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Shaughnessey No.

EEB REVIEW

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TYPE PRODUCTS(S): I, D, H, F, N, R, S _____
MRID NO(S). _____
DATA ACCESSION NO (S). 4128810-01,02,03,04
PRODUCT MANAGER NO. 74
PRODUCT NAME(S) Metalaxyl
COMPANY NAME Ciba-Geigy
SUBMISSION PURPOSE Reviews of estuarine/marine studies

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% A.I.
<u>113501</u>	<u>Metalaxyl</u>	<u>96.1%</u>
	<u>Ridomil 2E</u>	<u>25.2%</u>



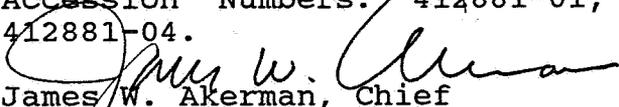
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 10 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Reviews of acute toxicity test for estuarine/marine invertebrates, (mollusc 96-hour flow-through shell deposition study and shrimp 96-hour acute toxicity test) for Metalaxyl and Ridomil 2E (25.2% metalaxyl a.i.) Accession Numbers. 412881-01, 412881-02, 412881-03, 412881-04.

FROM: 
James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H-7507C)

TO: Lois Rossi (PM74)
Reregistration Branch
Special Review and Reregistration Division (H-7508W)

Attached are the Ecological Effects Branch reviews of the acute toxicity tests for estuarine/marine invertebrates, (mollusc 96-hour flow-through shell deposition study and shrimp 96-hour acute toxicity test) for metalaxyl (96.1% a.i.) and ridomil 2E (25.2% metalaxyl a.i.). Regarding the two mollusc studies, 412881-01 is classified as supplemental and 412881-02 is classified as invalid. Regarding the two shrimp studies, 412881-03 is classified as core and 412881-04 is classified as supplemental. When comparing the technical grade to the formulation, the formulation appears to be more soluble since the solvent was used only in the studies using metalaxyl tgai. Furthermore, the toxicity results for the formulation are expressed in terms of the technical grade and the formulation appears to be more toxic than the technical grade.

These studies were submitted in response to data requirements listed in the Registration Standard of September 1988: 72-3 (b) (tgai and tep) and 72-3 (c) (tgai and tep). However, only the 72-3 (c) tgai requirement was fulfilled.

Should you have any questions concerning this study, please contact Nimish Vyas (557-0577) of my staff.

DATA EVALUATION RECORD

1. Chemical: Metalaxyl
2. Test Material: Ridomil 2E (25.2% metalaxyl a.i.)
3. Study Type: Acute toxicity test for estuarine and marine organisms (Shrimp 96-hour acute toxicity test).

Test Species: Mysid shrimp Mysidopsis bahia

MRID/Accession number 41288104

4. Study ID: Acute Toxicity to Mysid shrimp Mysidopsis bahia under flow-through conditions. Conducted by Springborn Laboratories, Inc. 790 Main Street Wareham, MA 02571 for Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419. Ridomil 2E, lot #71111ML5598, FL#871597.

5. Reviewed by: Nimish Vyas
Biologist
EEB/EFED

Signature: *Nimish Vyas*
Date: 2/13/91

6. Approved by: Norman Cook
Head, Section 2
EEB/EFED

Signature: *Norman J. Cook*
Date: 2-12-91

7. Conclusion: The study is scientifically sound however, it is classified as supplemental. Based on the results of this study, this chemical is considered to be highly toxic to mysid shrimp (LC₅₀ value of 0.73 mg A.I./L with the 95% C.I. of 0.64 - 0.86 mg A.I./L. and a slope of 4.52.).

96 hrs
(5/1/91)

8. Recommendations: A satisfactory response to the Ecological Effects Branch justifying the points listed in 14 a. would make this study repairable to core.

9. Background Information: NA.

10. Discussion of Individual Tests: NA

11. Materials and Methods:

- a. Test Animals: Shrimp were obtained from laboratory cultures maintained at Springborn Laboratories, Inc. Shrimp were fed brine shrimp nauplii twice daily and Hatchfry Encapsulon[®] three times weekly. The culture was held at 25[±].1°C. Shrimp were held on a 16 hrs light/8 hrs dark photoperiod.

- b. Test System: Natural, unfiltered seawater was used for dilution water. Each glass aquarium measured 39 X 20 X 25 cm. The flow rate of the test solution was equivalent to 7 volume additions per 24 hours. The aquaria were placed in a water bath maintained at 25+/-1°C. The maximum loading concentration was < 3mg of biomass per liter of solution.
- c. Study Design: The range finding test included nominal Ridomil 2E concentrations from 5.0 - 0.58 mg A.I./L. 100% mortality was observed in the three highest levels (5.0, 3.3, and 2.1 mg A.I./L) and for the lower concentrations, mortality ranged from 20 - 40 %. The definitive test included the following nominal concentrations: control, 1.9, 1.2, 0.80, 0.52, 0.34, and 0.22 mg A.I./L. Twenty shrimp (<24 hours old) were used at each treatment level. Temperature, pH, salinity, and DO were measured daily in each replicate aquarium. Temperature was measured continuously ds one replicate of the highest concentration. Water was collected for analysis prior to the initiation of the definitive study from the both replicates of the control, high, middle, and low concentrations and on test days 0 and 4 from all test levels (including controls). During the experiment, the shrimp were fed live brine shrimp nauplii twice daily.
- d. Statistics: The LC₅₀ value and the 95% confidence limits were determined by using a computer program developed by Stephan (1977, 1982) The NOEL was also determined.

12. Reported Results:

The LC₅₀ was reported as 0.75 mg A.I./L with the 95% C.I. of 0.65 - 0.89 mg A.I./L. The no-effect-level was 0.29 mg A.I./L. pH ranged from 8.0 - 8.1 units. The DO ranged from 6.9 mg/L - 5.6 mg/L. The temperature ranged from 24 - 25°C. And the salinity ranged from 31 - 32°/oo.

Analysis of the exposure solutions for 0.52 mg A.I./L on day 0 resulted in measurements below the analytical limit of detection for this study. It is also reported in this study on page 18 that analysis of the solution for the B replicate of 0.34 mg A.I./L on day 0 resulted in measurements below the analytical limit of detection for this study.

The mean measured concentrations for the definitive study were reported as follows: 1.7, 1.2, 0.63, 0.38, 0.29, 0.28 mg A.I./L and control.

13. Study Author's Conclusions/Quality Assurance Measures:

The LC₅₀ was reported as 0.75 mg A.I./L with the 95% C.I. of 0.65 - 0.89 mg A.I./L. The no-effect-level was 0.29 mg A.I./L.

Quality Assurance and Good Lab Practice Statements were included in the report.

14. Reviewer's Discussion and Interpretation of the Results:

a. Test Procedure: Test procedure was in accordance with the protocols recommended by the Guidelines. However, the following deviations were noted:

There was no mention of the size of shrimp tested; the Guidelines require this.

There was no mention if any shrimp died within 48 hrs prior to testing; the Guidelines require this.

There was no 15 - 30 minute photoperiod transition period; the Guidelines require this.

The B replicate at 96 hrs for 0.52 mg A.I./L (nominal) was lower than the A replicate. Under a flow through system, both replicates should have similar measured concentrations. The registrant should provide an explanation for why this occurred and how it could be prevented.

The 0 hr measurements for 0.52 mg A.I./L were below detection limits. The registrant should provide an explanation for why this occurred and how it could be prevented.

There is not much difference between the mean measured concentrations of the 0.34 and 0.22 mg A.I./L (nominal). The Guidelines require a dose regime which exposes each treatment group to a concentration of toxicant that is at least 60% of the next highest concentration. The registrant must explain why this was not the case and how it could be prevented.

If the < 0.11 mg A.I./L was considered as the limit of detection for this study (as in the cases of 0 hr 0.52 and 0.22 mg A.I./L and the control, why then is < 0.054 mg A.I./L the limit of detection for the control at 96 hrs. measurement? Why were the 0 hr measurements not taken with the < 0.054 mg A.I./L detection limit? (attachment 1).

Page 18 reports that analytical measurements for the B replicate of the 0.34 mg A.I./L group at day 0 were below the analytical limit of detection. However, on page 25, table 2 lists 0.22 mg A.I./L level as below the analytical limit of detection, and not the 0.34 mg A.I./L. Which treatment level is really below the analytical limit of detection?

b. Statistical Analysis: EEB analyzed the mortality data using the TOXANAL program and resulted in an LC_{50} value of 0.73 mg A.I./L with the 95% C.I. of 0.64 - 0.86 mg A.I./L. and a slope of 4.52. (see attachment 2).

c. Discussion and Results: The study is scientifically sound however, because of the items listed in 14 a. above, it is classified as supplemental.

d. Adequacy of the Study:

1. Classification: supplemental

2. Rational: See 14 a.

3. Repairability: A satisfactory response to the Ecological Effects Branch justifying the points listed in 14 a. would make this study repairable to core.

4. Descriptive Conclusion: Based on the results of this study, this chemical is considered to be highly toxic to mysid shrimp.

METALAXYL

Page 7 is not included in this copy.

Pages _____ through _____ 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.

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY. THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

Nimish Vyas rydomil 2E shrimp acute tox

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*****
CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED     DEAD        DEAD        PROB. (PERCENT)
1.7        20              20          100         9.536742E-05
1.2        20              14          70          5.765915
.63        20              6           30          5.765915
.38 0.2 20 10          2           10          2.012253E-02
.29        20              1           5           2.002716E-03
.28        20              0           0           9.536742E-05
```

THE BINOMIAL TEST SHOWS THAT .38 AND 1.7 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 .8694825

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	4.070143E-02		.7378511	.6418638

.865906

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	7.340176E-02	1

.4458987

SLOPE = 4.524779
95 PERCENT CONFIDENCE LIMITS = 3.298891 AND 5.750666

LC50 = .7925792
95 PERCENT CONFIDENCE LIMITS = .673759 AND .9478839

LC10 = .4153039
95 PERCENT CONFIDENCE LIMITS = .3158186 AND .5011875

DATA EVALUATION RECORD

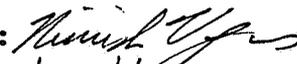
1. Chemical: Metalaxyl
2. Test Material: Metalaxyl technical 96.1% A.I.
3. Study Type: Acute toxicity test for estuarine and marine organisms (Shrimp 96-hour acute toxicity test).

Test Species: Mysid shrimp Mysidopsis bahia

MRID/Accession number 41288103

4. Study ID: Acute Toxicity to Mysid shrimp Mysidopsis bahia under flow-through conditions. Conducted by Springborn Laboratories, Inc. 790 Main Street Wareham, MA 02571 for Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419. Metalaxyl technical, Batch code #EN603107, FL#861650.

5. Reviewed by: Nimish Vyas
Biologist
EEB/EFED

Signature: 

Date: 2/13/91

6. Approved by: Norman Cook
Head, Section 2
EEB/EFED

Signature: 

Date: 2.12.91

7. Conclusion: The study is scientifically sound despite its deviations from the Guidelines and is classified as core. Based on the results of this study, (LC₅₀ = 25.7 mg A.I./L metalaxyl technical with 95% C.I. of 21-32 mg A.I./L metalaxyl technical and a slope of 3.25.) metalaxyl technical may be considered to be slightly toxic to mysid shrimp.

8. Recommendations: N/A

9. Background Information: NA

10. Discussion of Individual Tests: NA

11. Materials and Methods:

- a. Test Animals: Shrimp were obtained from laboratory cultures maintained at Springborn Laboratories, Inc. Shrimp were fed brine shrimp nauplii twice daily and Hatchfry Encapsulon^R three times weekly. The culture was held at 25⁺/1°C. Shrimp were held on a 16 hrs light/8 hrs dark photoperiod.

- b. Test System: Natural, unfiltered seawater was used for dilution water. Each glass aquarium measured 39 X 20 X 25 cm. The flow rate of the test solution was 75mL/min. or 6.5 volume replacements every 24 hours. The aquaria were placed in a water bath maintained at 25+/-1°C. The maximum loading concentration was < 3mg of biomass per liter of solution.
- c. Study Design: The range finding test included the following concentrations: 60, 39, 25, 16 and 11 mg a.i./L metalaxyl technical with the following mortalities: 100, 70, 20, 0, 0, percent, respectively. Acetone was used as the solvent. The definitive test included the following concentrations: control, solvent control, 11, 16, 25, 39, and 60 mg A.I./L metalaxyl technical. Twenty shrimp were used at each treatment level. Temperature, pH, salinity, and DO were measured daily in each replicate aquarium. Temperature was measured continuously is one replicate of the highest concentration. Water was collected for analysis prior to the initiation of the definitive study from the both replicates of the control, high, middle, and low concentrations and on test days 0 and 4 from all test levels (including controls). During the experiment, the shrimp were fed live brine shrimp nauplii twice daily.
- d. Statistics: The LC₅₀ value and the 95% confidence limits were determined by using a computer program developed by Stephan (1977, 1982) The NOEL was also determined.

12. Reported Results:

The LC₅₀ was reported as 25 mg A.I./L metalaxyl technical with the 95% C.I. of 21 - 30 mg A.I./L metalaxyl technical. The no-effect-level was 11 mg A.I./L metalaxyl technical. pH ranged from 8.0 - 8.1 units. The DO ranged from 7.3 mg/L - 5.5 mg/L. The temperature ranged from 23 - 25°C. And the salinity was 31‰.

A small amount of precipitate was observed at the end of the syringe tubing used to deliver the toxicant stock to the diluter mixing chamber. The precipitate was removed to prevent diluter malfunction.

Analysis of the quality control samples at 0-hr. for two of the three samples were outside the acceptable range established by this laboratory.

13. Study Author's Conclusions/Quality Assurance Measures:

The LC₅₀ was reported as 25 mg A.I./L metalaxyl technical with the 95% C.I. of 21 - 30 mg A.I./L metalaxyl technical. The no-effect-level was 11 mg A.I./L metalaxyl technical. See attachment 1 for details.

Quality Assurance and Good Lab Practice Statements were included in the report.

14. Reviewer's Discussion and Interpretation of the Results:

a. Test Procedure: Test procedure was in accordance with the protocols recommended by the Guidelines. However, the following deviations were noted:

The temperature deviated by more than 1 degree. There is also a discrepancy in the range of temperature between the text on pages 18 and 20 and table 1.

There was no mention of the size of shrimp tested.

There was no mention if any shrimp died within 48 hrs prior to testing.

There was no 15 - 30 minute photoperiod transition period.

b. Statistical Analysis: EEB analyzed the mortality data using the TOXANAL program and resulted in an LC₅₀ value of 25.7 mg A.I./L metalaxyl technical with 95% C.I. of 21-32 mg A.I./L metalaxyl technical and a slope of 3.25.

c. Discussion and Results: The study is scientifically sound despite its deviations from the Guidelines and is classified as core.

d. Adequacy of the Study:

1. Classification: core

2. Rational: N/A

3. Repairability: N/A

4. Descriptive Conclusion: Based on the results of this study, metalaxyl technical may be considered to be slightly toxic to mysid shrimp.

Attachment 1

Nimish Vyas metalaxyl shrimp. acute 96hrs

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
52	20	16	80	.5908966
34	20	14	70	5.765915
21	20	8	40	25.17223
12	20	5	25	2.069473
11	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 12 AND 52 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 24.60712

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	8.469117E-02		24.72897	20.96265

29.97984

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	.1256677	1

GOODNESS OF FIT PROBABILITY
.17189

SLOPE = 3.2508
95 PERCENT CONFIDENCE LIMITS = 2.098404 AND 4.403197

LC50 = 25.67313
95 PERCENT CONFIDENCE LIMITS = 20.9977 AND 32.41925

LC10 = 10.44253
95 PERCENT CONFIDENCE LIMITS = 6.355701 AND 13.6824

DATA EVALUATION RECORD

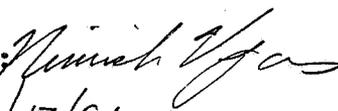
1. Chemical: Metalaxyl
2. Test Material: Metalaxyl technical 96.1% A.I.
3. Study Type: Acute toxicity test for estuarine and marine organisms (Mollusc 96-hour flow-through shell deposition study).

Test Species: Eastern oyster Crassostrea virginica

MRID number 412881-01

4. Study ID: Acute Toxicity of Eastern Oyster (Crassostrea virginica under flow-through conditions. Conducted by Springborn Laboratories, Inc. 790 Main Street Wareham, MA 02571 for Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419. Metalaxyl technical, batch #EN603107, lot # FL 861650.

5. Reviewed by: Nimish Vyas
Biologist
EEB/EFED

Signature: 
Date: 2/13/91

6. Approved by: Norman Cook
Head, Section 2
EEB/EFED

Signature: 
Date: 2.12.91

7. Conclusion: The study is scientifically sound and is classified as supplemental. Based on the results submitted by the author, metalaxyl technical may be considered to be moderately toxic to oyster shell deposition with an EC₅₀ value of 4.6 mg/L metalaxyl technical with 95% C.I. limits of 2.8 - 11.0 mg/L metalaxyl technical and a slope of 2.98.

8. Recommendations: Submission of raw new shell deposition data per oyster is required to upgrade this study to core.

9. Background Information: NA

10. Discussion of Individual Tests: NA

11. Materials and Methods:

- a. Test Animals: Oysters were obtained from Aquacultural Research Corporation (ARC), Dennis, MA. At ARC, the oysters were reared in natural flowing seawater. The temperature range of culture water was 19-22°C, pH was 7.6 - 8.0, salinity was 31-31‰ and dissolved oxygen was 84 - 96% of saturation. Oysters were fed a

combination of marine algae continuously. Oysters were held here from the fourteenth to the third day prior to the testing.

Oysters were transported to Springborn Laboratories, Inc. and were outside of water for approximately 1.5 hrs. Oysters were held prior to the test in a wooden epoxy-painted tray through which seawater was pumped. The oysters were of the same age (<1 year) and size (mean valve height of 33 (+/-2) mm. During the acclimation period and throughout the testing period, oysters were fed a supplementary algal diet of Isochrysis galbana, Parke, clone Y-ISO and Tetraselmis maculata. 48-hours prior to test initiation, the oysters were held in water at the temperature of 19-21°C, salinity of 31-33‰, pH of 7.6-7.9, and DO of 81-96% of saturation. No mortality occurred during the holding period.

- b. Test System: Natural, unfiltered seawater was used for dilution and control water. The glass aquarium measured 60 X 30 X 30. The flow rate of the test solution was 75mL/min. or 6.0 volume replacements every 24 hours. The aquaria were placed in a water bath maintained at 20+/-2°C. The photoperiod provided 16 hours Light and 8 hours dark.
- c. Study Design: The range finding test included the following concentrations: control, solvent control, 100, 20, 4.0, 0.80, and 0.16 mg A.I./L metalaxyl technical. Acetone was used as the solvent. The definitive test included the following concentrations: control, solvent control, 10, 6.0, 3.6, 2.2, and 1.3 mg A.I./L metalaxyl technical. Temperature, pH, salinity, and DO were measured daily in each replicate aquarium. Temperature was measured continuously as one replicate of the highest concentration. Water was collected for analysis prior to the initiation of the definitive study from the both replicates of the high, middle, and low concentrations and on test days 0 and 4 from all test levels (including controls).

Twenty-four hours prior to testing 3 - 5 mm of the new shell growth was removed by grinding the shell to a blunt edge. Immediately prior to the initiation of the test, the outer shell edge was buffed. 20 oysters were placed in each aquaria with their valve inflow openings facing toward the flow of water. Two replicates per treatment were tested resulting in 40 oysters per treatment. During the exposure, oysters received supplemental feeding of algae (Isochrysis galbana, Tetraselmis maculata). At the termination of the test, new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

- d. Statistics: The EC₅₀ value and the 95% confidence limits were determined by fitting the untransformed and transformed data to a best fit linear regression curve based on least squares. The NOEL was determined using the William's Test coupled with Bartlett's test. The Kruskal-Wallis test was used if the data was non-parametric.

12. Reported Results:

The EC₅₀ was reported as 4.6 mg A.I./L metalaxyl technical with the 95% C.I. of 2.0 - 13 mg A.I./L metalaxyl technical. The no-effect-level was 1.4 mg A.I./L metalaxyl technical. See attachment 1 for details. pH ranged from 7.7 - 8.0 units. The DO ranged from 7.4 mg/L - 5.8 mg/L. The temperature ranged from 20 - 21°C. And the salinity ranged from 31 - 33‰.

13. Study Author's Conclusions/Quality Assurance Measures:

The EC₅₀ was reported as 4.6 mg A.I./L metalaxyl technical with the 95% C.I. of 2.0 - 13 mg A.I./L metalaxyl technical. The no-effect-level was 1.4 mg A.I./L metalaxyl technical.

Quality Assurance and Good Lab Practice Statements were included in the report.

14. Reviewer's Discussion and Interpretation of the Results:

a. Test Procedure: Test procedure was in accordance with the protocols recommended by the Guidelines. However, the following deviations were noted:

1. Raw data on shell deposition per oyster was not included.

2. The photoperiod did not include a transition period.

b. Statistical Analysis: EEB confirmed the author's statistical analysis using the Toxanal program. The resulting EC₅₀ value is 4.6 mg/L metalaxyl technical with 95% C.I. limits of 2.8 - 11.0 mg/L metalaxyl technical. The slope is reported as 2.97 with 95% C.I. limits of 0.90 - 5.05. (attachment 2). EEB was not able to determine a NOEL, LOEL, and MATC because raw data on shell deposition was not presented.

c. Discussion and Results: The study is scientifically sound but is classified as supplemental. Raw data on shell deposition was not provided and therefore, EEB

cannot confirm the NOEL, MATC, and LOEL values. Based on the results submitted by the author, metalaxyl technical may be considered to be moderately toxic to oyster shell deposition with an EC₅₀ value of 4.6 mg/L metalaxyl technical with 95% C.I. limits of 2.8 - 11.0 mg/L metalaxyl technical and a slope of 2.98.

d. Adequacy of the Study:

1. Classification: supplemental

2. Rational: raw data on shell deposition was not provided.

3. Repairability: Submission of raw data on shell deposition per oyster is required to upgrade this study to core.

4. Descriptive Classification: Based on the results submitted by the author, metalaxyl technical may be considered to be moderately toxic to oyster shell deposition.

METAXYL

Page 17 is not included in this copy.

Pages _____ through _____ 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.
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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.

n vyas metalaxyl shell depositon

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.399999		40	33	82.5
5.6	40	24	60.00001	0
3.1	40	9	22.5	0
1.8	40	9	22.5	0
1.4	40	0	0	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 4.809205

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1124777	4.711065	3.818192	5.997597

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	.485011	3.049005

GOODNESS OF FIT PROBABILITY
2.739877E-02

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

SLOPE = 2.978905
95 PERCENT CONFIDENCE LIMITS = .9043142 AND 5.053495

LC50 = 4.666821
95 PERCENT CONFIDENCE LIMITS = 2.834599 AND 11.02317

LC10 = 1.748598
95 PERCENT CONFIDENCE LIMITS = .2406667 AND 2.868743

DATA EVALUATION RECORD

1. Chemical: Metalaxyl
2. Test Material: Ridomil 2E (25.2% metalaxyl a.i.)
3. Study Type: Acute toxicity test for estuarine and marine organisms (Mollusc 96-hour flow-through shell deposition study).

Test Species: Eastern oyster Crassostrea virginica

MRID number 412881-02

4. Study ID: Acute Toxicity of Eastern Oyster (Crassostrea virginica under flow-through conditions. Conducted by Springborn Laboratories, Inc. 790 Main Street Wareham, MA 02571 for Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419. Ridomil 2E, lot #71111ML5598, FL#871597.

5. Reviewed by: Nimish Vyas
Biologist
EEB/EFED

Signature: 
Date: 2/13/91

6. Approved by: Norman Cook
Head, Section 2
EEB/EFED

Signature: 
Date: 2-12-91

7. Conclusion: The study is classified as invalid. The guidelines recommend a minimum of 2.0 mm of new shell deposition in the control group at the end of 96 hours. The growth of new shell is primarily linear during the first week and the rate of deposition is an index of the animals' reaction to ambient water quality. The results of this study indicate new shell deposition of 1.3 mm for the control group. Furthermore, EEB was not able to determine a NOEL, LOEL, and MATC because raw data on shell deposition was not presented. Based on the results submitted by the author, ridomil 2E may be considered to be highly toxic to oyster shell deposition with an EC₅₀ value of 0.69 mg/L and 95% CI of 0.616 - 0.798 mg/L ridomil 2E.
8. Recommendations: This study must be repeated such that ambient water quality allows for a minimum of 2.0 mm of new shell deposition and raw shell deposition data per oyster must be submitted.

9. Background Information: NA
10. Discussion of Individual Tests: NA
11. Materials and Methods:

- a. **Test Animals:** Oysters were obtained from Aquacultural Research Corporation (ARC), Dennis, MA. At ARC, the oysters were reared in natural flowing seawater. The temperature range of culture water was 20.5-24.0°C, pH was 7.7 - 8.0, salinity was 30-31‰ and dissolved oxygen was 84 - 98% of saturation. Oysters were feed a combination of marine algae continuously. Oysters were held here from the fourteenth to the third day prior to the testing.

Oysters were transported to Springborn Laboratories, Inc. and were outside of water for approximately 1.5 hrs. Oysters were held prior to the test in a wooden epoxy-painted tray through which seawater was pumped. The oysters were of the same age (<1 year) and size (mean valve height of 36 (+/-9) mm. During the acclimation period and throughout the testing period, oysters were fed a supplementary algal diet of Isochrysis galbana, Parke, clone Y-ISO and Tetraselmis maculata. 72-hours prior to test initiation, the oysters were held in water at the temperature of 21°C, salinity of 32‰, pH of 7.7-8.0, and DO of 84-88% of saturation. No mortality occurred during the holding period.

- b. **Test System:** Natural, unfiltered seawater was used for dilution and control water. The glass aquarium measured 60 X 30 X 30. The flow rate of the test solution was 75mL/min. or 6.0 volume replacements every 24 hours. The aquaria were placed in a water bath maintained at 20+/-2°C. The photoperiod provided 16 hours Light and 8 hours dark.
- c. **Study Design:** The range finding test included the following concentrations: control, 10, 2.0, .40, 0.080, and 0.016 mg A.I./L ridomil 2E. The definitive test included the following concentrations: control, 2.0, 1.2, 0.72, 0.43, and 0.26 mg A.I./L ridomil 2E. Temperature, pH, salinity, and DO were measured daily in each replicate aquarium. Temperature was measured continuously is one replicate of the highest concentration. Water was collected for analysis prior to the initiation of the definitive study from the both replicates of the high, middle, and low concentrations and on test days 0 and 4 from all test levels (including controls).

Forty-eight hours prior to testing 3 - 5 mm of the new

shell growth was removed by grinding the shell to a blunt edge. Immediately prior to the initiation of the test, the outer shell edge was buffed. 20 oysters were placed in each aquaria with their valve inflow openings facing toward the flow of water. Two replicates per treatment were tested resulting in 40 oysters per treatment. During the exposure, oysters received supplemental feeding of algae (Isochrysis galbana, Tetraselmis maculata). At the termination of the test, new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

- d. Statistics: The EC₅₀ value and the 95% confidence limits were determined by fitting the untransformed and transformed data to a best fit linear regression curve based on least squares. The NOEL was determined using the William's Test coupled with Bartlett's test. The Kruskal-Wallis test was used if the data was non-parametric.

12. Reported Results:

The EC₅₀ was reported as 0.69 mg A.I./L ridomil 2E with the 95% C.I. of 0.56 - 0.86 mg A.I./L ridomil 2E. The no-effect-level was 0.39 mg A.I./L ridomil 2E. See attachment 1 for details. pH ranged from 7.8 - 8.0 units. The DO ranged from 7.2 mg/L - 6.2 mg/L. The temperature ranged from 21 - 22°C. And the salinity ranged from 31 - 35‰.

13. Study Author's Conclusions/Quality Assurance Measures:

The EC₅₀ was reported as 0.69 mg A.I./L ridomil 2E with the 95% C.I. of 0.56 - 0.86 mg A.I./L ridomil 2E. The no-effect-level was 0.39 mg A.I./L ridomil 2E.

Quality Assurance and Good Lab Practice Statements were included in the report.

14. Reviewer's Discussion and Interpretation of the Results:

a. Test Procedure: Test procedure was in accordance with the protocols recommended by the Guidelines. However, the following deviations were noted:

1. The results of this study indicate new shell deposition of 1.3 mm for the control group. The guidelines recommend a minimum of 2.0 mm of new shell deposition in the control group at the end of 96 hours.

2. Raw data on shell deposition per oyster was not included.

3. The photoperiod did not include a transition period.

b. Statistical Analysis: EEB confirmed the EC_{50} value presented by the author as 0.69 mg/L with 95% CI of 0.616 - 0.798 mg/L ridomil 2E. The slope is reported as 3.74 with 95% C.I. limits of 2.84 - 4.63 (attachment 2). EEB was not able to determine a NOEL, LOEL, and MATC because raw data on shell deposition was not presented.

c. Discussion and Results: The study is classified as invalid. The guidelines recommend a minimum of 2.0 mm of new shell deposition in the control group at the end of 96 hours. The results of this study indicate new shell deposition of 1.3 mm for the control group. The growth of new shell is primarily linear during the first week and the rate of deposition is an index of the animals' reaction to ambient water quality. Furthermore, EEB was not able to determine a NOEL, LOEL, and MATC because raw data on shell deposition was not presented.

d. Adequacy of the Study:

1. Classification: invalid

2. Rational: See 14 a. above.

3. Repairability: This study must be repeated such that ambient water quality allows for a minimum of 2.0 mm of new shell deposition and raw new shell deposition data per oyster must be submitted.

4. Descriptive Conclusion: Based on the results submitted by the author, ridomil 2E may be considered to be highly toxic to oyster shell deposition.

METALAX VL

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Attachment 2

n vyas ridomil 2E shell depostion

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.5	40	36	90	0
1	40	28	70	0
.57	40	15	37.5	0
.39	39	9	23.07692	0
.34	40	3	7.500001	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 .705989

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	4.427887E-02		.691686	.6163336
.7831242				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	5.688269E-02	1
.6041272		

SLOPE = 3.741486
95 PERCENT CONFIDENCE LIMITS = 2.849138 AND 4.633834

LC50 = .6979032
95 PERCENT CONFIDENCE LIMITS = .6160378 AND .7980988

LC10 = .3194169
95 PERCENT CONFIDENCE LIMITS = .2458783 AND .3811745
