6/15/88

Accession Number 404892-06

DATA EVALUATION RECORD

1. CHEMICAL: ROVRAL 50 WP

2. TEST MATERIAL: Formulation: ROVRAL 50 WP, 50 Percent Iprodione as active ingredient. A tan-colored powder.

3. STUDY TYPE: Acute Toxicity Test For Freshwater Invertebrate, Flow-Through.
Species Tested: Daphnia magna

4. CITATION: Surprenant, D.C. 1988. Acute Toxicity of ROVRAL 50 WP to Daphnids (Daphnia magna) Under Flow-through Conditions, Bionomics Report # 87-12-2597. Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. Submitted by Rhone-Poulenc Ag Co., Research Triangle Park, North Carolina. Report #87-12-2597. Accession Number 404892-06.

5. REVIEWED BY:

Brian A. Wade
Aquatic Toxicologist
Hunter Environmental Services, Inc.

Signature: Brian A. Wade
Date: June 15, 1988

6. APPROVED BY:

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
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Signature: Prapimpan Kosalwat
Date: 6-15-88

Henry T. Carven
Supervisor, EEB/HED
USERPA

Signature:
Date:

7. CONCLUSIONS: This study is scientifically sound, but does not fulfill the guideline requirements for an acute toxicity test for a freshwater invertebrate species. As a result, this study has been rated supplemental. The 48 hour EC50 value for Rovral 50 WP, as Iprodione, was 0.36 mg A.I./L. Therefore, Rovral 50 WP is classified as highly toxic to Daphnia magna. The NOEC was 0.13 mg A.I./L.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna were obtained from cultures maintained at Springborn's Wareham, Mass. laboratories. The cultures were maintained with water classified as hard by ASTM (total hardness of 160-180 mg/L as $CaCO_3$), but the test system water had a total hardness of 30 mg/L as $CaCO_3$. To avoid potential problems with this, first instar daphnids were slowly acclimated (approximately 3 hours) to the lower hardness prior to test initiation.

B. Test System: The test was conducted under flow-through conditions with an intermittent-flow serial diluter with a 60 percent dilution factor. The dilution water used was characterized as having a total hardness and alkalinity of 30 mg/L as $CaCO_3$, a pH of 7.3, and a specific conductivity of 110 umhos/cm. The test was run for 48 hours with test solutions maintained at 20 $\pm$ 1 ^{o}C. The photoperiod utilized was 16 hours light and 8 hours light.

C. Dosage: 48-hour acute flow-through test.

D. Design: Twenty Daphnia magna were tested per test vessel , four vessels per treatment/control, for a total of eighty animals per exposure level. A control and nominal ROVRAL 50 concentrations (as active ingredient) of 0.23, 0.39, 0.65, 1.1, and 1.8 mg/L were maintained. The mean measured concentrations were 0.13, 0.34, 0.58, 0.90, and 1.6 mg/L.

Dissolved oxygen concentration, temperature, and pH were measured once daily in one replicate vessel of each treatment level and the control. Total hardness, alkalinity, and specific conductance were measured at test initiation in one replicate vessel of each treatment level and the control.

E. Statistics: The computer program developed by Stephan et al. was used to calculate the EC50 values.

12. REPORTED RESULTS: Water quality parameters (pH, dissolved oxygen, temperature, specific conductance, hardness and alkalinity) measured were consistent throughout the exposure period and were unaffected by the concentrations of ROVRAL 50 WP tested.

The results of the 48-hour exposure are presented in Table 2 (attached). The 48-hour EC50 (95% confidence interval) for ROVRAL 50 WP and Daphnia magna was calculated to be 0.36 (0.34-0.39) mg A.I./L, based upon mean measured concentrations. A no-observed-effect concentration (NOEC) was determined to be 0.13 mg A.I./L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 48-hour EC50 for Daphnia magna exposed to ROVRAL 50 WP under flow-through conditions was 0.36 mg/L with 95% confidence limits of 0.34 and 0.39 mg A.I./L. The NOEC was 0.13 mg A.I./L.

The data were audited by the laboratory's Quality Assurance Unit to assure compliance with protocols, standard operating procedures and pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guideline, except concerning the test substance lot or batch number, which was not provided and the lack of a formulation inert control.

The test daphnids were acclimated to lower water hardness (from 160 mg/L to 30 mg/L as $CaCO_3$) within 3 hours which is considered a short time for that big difference in water hardness. Because very young animals ($\leq$ 24 hours old) were used in the test, the parents of the test daphnids should have been reared in the test water so the test animals could be acclimated since the time of birth.

The test temperature was controlled by a water bath. Therefore, temperature measurements should have been recorded every six hours as recommended by the SEP.

B. Statistical Analysis: The reviewer used the computer program developed by Stephan et al. to calculate the EC50. The calculations are attached. The calculated 48-hour EC50 value of 0.34 mg/L with confidence limits of 0.31 and 0.36 mg/L closely matches the study author's EC50 values.

C. Discussion/Results: The reported 48-hour EC50 value of 0.36 mg/L for Daphnia magna classifies ROVRAL 50 WP as highly toxic. The NOEC was determined to be 0.13 mg A.I./L.

D. Adequacy of the Study:

(1) Classification : Supplemental
(2) Rationale : Lack of appropriate formulation inert control.
(3) Repairability : Submission of inert control data.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes. June 13, 1988

No. 404892-06 Chemical Name ROVAL Chemical Class ____ Page 1 of ____

Study/Species/Lab/ Succession	Chemical % a.i	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Sol. Contr. Mort.(%)=		
Lab	____	96-Hour Dose Level ppm /(%Mortality) Temperature = ( ), ( ), ( ), ( ), ( )	____	____
Acc		Comments:		
96-Hour LC_{50}		LC50 = 0.36ppm (95% C.L. 0.34-0.39) Con. Mor(%)= 1%		
Species Daphnia magna		Slope= N/A # Animals/Level= 80 Sol. Con. Mor.(%)= N/A		
Lab Springborn Life Science	50	96-Hour Dose Level ppm /(%Mortality) Temp.= 20± 1oC	BAW 6/13/88	Supp
Acc. 404892-06		0.13 (0), 0.34 (39), 0.58 (98), 0.90 (100) 1.6 (100)		
		Comments: Reported as mean measured concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Sol. Contr. Mort.(%)=		
Lab	____	96-Hour Dose Level ppm /(%Mortality) Temp.= ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(%)
1.6	80	80	100	8.271806E-23
.9	80	80	100	8.271806E-23
.58	80	78	97.5	2.680892E-19
.34	80	31	38.75	7.175819E-04
.13	80	0	0	8.271806E-23

THE BINOMIAL TEST SHOWS THAT .34 AND .58 CAN BE
USED AS STATISCALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.99928 PERCENT.
AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .3693951

>>>>>>>>RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.330038E-02	.3363596	.312142	.3633962

>>>>>>>RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
14	4.576388E-04	2.141317E-03	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 9.701809
95 PERCENT CONFIDENCE LIMITS = 9.494262 AND 9.909355

LC50 = .3639104
95 PERCENT CONFIDENCE LIMITS = .362256 AND .3655619
LC1 = .2094903
95 PERCENT CONFIDENCE LIMITS = .2066729 AND .2122327

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(%)
1.6	80	80	100	8.271806E-23
.9	80	30	37.5	7.175819E-04
.58	80	11	13.75	1.024758E-09
.34	80	22	27.5	7.170138E-04
.13	80	0	0	8.271806E-23

THE BINOMIAL TEST SHOWS THAT .9 AND 1.6 CAN BE
USED AS STATISCALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.99928 PERCENT.
AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .9788702

>>>>>>>>RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	2.950227E-02	.874586	.7921317	.9770438

>>>>>>>RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.982896	21.16483	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.348138
95 PERCENT CONFIDENCE LIMITS =-1.366553 AND 8.062829

LC50 = .8032223
95 PERCENT CONFIDENCE LIMITS = 0 AND + INFINITY
LC1 = .1621382
95 PERCENT CONFIDENCE LIMITS = 0 AND .455992

Table 2. Concentrations tested and corresponding cumulative number of immobilized organisms during the 48-hour exposure of daphnids (Daphnia magna) to ROVRAL 50 WP. Measured concentrations are expressed based on the concentration of active ingredient (IPRODIONE).

Mean Measured Concentration (mg A.I./L)	Cumulative Number of Immobilized Organisms (%)									
	24-Hour					48-Hour				
	A	B	C	D	Mean	A	B	C	D	Mean
Control	0	0	0	0	0	5	0	0	0	1
0.13	0	0	0	0	0[a]	0	0	0	0	0
0.34	75	5	15	15	28[b]	75	15	30	35	39[d]
0.58	5	15	5	30	14[c]	100	90	100	100	98[d]
0.90	15	30	25	80	38[c]	100	100	100	100	100
1.6	100	100	100	100	100	100	100	100	100	100

[a]One daphnid was entrapped on the surface of the exposure solution.

[b]One daphnid was lethargic.

[c]All daphnids were lethargic.

[d]All surviving daphnids were swimming erratically on the bottom of the test vessel.