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

AUG 26 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Request to Upgrade Three Acute Toxicity Tests for Estuarine and Marine Organisms for Metalaxyl (Chemical No. 113501)

FROM: Douglas Urban, Acting Branch Chief for H.T. Craven 8/12/91
Ecological Effects Branch
Environmental Fate and Effects Division

TO: Lois Rossi, PM 74
Reregistration Branch
Special Review and Reregistration Division (H 7508 W)

The Ecological Effects Branch has reviewed the subject request to upgrade three Acute toxicity tests for Estuarine and Marine Organisms for the active ingredient Metalaxyl (Chemical No 113501). The results of the review are summarized below.

Mollusc 96-hour Flow-through Shell Deposition (72-3 (b)

Test Substance: Metalaxyl Technical 96.1% a.i.
MRID #: 41288101

This study was previously classified as supplemental because raw data was not submitted to confirm the NOEL, MATC, and LOEL values. The raw data now submitted has allowed us to confirm these values, and this study is now classified as core.

Shrimp 96-hour Acute Toxicity Tests (72-3 (c)

Test Substance: Ridomil 2E (25.2% a.i.)
MRID #: 41288104

Registrant was asked to respond to a number of points about this study to reclassify this study from supplemental to core. Responses to deviations from the Guidelines for the size of the shrimp tested, the mortality figures prior to testing, and the lack of a 15 - 30 minute photoperiod transition period could be justified in reclassifying this study from supplemental to core. However, aberrations for several variations in measured concentrations could not be adequately explained to justify the reclassification of this study to core. Therefore, a new study is required.

Mollusc 96-hour Flow-through Shell Deposition (72-3 (b)

Test Substance: Ridomil 2E (25.2% a.i.)
MRID #: 41288102

This study was classified as invalid due to the new shell deposition of 1.3 mm for the control group at the end of 96 hours. The guidelines currently recommend a minimum of 2.0 mm of new shell deposition at the end of 96 hours. As the registrant cited, the minimum shell deposition rate was established by EPA at 2.0 mm and with an expected deposition to as much as 4.0 mm. In addition, a linear growth pattern would be expected during the first week, and any retarded growth may be due to the ambient water quality. Therefore, the classification for this study remains invalid and this study must be repeated.

Amended Data Evaluation Records are attached. If there are any questions concerning this review please contact Bill Evans of my staff on 557-2115.

AMENDMENT TO
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: 41288101

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. Completed October 30, 1989. Metalaxyl technical, batch #EN603107, lot # FL 861650.

5. Reviewed by: William Evans, Biologist, EEB/EFED — Signature: William Evans — Date: 8/14/91

6. Approved by: Ann Stavola, Head, Section 5, EEB/EFED — Signature: Ann Stavola — Date: 8/14/91

7. Conclusion: The study is scientifically sound and is classified as core. Based on the results submitted by the author, metalaxyl technical may be considered to be moderately toxic to oyster shell deposition with an EC_{50} value of 4.6 mg/L metalaxyl technical and a slope of 2.98.

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

   a. Test Procedure: Test procedure was in accordance with the protocols recommended by the Guidelines with only the following minor deviation.

      1. The photoperiod did not include a transition period.
         SLI responded that guidelines state that a transition period should be provided, but that there is no significant influence on an acute study.

         EEB accepts SLI's deviation from the guideline.

   b. Statistical Analysis: EEB confirmed the author's statistical analysis for the EC_{50} value using the Toxanal program. The resulting EC_{50} value is 4.6 mg/L metalaxyl

The NOEL, LOEL, and MATC were determined with the SAS program (see attachment). The following values are:

NOEL = 1.3 mg A.I./L
LOEL = 2.2 mg A.I./L
MATC = 1.75 mg A.I./L

c. Discussion and Results: The study is scientifically sound and is classified as core despite the minor deviation from the guidelines. Based on the results submitted by the author, metalaxyl technical may be considered to be moderately toxic to oyster shell deposition with an EC_{50} 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: core

2. Rational: Raw data on shell deposition were provided to confirm NOEL, LOEL, and MATC values.

3. Repairability: N/A

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

General Linear Models Procedure

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	133.1301625	22.1883604	28.33	0.0001
Error	272	213.0491923	0.7832691		
Corrected Total	278	346.1793548			

ZOOM I

PROGRAM EDITOR
Command ===>

Duncan Grouping	concentration ppm (mg/l)	Mean	N	TRT	
A	1.3	2.360	40	c	← NOEC
A					
A	Solvent Control	2.290	40	b	
A					
A	Control	2.250	40	a	
B	2.2	1.823	40	d	← LOEC
B					
B	3.6	1.750	40	e	
C	6.0	0.927	40	f	
D	10	0.408	39	g	

Attachment 2

n vyas metalaxyl shell depositon

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.399999		40	33	82.5
0				
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	GOODNESS OF FIT PROBABILITY
4	.485011	3.049005	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

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AMENDMENT TO
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: 41288102

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. Completed October 30, 1989. Ridomil 2E, lot #71111ML5598, lot # FL#871597.

5. Reviewed by: William Evans
   Biologist
   EEB/EFED
   Signature: William Evans
   Date: 8/14/91

6. Approved by: Ann Stavola
   Head, Section 5
   EEB/EFED
   Signature: Ann Stavola
   Date: 8/14/91

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, deviations were noted in the Data Evaluation Review dated 2/12/91. The registrant's representative, Springborn Laboratories, Inc. (SLI), responded to these deviations. These deviations are discussed below.

      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 the 96-hour period.

         SLI recommends greater latitude be given for minimum shell deposition in group. Specifically, they would like performance criteria to be more general and read "control oysters are expected to deposit mean shell growth of between 1.5 and 3.5 mm during a 96-hour exposure period. The result of tests with mean control deposition less than 2.0 mm should be examined more closely to ensure

2. There was no mention if any shrimp died within 48 hours prior to testing. The guidelines require this.

   SLI responded that mortality is difficult to access due to the small size and cannibalistic behavior of the organisms. During the study only 1 mortality was observed among the 20 organisms used as the control.

   EEB accepts SLI response to this deviation from the guidelines.

3. There was no 15-30 minute photoperiod transition period as required by guidelines.

   SLI responded that guidelines state that a transition period <u>should</u> be provided, but that there is no significant influence on an acute study.

   EEB accepts SLI response to this deviation from the guidelines.

4. The B replicate at 96-hours for 0.52 mg A.I./L (nominal treatment level) was lower than the A replicate at 96 hours. Under flow-through conditions, both replicate vessels should result in similar measured test material concentrations. The registrant should provide an explanation for why this occurred and how it could be prevented.

   SLI responded that it appeared that the variability was not due to the material's behavior under the maintained exposure conditions due to the results of the diluter system performance records. Although the variability could not be defined, the treatment level did not bracket the calculated LC_{50}.

   Although the treatment level did not bracket the LC_{50} the variability was not explained. Perhaps if this variability in measured concentrations was the sole deviation from the guidelines EEB might be more willing to accept this deviation. However, as other significant deviations have also been noted, EEB can not accept this and other major deviations.

5. The 0-hour measurement for 0.52 mg/A.I. were below detection limits. An explanation is needed for why this occurred and how it could be prevented.

   SLI responded with the same explanation as discussed in 4. above--i.e. the diluter system functioned properly throughout the test and the lower than expected concentration could not be explained.

However, the concentration did not bracket the calculated LC_{50}.

6. There is not much difference between the mean measured concentrations of the 0.34 and 0.22 mg/L (nominal) treatment level. 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.

   SLI responded that the behavior of the test material of minor deviations in the diluter system performance often result in some variability from the selected gradient. The primary objective is to provide a span of treatment levels which elicits a dose-response sufficient to calculate an LC_{50} value which accurately defines the acute toxicity of the test substance to exposed species.

   Although the treatment level did not bracket the LC_{50} the variability was not explained. Perhaps if this variability in measured concentrations was the sole deviation from the guidelines. EEB might be more willing to accept this deviation. However, as other significant deviations have also been noted, EEB can not accept this and other major deviations.

7. If the <0.11 mg A.I./L was considered as the limit of detection, as stated for hour measurement, why then is 0.054 mg A.I./L the limit of detection for the 96-hour measurements? Why were the 0-hour measurements not taken with the < 0.054 mg A.I./L detection limit?

   SLI responded that the analytical detection limit established at 0-hour was based on the predicted nominal concentration. Modifications were made prior to analyses of the solution at 96-hours to lower the analytical detection limit. In addition, differences between the detection limits for 0 and 96 hours was only 2X, and analysis using the 0.054 mg A.I./L may have defined the lower than expected treatment levels, but this values would not have significantly altered the LC_{50}s.

   Although the treatment level did not bracket the LC_{50} the variability was not explained. Perhaps if this variability in measured concentrations was the sole deviation from the guidelines. EEB might be more willing to accept this deviation. However, as other significant deviations have also been noted, EEB can not accept this and other major deviations.

EEB can not accept this and other major deviations.

8. Page 18 of the study reports that analytical measurements for the B replicate of the 0.34 mg/L treatment level was below the established analytical detection limits on day 0. However, on page 25, table 2 lists the B replicate of the 0.22 mg/L level as below the analytical limit of detection, and not the 0.34 mg/L. Which level is really below the analytical limit of detection?

   SLI responded that this was a report preparation error. The statement on page 25 is correctly defined as below the analytical detection limit.

   EEB accepts SLI corrections/clarification.

c. The study is scientifically sound, however, due to the aberrations related to variation in measured concentrations the study classification remains as supplemental.

d. Adequacy of the Study:

   1. Classification: supplemental

   2. Rational: See 14 a.

   3. Repairability: This study must be repeated due to the aberrations related to variation in measured concentrations.

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

AMENDMENT TO
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: Eastern oyster Mysidopsis bahia

   MRID 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. Completed October 30, 1989. Ridomil 2E, lot #71111ML5598, lot # FL#871597.

5. Reviewed by: William Evans
   Biologist
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   Signature: William Evans
   Date: 8/14/91

6. Approved by: Ann Stavola
   Head, Section 5
   EEB/EFED
   Signature: Ann Stavola
   Date: 8/14/91

8. Recommendations: In order to satisfy core requirements this study must be repeated.

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, deviations were noted in the Data Evaluation Review dated 2/12/91. The registrant's representative, Springborn Laboratories, Inc. (SLI), responded to these deviations. These deviations are discussed below.

      1. There was no mention of the size of shrimp tested as required in the guidelines.

         SLI responded that juvenile shrimp $\leq$ 24 hours old were used and sensitivity of juvenile shrimp wight measurements are generally not performed. SLI estimated the weight at 0.001 g/L based on the average wet weight of an adult mysid shrimp at 5.11 mg.

         EEB accepts SLI's response to this minor deviation from the guidelines.

statistical significance and reliability of the biological observance". It is further stated that literature values have been correlated and estimated to range from 1.5 to 3.5 mm.

EEB believes that a minimum shell deposition of 2.0 mm can be obtained for control groups. The minimum shell deposition was established at 2.0 mm with an expected deposition to as much as 4.0 mm. Therefore, shell growth between 2.0 - 4.0 mm would be expected. Further, the 1.3 mm growth falls below the 1.5 mm minimum range of literature values sited by SLI.

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

Although raw data was included, due to the unacceptable deviation from guidelines discussed in 14. a. 1. the raw data was not used to determine the NOEL, LOEL, and MATC.

3. The photoperiod did not include a transition period.

SLI responded that guidelines state that a transition period should be provided, but that there is no significant influence on an acute study.

EEB accepts SLI's deviation from the guideline.

d. Adequacy of the Study:

1. Classification: invalid

2. Rational: See 14 a. above.

3. Repairability: This study must be repeated such that the ambient water quality allows for a minimum of 2.0 mm of new shell deposition.

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