

US EPA ARCHIVE DOCUMENT

11-2-93
[Handwritten signature]

DP Barcode : D180647
 PC Code No : 113501
 EEB Out : NOV 2 1993

To: Linda Propst
 Product Manager 53
 Special Review and Reregistration Division (H7508W)

From: Anthony Maciorowski, Chief
 Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 113501
 Chemical Name : Metalaxyl
 Type Product : Fungicide
 Product Name : Ridomil 2E
 Company Name : Ciba-Geigy
 Purpose : Review Oyster Dep. study, data gaps?

Action Code : 627 Date Due : 11/12/92
 Reviewer : Dana Lateulere

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)	423781-01	Y	123-1(B)		
71-5(A)			72-3(F)	423781-01	Y	123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
 P=Partial (Study partially fulfilled Guideline but additional information is needed)
 S=Supplemental (Study provided useful information but Guideline was not satisfied)
 N=Unacceptable (Study was rejected)/Nonconcur

DP Barcode :D179489
 PC Code No : 113501
 EEB Out : NOV 2 1993

To: Linda Propst
 Product Manager 53
 Special Review and Reregistration Division (H7508W)

From: Anthony Maciorowski, Chief
 Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 113501
 Chemical Name : Metalaxyl
 Type Product : Fungicide
 Product Name : Ridomil 2E
 Company Name : Ciba-Geigy
 Purpose : Review Acute Mysid with TEP

Action Code : 627 Date Due : 10/14/92
 Reviewer : Dana Lateulere

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)	423375-01	Y	123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
 P=Partial (Study partially fulfilled Guideline but additional information is needed)
 S=Supplemental (Study provided useful information but Guideline was not satisfied)
 N=Unacceptable (Study was rejected)/Nonconcur

2



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

File

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Estuarine TEP Testing for Metalaxyl, D180647 and D179489.

To: Linda Propst
PM 53
Special Review and Reregistration Division, H7508W

From: *for* Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division, H7507C

Douglas J. Urban 11/1/93

EEB has completed a review of the Estuarine Toxicity testing submitted by Ciba-Geigy for Ridomil 2E, active ingredient Metalaxyl. The following is a summary of those reviews:

1). Machado, M.W. 1992. Ridomil®2E - Acute Toxicity to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. SLI Report No. 92-4-4196. Performed by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 423375-01.

This study is scientifically sound and meets the guideline requirements for an acute flow-through estuarine shrimp toxicity study using a technical end product (TEP), #72-3f. Based on mean measured concentrations, the 96-hour LC₅₀ of Ridomil®2E (a formulated product) for Mysidopsis bahia was 5.98 mg/L (1.5 mg a.i./l). Therefore, Ridomil®2E is classified as moderately toxic to mysid shrimp. The NOEC was 2.36 mg/L (0.59 mg a.i./l).

2). Dionne, E. 1992. (Ridomil 2E) - Acute Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions. SLI Report No. 92-6-4291. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 423781-01.

This study is scientifically sound and meets the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test using a technical end use product (TEP), #72-3e. Based on mean measured concentrations, the 96-hour EC₅₀ of Ridomil 2E (a formulated product) was 4.44 mg/L (1.1 mg a.i./l). Therefore, Ridomil 2E is classified as moderately toxic to Crassostrea virginica. The NOEC was 1.80 mg/L (0.45 mg a.i./l).



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

3

As requested, the following is a list of unfulfilled data requirements for metalaxyl:

- #72-3c Estuarine Shrimp (TGAI)
- #72-3b Estuarine Mollusc (TGAI)
- #141-1 Honey bee Acute contact

Questions regarding this review, please contact Dana Lateulere at 308-2856.

file

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Metalaxyl. Shaughnessey No. 113501.
- 2. **TEST MATERIAL:** Ridomil 2E; CAS No. 57837-19-1; Lot No. FL No. 892783; 25.0% metalaxyl (the active ingredient); a brown liquid.
- 3. **STUDY TYPE:** 72-3 ϕ , Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).
- 4. **CITATION:** Dionne, E. 1992. (Ridomil 2E) - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions. SLI Report No. 92-6-4291. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 423781-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Rosemary Graham Mora
Date: 9/21/92
Doreen Kater
3/11/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Louis M. Rifici
9/23/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:

Ann Standa
10/28/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. Based on mean measured concentrations, the 96-hour EC₅₀ of Ridomil 2E (a formulated product) was 4.44 mg/L. Therefore, Ridomil 2E is classified as moderately toxic to *Crassostrea virginica*. The NOEC was 1.80 mg/L.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Eastern oysters (*Crassostrea virginica*) were obtained from P. Cummins Oyster Co., Pasadena, MD. Once received in the laboratory, the animals were held in wooden epoxy-painted trays in flowing seawater and examined for suitability in the test. The oysters were maintained at 15-22°C for 22 days prior to test initiation. During this acclimation period, the salinity was adjusted from 15 to 28 parts per thousand (ppt) in the first 8 days and was maintained at 31-32 ppt for the remainder of the acclimation period. The oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata*.

The oysters were of similar age and size and had a mean valve height of 41 ±5 mm. Less than one-percent mortality occurred during the acclimation period.

- B. Test System: The test was conducted in a constant-flow serial diluter system (60% dilution factor). This system provided a flow rate of 75 ml/minute to each aquarium, providing six volume replacements/day. The glass test aquaria (60 x 30 x 30 cm) were each equipped with a 10-cm standpipe to regulate solution volume at 18 l. Circulation (flow rate of 1.75 l/minute) within each aquarium provided an even distribution of algae and test solution. The flow rate of the recirculated volume of test solution was 5 l/oyster/hour.

A temperature-controlled water bath was used to maintain test temperature (20 ±2°C). The test was conducted under fluorescent lighting on a 16-hour light and 8-hour dark photoperiod. Sudden transition from light to dark and dark to light were avoided.

The dilution water was natural, unfiltered seawater collected from Cape Cod Canal, Bourne, MA. The seawater had a pH of 7.8 and a salinity of 31 ppt.

The test material, identified as containing 241 mg a.i./ml, was delivered, along with dilution water, to the chemical mixing chamber of the diluter. The concentration of solution in the mixing chamber was equivalent to the highest nominal test concentration (2.0 mg a.i./l) and was proportionally diluted to produce the remaining test concentrations.

- C. **Dosage:** Ninety-six-hour flow-through acute test. Based on preliminary testing, five nominal test concentrations (0.26, 0.43, 0.72, 1.2, and 2.0 mg a.i./l) were selected for this study. A dilution water control was also included.
- D. **Design:** One day prior to test initiation, 3-5 mm of the new peripheral shell growth of each oyster were removed by grinding the shell to a blunt edge. Immediately prior to test initiation, the outer shell edge was buffed to remove any new shell deposition.

The test was initiated by impartially selecting and placing 20 oysters in each of two replicate aquaria per treatment (i.e., 40 oysters/treatment). During the exposure period, the oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata* three times daily.

At test initiation and every 24 hours thereafter, the oysters were observed for visible abnormalities and the physical characteristics of the test solutions were observed. The oysters were removed from the test containers after 96 hours of continuous exposure and new shell growth of each oyster was measured to the nearest 0.1 mm using a calibrated micrometer.

The temperature, salinity, pH, and dissolved oxygen concentration were measured daily in each aquarium. The temperature was also continuously monitored in one replicate of the control.

Chemical analysis of metalaxyl (the active ingredient of Ridomil 2E) was performed, using gas chromatography, on each test solution collected on days 0 and 4 to verify the test concentrations.

- E. **Statistics:** The EC_{50} values (with 95% confidence limits) were determined by fitting untransformed and transformed data to a best fit linear regression curve based on least squares. Four linear regression curves were computed and the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination (i.e., r^2). This regression equation was then applied to calculate the EC_{50} and 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981). A computer program was used to assist in the calculation.

Williams' test method (1971, 1972) coupled with Bartlett's test for homogeneity of variance was used to determine the NOEC.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.27, 0.45, 0.63, 1.2, and 2.0 mg a.i./l which averaged 99% of nominal concentrations (Table 2, attached). No undissolved test material was observed in the test chambers or the diluter system.

No mortalities or sublethal effects were noted. Based on mean measured concentrations and new shell growth relative to the control, the EC₅₀ was 1.1 mg a.i./l (Table 3, attached). The slope of the dose-response curve was 3.3. The NOEC was 0.45 mg a.i./l.

During the test period, the pH was 7.8-8.0, the dissolved oxygen concentration was 7.0-7.6 mg/l, the temperature was 20-22°C, and the salinity was 31 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"Based on US EPA (1985) criteria, Ridomil 2E would be classified as moderately toxic to the Eastern oyster."

Good Laboratory Practice and quality assurance statements were included in the report, indicating that the study was conducted in accordance with the EPA Good Laboratory Practice regulations (40 CFR Part 160) except for the stability, characterization, and verification of the test substance identity.

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

- A. **Test Procedure:** The test procedures were generally in accordance with the SEP, but deviated as follows:

The report did not indicate whether the technical grade on the test material was used. The percentage of active ingredient was 25.0%, therefore, it is likely that a formulated product was used; in which case, a control containing the inert material present in the formulation should have been included in the study.

In this study, the flow rate of the "recirculating" test solution was about 5 l/oyster/hour. According to protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 l of "once-through" flow through test solution per hour. However, for this study, it is probably acceptable since a supplemental algal diet was added

and control oysters met the minimum new shell growth requirement (2 mm).

- B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the 96-hour EC_{50} . ~~The 96-hour EC_{50} was 1.1 mg a.i./l with a 95% confidence interval of 0.99-1.2 mg a.i./l. These results are similar to those of the author (printouts, attached).~~

The new shell growth data (squareroot transformed) met the assumptions of homogeneity of variance and normality (Hartley and chi-square test, respectively). Therefore, the reviewer determined the NOEC using Williams' test (printouts, attached). Based on new shell growth relative to the control oysters, the NOEC was mg ~~the same NOEC as author reported.~~

- C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. Based on mean measured concentrations, the 96-hour EC_{50} of Ridomil 2E (a formulated product) for *Crassostrea virginica* was 4.44 mg/L. Therefore, Ridomil 2E is classified as moderately toxic to the *Oyster*. The NOEC was 1.80 mg/L. DR.

- D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, September 11, 1992.

METALAXYL

Page _____ is not included in this copy.

Pages 10 through 11 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
 - Identity of product impurities.
 - Description of the product manufacturing process.
 - Description of quality control procedures.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
 - Information about a pending registration action.
 - FIFRA registration data.
 - The document is a duplicate of page(s) _____.
 - The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

LATEULERE RIDOMIL 2E OYSTER SHELL INHIBITION OF GROWTH

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8	100	70	70	9.536742E-05
4.8	100	60	60.00001	5.765915
2.52	100	30	30	2.069473
1.8	100	0	0	.5908966
1.08	100	5	5	.1288414

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.883941

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.0188679	4.444225	4.098764	4.861164

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
GOODNESS OF FIT PROBABILITY		
4	.5315448	7.498317

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 = 2.962115
 95 PERCENT CONFIDENCE LIMITS = .8025225 AND 5.121707

LC50 = 4.624152
 95 PERCENT CONFIDENCE LIMITS = 2.84582 AND 13.57856

LC10 = 1.722996
 95 PERCENT CONFIDENCE LIMITS = .2032579 AND 2.808918

Metalaxyl C. virginica

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2	100	72	72	0
1.2	100	61	61	0
.63	100	27	27	0
.45	100	1	1	0
.27	100	4	4	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .9782338

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.0241093	1.086186	.9948254	1.197328

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.3864385	5.740397	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 = 3.14106
 95 PERCENT CONFIDENCE LIMITS = 1.188446 AND 5.093675

LC50 = 1.14265
 95 PERCENT CONFIDENCE LIMITS = .7689378 AND 2.281883

LC10 = .4503924
 95 PERCENT CONFIDENCE LIMITS = .1231027 AND .6854341

Metalaxyl: GROWTH OF EXPOSED EASTERN OYSTERS
File: c:42378101.oys Transform: SQUARE ROOT(Y)

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	16.080	58.080	91.680	58.080	16.080
OBSERVED	18	49	89	76	8

Calculated Chi-Square goodness of fit test statistic = 11.3163

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Metalaxyl: GROWTH OF EXPOSED EASTERN OYSTERS
File: c:42378101.oys Transform: SQUARE ROOT(Y)

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 1.85
Closest, conservative, Table H statistic = 3.6 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 30
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 39.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Metalaxyl: GROWTH OF EXPOSED EASTERN OYSTERS
 File: c:42378101.oys Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	40	1.980	1.347	1.347
2	0.272 mg/l	40	1.895	1.308	1.308
3	0.448 mg/l	40	1.960	1.291	1.291
4	0.626 mg/l	40	1.440	1.132	1.132
5	1.22 mg/l	40	0.773	0.753	0.753
6	2.03 mg/l	40	0.555	0.536	0.536

Metalaxyl: GROWTH OF EXPOSED EASTERN OYSTERS
 File: c:42378101.oys Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	1.347				
0.272 mg/l	1.308	0.373		1.66	k= 1, v=234
0.448 mg/l	1.291	0.536		1.73	k= 2, v=234
0.626 mg/l	1.132	2.063	*	1.75	k= 3, v=234
1.22 mg/l	0.753	5.703	*	1.77	k= 4, v=234
2.03 mg/l	0.536	7.781	*	1.77	k= 5, v=234

s = 0.466

Note: df used for table values are approximate when v > 20.

TITLE: Metalaxyl: GROWTH OF EXPOSED EASTERN OYSTERS

FILE: c:42378101.oys

TRANSFORM: SQUARE ROOT(Y)

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.4000	1.1832
1	Control	2	1.1000	1.0488
1	Control	3	3.3000	1.8166
1	Control	4	2.8000	1.6733
1	Control	5	1.5000	1.2247
1	Control	6	2.0000	1.4142
1	Control	7	1.2000	1.0954
1	Control	8	0.6000	0.7746
1	Control	9	2.1000	1.4491
1	Control	10	2.8000	1.6733
1	Control	11	2.0000	1.4142
1	Control	12	1.6000	1.2649
1	Control	13	0.5000	0.7071
1	Control	14	1.3000	1.1402
1	Control	15	0.6000	0.7746
1	Control	16	2.8000	1.6733
1	Control	17	1.5000	1.2247
1	Control	18	1.2000	1.0954
1	Control	19	3.4000	1.8439
1	Control	20	2.5000	1.5811
1	Control	21	3.0000	1.7321
1	Control	22	2.3000	1.5166
1	Control	23	3.4000	1.8439
1	Control	24	0.6000	0.7746
1	Control	25	3.8000	1.9494
1	Control	26	2.2000	1.4832
1	Control	27	3.1000	1.7607
1	Control	28	3.6000	1.8974
1	Control	29	2.0000	1.4142
1	Control	30	3.3000	1.8166
1	Control	31	1.4000	1.1832
1	Control	32	1.7000	1.3038
1	Control	33	2.1000	1.4491
1	Control	34	0.0000	0.0000
1	Control	35	2.4000	1.5492
1	Control	36	1.3000	1.1402
1	Control	37	0.8000	0.8944
1	Control	38	0.8000	0.8944
1	Control	39	2.2000	1.4832
1	Control	40	3.0000	1.7321
2	0.272 mg/l	1	1.2000	1.0954
2	0.272 mg/l	2	0.4000	0.6325
2	0.272 mg/l	3	1.5000	1.2247
2	0.272 mg/l	4	0.9000	0.9487
2	0.272 mg/l	5	4.2000	2.0494
2	0.272 mg/l	6	3.5000	1.8708
2	0.272 mg/l	7	1.1000	1.0488
2	0.272 mg/l	8	3.2000	1.7889
2	0.272 mg/l	9	1.3000	1.1402
2	0.272 mg/l	10	2.5000	1.5811

17

2	0.272	mg/l	11	2.1000	1.4491
2	0.272	mg/l	12	2.6000	1.6125
2	0.272	mg/l	13	3.8000	1.9494
2	0.272	mg/l	14	3.7000	1.9235
2	0.272	mg/l	15	1.6000	1.2649
2	0.272	mg/l	16	0.8000	0.8944
2	0.272	mg/l	17	1.5000	1.2247
2	0.272	mg/l	18	2.1000	1.4491
2	0.272	mg/l	19	0.8000	0.8944
2	0.272	mg/l	20	2.2000	1.4832
2	0.272	mg/l	21	2.8000	1.6733
2	0.272	mg/l	22	1.4000	1.1832
2	0.272	mg/l	23	2.7000	1.6432
2	0.272	mg/l	24	0.6000	0.7746
2	0.272	mg/l	25	0.4000	0.6325
2	0.272	mg/l	26	2.5000	1.5811
2	0.272	mg/l	27	0.7000	0.8367
2	0.272	mg/l	28	1.4000	1.1832
2	0.272	mg/l	29	1.5000	1.2247
2	0.272	mg/l	30	2.7000	1.6432
2	0.272	mg/l	31	0.9000	0.9487
2	0.272	mg/l	32	1.6000	1.2649
2	0.272	mg/l	33	2.4000	1.5492
2	0.272	mg/l	34	3.0000	1.7321
2	0.272	mg/l	35	2.8000	1.6733
2	0.272	mg/l	36	1.7000	1.3038
2	0.272	mg/l	37	2.9000	1.7029
2	0.272	mg/l	38	0.6000	0.7746
2	0.272	mg/l	39	2.2000	1.4832
2	0.272	mg/l	40	0.0000	0.0000
3	0.448	mg/l	1	4.6000	2.1448
3	0.448	mg/l	2	2.6000	1.6125
3	0.448	mg/l	3	1.4000	1.1832
3	0.448	mg/l	4	2.9000	1.7029
3	0.448	mg/l	5	1.1000	1.0488
3	0.448	mg/l	6	5.7000	2.3875
3	0.448	mg/l	7	2.3000	1.5166
3	0.448	mg/l	8	2.4000	1.5492
3	0.448	mg/l	9	1.5000	1.2247
3	0.448	mg/l	10	2.6000	1.6125
3	0.448	mg/l	11	1.4000	1.1832
3	0.448	mg/l	12	3.5000	1.8708
3	0.448	mg/l	13	0.5000	0.7071
3	0.448	mg/l	14	0.3000	0.5477
3	0.448	mg/l	15	3.1000	1.7607
3	0.448	mg/l	16	0.4000	0.6325
3	0.448	mg/l	17	1.3000	1.1402
3	0.448	mg/l	18	0.0000	0.0000
3	0.448	mg/l	19	2.9000	1.7029
3	0.448	mg/l	20	0.4000	0.6325
3	0.448	mg/l	21	0.0000	0.0000
3	0.448	mg/l	22	2.9000	1.7029
3	0.448	mg/l	23	1.1000	1.0488
3	0.448	mg/l	24	1.2000	1.0954
3	0.448	mg/l	25	1.9000	1.3784
3	0.448	mg/l	26	4.9000	2.2136
3	0.448	mg/l	27	3.0000	1.7321
3	0.448	mg/l	28	1.8000	1.3416
3	0.448	mg/l	29	2.6000	1.6125
3	0.448	mg/l	30	1.1000	1.0488

3	0.448 mg/l	31	3.0000	1.7321
3	0.448 mg/l	32	3.0000	1.7321
3	0.448 mg/l	33	0.5000	0.7071
3	0.448 mg/l	34	1.7000	1.3038
3	0.448 mg/l	35	1.3000	1.1402
3	0.448 mg/l	36	0.7000	0.8367
3	0.448 mg/l	37	1.8000	1.3416
3	0.448 mg/l	38	0.4000	0.6325
3	0.448 mg/l	39	3.6000	1.8974
3	0.448 mg/l	40	1.0000	1.0000
4	0.626 mg/l	1	0.7000	0.8367
4	0.626 mg/l	2	1.8000	1.3416
4	0.626 mg/l	3	1.4000	1.1832
4	0.626 mg/l	4	1.1000	1.0488
4	0.626 mg/l	5	1.1000	1.0488
4	0.626 mg/l	6	2.5000	1.5811
4	0.626 mg/l	7	0.0000	0.0000
4	0.626 mg/l	8	1.7000	1.3038
4	0.626 mg/l	9	0.5000	0.7071
4	0.626 mg/l	10	0.4000	0.6325
4	0.626 mg/l	11	1.8000	1.3416
4	0.626 mg/l	12	2.3000	1.5166
4	0.626 mg/l	13	1.3000	1.1402
4	0.626 mg/l	14	4.2000	2.0494
4	0.626 mg/l	15	1.7000	1.3038
4	0.626 mg/l	16	1.2000	1.0954
4	0.626 mg/l	17	2.5000	1.5811
4	0.626 mg/l	18	1.0000	1.0000
4	0.626 mg/l	19	1.5000	1.2247
4	0.626 mg/l	20	2.5000	1.5811
4	0.626 mg/l	21	1.3000	1.1402
4	0.626 mg/l	22	0.9000	0.9487
4	0.626 mg/l	23	1.6000	1.2649
4	0.626 mg/l	24	1.2000	1.0954
4	0.626 mg/l	25	2.0000	1.4142
4	0.626 mg/l	26	1.3000	1.1402
4	0.626 mg/l	27	0.4000	0.6325
4	0.626 mg/l	28	1.7000	1.3038
4	0.626 mg/l	29	0.0000	0.0000
4	0.626 mg/l	30	2.9000	1.7029
4	0.626 mg/l	31	1.7000	1.3038
4	0.626 mg/l	32	1.3000	1.1402
4	0.626 mg/l	33	1.0000	1.0000
4	0.626 mg/l	34	1.2000	1.0954
4	0.626 mg/l	35	2.0000	1.4142
4	0.626 mg/l	36	1.1000	1.0488
4	0.626 mg/l	37	0.7000	0.8367
4	0.626 mg/l	38	2.3000	1.5166
4	0.626 mg/l	39	0.3000	0.5477
4	0.626 mg/l	40	1.5000	1.2247
5	1.22 mg/l	1	1.6000	1.2649
5	1.22 mg/l	2	1.2000	1.0954
5	1.22 mg/l	3	1.9000	1.3784
5	1.22 mg/l	4	1.4000	1.1832
5	1.22 mg/l	5	0.8000	0.8944
5	1.22 mg/l	6	1.0000	1.0000
5	1.22 mg/l	7	0.0000	0.0000
5	1.22 mg/l	8	0.3000	0.5477
5	1.22 mg/l	9	0.0000	0.0000
5	1.22 mg/l	10	0.7000	0.8367

5	1.22 mg/l	11	0.6000	0.7746
5	1.22 mg/l	12	0.4000	0.6325
5	1.22 mg/l	13	1.0000	1.0000
5	1.22 mg/l	14	1.5000	1.2247
5	1.22 mg/l	15	0.0000	0.0000
5	1.22 mg/l	16	1.1000	1.0488
5	1.22 mg/l	17	0.7000	0.8367
5	1.22 mg/l	18	0.0000	0.0000
5	1.22 mg/l	19	0.8000	0.8944
5	1.22 mg/l	20	0.0000	0.0000
5	1.22 mg/l	21	1.9000	1.3784
5	1.22 mg/l	22	1.2000	1.0954
5	1.22 mg/l	23	1.1000	1.0488
5	1.22 mg/l	24	0.6000	0.7746
5	1.22 mg/l	25	0.4000	0.6325
5	1.22 mg/l	26	0.0000	0.0000
5	1.22 mg/l	27	1.0000	1.0000
5	1.22 mg/l	28	1.5000	1.2247
5	1.22 mg/l	29	0.0000	0.0000
5	1.22 mg/l	30	0.6000	0.7746
5	1.22 mg/l	31	0.6000	0.7746
5	1.22 mg/l	32	0.0000	0.0000
5	1.22 mg/l	33	0.5000	0.7071
5	1.22 mg/l	34	0.6000	0.7746
5	1.22 mg/l	35	0.9000	0.9487
5	1.22 mg/l	36	1.9000	1.3784
5	1.22 mg/l	37	0.5000	0.7071
5	1.22 mg/l	38	0.0000	0.0000
5	1.22 mg/l	39	1.1000	1.0488
5	1.22 mg/l	40	1.5000	1.2247
6	2.03 mg/l	1	0.0000	0.0000
6	2.03 mg/l	2	0.3000	0.5477
6	2.03 mg/l	3	1.0000	1.0000
6	2.03 mg/l	4	0.0000	0.0000
6	2.03 mg/l	5	0.0000	0.0000
6	2.03 mg/l	6	0.4000	0.6325
6	2.03 mg/l	7	0.0000	0.0000
6	2.03 mg/l	8	0.3000	0.5477
6	2.03 mg/l	9	0.8000	0.8944
6	2.03 mg/l	10	1.1000	1.0488
6	2.03 mg/l	11	0.0000	0.0000
6	2.03 mg/l	12	0.0000	0.0000
6	2.03 mg/l	13	0.0000	0.0000
6	2.03 mg/l	14	1.6000	1.2649
6	2.03 mg/l	15	1.0000	1.0000
6	2.03 mg/l	16	1.9000	1.3784
6	2.03 mg/l	17	0.4000	0.6325
6	2.03 mg/l	18	0.0000	0.0000
6	2.03 mg/l	19	0.0000	0.0000
6	2.03 mg/l	20	0.8000	0.8944
6	2.03 mg/l	21	0.4000	0.6325
6	2.03 mg/l	22	1.1000	1.0488
6	2.03 mg/l	23	0.0000	0.0000
6	2.03 mg/l	24	0.0000	0.0000
6	2.03 mg/l	25	0.0000	0.0000
6	2.03 mg/l	26	0.0000	0.0000
6	2.03 mg/l	27	0.0000	0.0000
6	2.03 mg/l	28	0.5000	0.7071
6	2.03 mg/l	29	0.0000	0.0000
6	2.03 mg/l	30	0.0000	0.0000

6	2.03 mg/l	31	0.5000	0.7071
6	2.03 mg/l	32	0.0000	0.0000
6	2.03 mg/l	33	2.1000	1.4491
6	2.03 mg/l	34	0.8000	0.8944
6	2.03 mg/l	35	1.0000	1.0000
6	2.03 mg/l	36	1.1000	1.0488
6	2.03 mg/l	37	1.0000	1.0000
6	2.03 mg/l	38	0.3000	0.5477
6	2.03 mg/l	39	3.2000	1.7889
6	2.03 mg/l	40	0.6000	0.7746

file

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Metalaxyl. Shaughnessey No. 113501.
- 2. **TEST MATERIAL:** Ridomil®2E; CAS No. 57837-19-1; Lot No. FL No. 892783; 25.0% metalaxyl (the active ingredient); a reddish-brown liquid.
- 3. **STUDY TYPE:** 72-3f, Estuarine Shrimp Acute Flow-Through Toxicity Test. Species Tested: Mysid (*Mysidopsis bahia*).
- 4. **CITATION:** Machado, M.W. 1992. Ridomil®2E - Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*) Under Flow-Through Conditions. SLI Report No. 92-4-4196. Performed by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 423375-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Rosemary Graham Mora
Date: *9/21/92*
Dana Katenberg
EEB/EFED
3/11/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Louis M Rifici
Date: *9/23/92*

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:

Henry T Craven
Date: *6/28/93*

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study using estuarine shrimp. ~~Based on mean measured concentrations, the 96-hour LC₅₀ of Ridomil®2E (a formulated product) for *Mysidopsis bahia* was 1.5 mg a.i./l. Therefore, Ridomil®2E is classified as moderately toxic to mysid shrimp. The NOEC was 0.59 mg a.i./l. The TEP LC₅₀ for Ridomil 2E is 5.98 mg/L, the NOEC was 2.36 mg/L.~~

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

22

11. MATERIALS AND METHODS:

A. **Test Animals:** The mysid shrimp (*Mysidopsis bahia*) used in this study were obtained from in-house cultures maintained in a 500-l tank under recirculating conditions with a photoperiod of 16 hours of light (intensity of 70-80 footcandles) and 8 hours of darkness. The culture water had a temperature of 24-26°C and a salinity of 31-32 parts per thousand (ppt). Mysids were fed live *Artemia salina* nauplii twice daily.

B. **Test System:** The test system consisted of a constant-flow serial diluter (60% dilution factor), a temperature-controlled water bath, and fourteen 19.5-l glass aquaria (39 X 20 X 25 cm). The solution volume in each aquarium fluctuated between 7 and 11 l. The flow rate to each aquarium (50 ml/minute) was adequate to provide 6.5 volume replacements/day.

The photoperiod during the test was the same as that used for culturing with a light intensity of 20-50 footcandles. Sudden transitions from light to dark and dark to light were avoided.

The dilution water (culture water) was filtered (20 and 5 μ m) natural seawater collected from the Cape Cod Canal, Bourne, MA. The dilution water had a salinity of 31-32 ppt and a pH of 7.8-7.9.

The test material, identified as containing 240 mg a.i./ml, was delivered, along with dilution water, to the chemical mixing chamber of the diluter. The concentration of solution in the mixing chamber was equivalent to the highest nominal test concentration (1.9 mg a.i./l) and was proportionally diluted to produce the remaining test concentrations.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of preliminary testing, six nominal concentrations were used (0.15, 0.25, 0.41, 0.69, 1.2, and 1.9 mg a.i./l). In addition, a dilution water control was included.

D. **Design:** Ten shrimp (≤ 24 hours old) were impartially selected and distributed into each of two replicate aquaria (20 shrimp/treatment level). Each replicate contained two retention chambers which each housed five mysids. At any given time, the organism loading rate was 0.00069 g/l/day.

Biological observations and observations of physical characteristics of the test solutions were noted at test initiation and every 24 hours thereafter. Dead shrimp were removed at each observation.

The dissolved oxygen concentration, pH, and temperature were measured daily in each replicate chamber. The temperature in the A replicate of the control solution was monitored continuously using a min/max thermometer. Salinity was recorded daily.

Chemical analysis for metalaxyl (the active ingredient of Ridomil®2E) was performed, using gas chromatography, on each test solution collected on days 0 and 4 to verify the test concentrations.

E. Statistics: The author used the computer program developed by Stephan (1977, 1982) to calculate the LC₅₀ value.

- 12. REPORTED RESULTS:** Mean measured concentrations were 0.19, 0.36, 0.59, 0.91, 1.2, and 2.1 mg a.i./l (Table 2, attached). The coefficients of variation averaged 7.2% for all mean measured concentrations.

Based on mortality, the 96-hour LC₅₀ (95% confidence interval) for *Mysidopsis bahia* exposed to Ridomil®2E was 1.5 (1.2-2.1) mg a.i./l (Table 3, attached). The NOEC was 0.59 mg a.i./l.

During the study, the pH was 7.7-7.9, the dissolved oxygen was 6.3-7.0 mg/l (91-101% of saturation), and the salinity was 31-32 ppt. Based on continuous monitoring, the temperature of the test solutions ranged from 24 to 26°C.

- 13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

"Based on the results of this study and on criteria established by US EPA (1985), Ridomil®2E would be classified as moderately toxic to mysid shrimp (*Mysidopsis bahia*)."

A Good Laboratory Practice Compliance Statement and a Quality Assurance Statement were included in the report, indicating that the study was in accordance with GLP regulations (40 CFR, Part 160).

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

A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following deviations:

The report did not indicate whether the technical grade of the test material was used. The percentage of active ingredient was 25.0%, therefore, it is likely that a formulated product was used; in which case, a control containing the inert material present in the formulation should have been included in the study.

During the study, the test solution temperature and salinity were 24-26°C and 31-32 ppt, respectively. The SEP recommends a temperature of 22 ±1°C and a salinity of 10-17 ppt for euryhaline shrimp species.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the LC₅₀ value and obtained the same results as those of the author (printout, attached).

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study using estuarine shrimp. The 96-hour LC₅₀ was 5.98 mg/L, (based on mean measured concentrations) which classifies Ridomil®2E (as moderately toxic to mysid shrimp (*Mysidopsis bahia*)).

D. Adequacy of the Study:

(1) Classification: Core for a formulated product.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, September 11, 1992.

METILANYL

Page is not included in this copy.

Pages 26 through 27 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
 - Identity of product impurities.
 - Description of the product manufacturing process.
 - Description of quality control procedures.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
 - Information about a pending registration action.
 - FIFRA registration data.
 - The document is a duplicate of page(s) .
 - The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

LATEULERE RIDOMIL ACUTE MYSID

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8.399999		20	20	100
9.536742E-05				
4.8	20	2	10	2.012253E-02
3.64	20	0	0	9.536742E-05
2.36	20	0	0	9.536742E-05
1.44	20	0	0	9.536742E-05
.76	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 4.8 AND 8.399999 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.976074

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

28

Metalakyl Mysidopsis bahia (MEAN % MORTALITY)

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.1	20	20	100	9.536742E-05
1.2	20	1	5	2.002716E-03
.91	20	0	0	9.536742E-05
.59	20	0	0	9.536742E-05
.36	20	0	0	9.536742E-05
.19	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.2 AND 2.1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.530153

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
