

Concentrations Tested (pp__)= _____

MATC =>____< ____pp__. Effected Parameter(s)_____

Conctr. Mort.(%)=_____ Sol. Contr. Mort.(%)=_____

Comments:

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Table A
Post Hatch

Nominal Conc. mg/l		Day 9 L	Day 9 D	Day 16 L	Day 16 D	Day 23 L	Day 23 D	Day 30 L	Day 30 D
2.5	A	3	-	2	-	2	-	2	-
	B	4	-	3	-	3	-	1	-
1.3	A	26	-	25	-	23	2	22	-
	B	21	1	21	-	21	2	19	-
0.63	A	40	-	40	-	40	3	33	1
	B	36	-	33	-	32	3	31	2
0.31	A	38	-	38	-	37	6	31	-
	B	39	-	39	-	39	5	36	2
0.16	A	39	-	39	-	39	1	34	1
	B	39	-	39	-	38	5	35	-
Control	A	40	-	40	-	40	3	36	-
	B	40	-	39	-	39	6	36	3
Solvent Control	A	40	-	40	-	40	3	36	1
	B	39	1	39	-	38	3	35	1

L= live larvae, D= deformed and dark larvae

Table B

Time to Hatch

Nominal Conc. mg/l		Day 3	Day 4	Day 5
2.5	A	-	16	44
	B	1	11	40
1.3	A	-	47	47
	B	1	51	48
0.63	A	3	49	45
	B	2	49	46
0.31	A	1	50	-
	B	1	49	-
0.16	A	-	45	-
	B	1	50	-
Control	A	-	45	-
	B	4	49	-
Solvent Control	A	-	46	-
	B	3	49	-