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128997
Shaughnessey No.

EEB REVIEW

DATE: IN 07-26-89 OUT 7-2-90

FILE OR REG. NO. 3125-GIG

PETITION OR EXP. NO. _____

DATE OF SUBMISSION 04-19-89

DATE RECEIVED BY EFED 07-26-89

RD REQUESTED COMPLETION DATA 07-20-90

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RD ACTION CODE/TYPE OF REVIEW 101

TYPE PRODUCTS(S): I, D, H, F, N, R, S Fungicide

DATA ACCESSION NO(S). _____

PRODUCT MANAGER NO. S. Lewis (27)

PRODUCT NAME(S) Folicur Technical 3125-GIG, Folicur 1.2 EC

3125 GIU, Elite 45 DF 3125-XXX

COMPANY NAME Mobay Corporation

SUBMISSION PURPOSE Proposed registration of new chemical for

use as technical and for following and uses: grasses grown

for seed, wheat, barley, peanuts and grapes

SHAUGHNESSEY NO. CHEMICAL AND FORMULATION % A.I.

_____ Tebuconazole _____

_____ _____ _____

_____ _____ _____



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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Tebuconazole data submitted to support registration

FROM: James W. Surprenant, Chief
Ecological Effects Branch
Environmental Fate and Effects Division

TO: Susan Lewis, PM 27
Fungicide/Herbicide Branch
Registration Division

Ecological Effects Branch has completed its review of ecotoxicity data for Tebuconazole technical submitted by Mobay Corporation. The following is a brief summary of the results of this review.

CITATION: Surprenant, D. C. 1988. Acute Toxicity of Technical Grade HWG 1608 to Mysid Shrimp (Mysidopsis bahia) Under Flow Through Conditions. Prepared by Springborn Life Sciences, Inc., Wareham, MA for Mobay Corporation, Stilwell, KS. MIRD No. 409959-02.

CONCLUSIONS: This study is scientifically sound and fulfills guideline requirements for a 96-hour acute flow-through study using an estuarine shrimp. The 96-hour LC50 value for Mysidopsis bahia exposed to technical grade HWG 1608 was 0.49 mg a.i./l, based on mean measured concentration. Therefore, technical grade HWG 1608 is classified as highly toxic to mysid shrimp. The NOEC was estimated to be < 0.30 mg a.i./L after 96 hours of exposure.

CITATION: Surprenant, D. C. 1988. Acute Toxicity of Technical Grade HWG 1608 to Eastern Oysters (Crassostrea virginica). Prepared by Springborn Life Sciences, Inc., Wareham, MA for Mobay Corporation, Stilwell, KS. MIRD No. 409959-03.

CONCLUSIONS: This study is scientifically sound and fulfills guideline requirements for an oyster shell deposition study. The 96-hour EC50 value for Eastern oyster exposed to technical grade HWG 1608 was 2.7 mg a.i./L based on mean measured concentration. Therefore, technical grade HWG 1608 is classified as moderately toxic to Eastern oysters. The NOEC was 1.7 mg a.i./L.

CITATION: Surprenant, D. C. 1988. Acute Toxicity of Technical Grade HWG 1608 to Sheepshead minnow (Cyprinodon variegatus) Under Flow

Through Conditions. Prepared by Springborn Life Sciences, Inc., Wareham, MA for Mobay Corporation, Stilwell, KS. MIRD No. 409959-04.

CONCLUSIONS: This study is scientifically sound and fulfills guideline requirements for a 96-hour acute flow-through toxicity study using estuarine fish. The 96-hour LC50, based upon mean measured concentrations, of technical grade HWG 1608 to sheepshead minnows (Cyprinodon variegatus) was 5.9 mg a.i./l. Therefore, technical grade HWG 1608 is classified as moderately toxic to sheepshead minnows. The NOEC was determined to be <1.2 mg a.i./L after 96 hours of exposure.

CITATION: Heimbach, F. 1987. Growth Inhibition of Green Algae (Scenedesmus subspicatus) by HWG 1608 (Tech.). Conducted by Bayer AG, Chemical Research Institute of Environmental Biology, Federal Republic of Germany. Submitted by Mobay Corporation, Stilwell, KS. MIRD No. 409959-08.

CONCLUSIONS: This study is scientifically sound. However, it does not fulfill guideline requirements for a growth and reproduction test using a green algal species because S. subspicatus is not a recommended species. Based on cell counts, the 4-day EC50 value of HWG 1608 for Scenedesmus subspicatus was 1.45 mg a.i./L nominal concentration. The NOEC was 0.32 mg a.i./l.

If you have any questions regarding these reviews, please contact Clyde Houseknecht at 557-4372.

DATA EVALUATION RECORD

1. **CHEMICAL:** Folicur (or Tebuconazole).
Shaughnessey Number: 128997.
2. **TEST MATERIAL:** Technical grade HWG 1608; Lot No. 86R0082I;
96.28% Active Ingredient; an off-white colored solid.
3. **STUDY TYPE:** Estuarine Invertebrate Acute Toxicity Test.
Species Tested: Mysid shrimp (Mysidopsis bahia).
4. **CITATION:** Surprenant D.C. 1988. Acute Toxicity of
Technical Grade HWG 1608 to Mysid Shrimp (Mysidopsis bahia)
Under Flow-Through Conditions. Prepared by Springborn Life
Sciences, Inc., Wareham, Massachusetts. SLS Report #87-7-
2436. SLS Study #274.1186.6127.515. Submitted by Mobay
Corporation, Stilwell, Kansas. MRID No. 409959-02.
5. **REVIEWED BY:**

Kimberly D. Rhodes Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Kimberly D. Rhodes</i> Date: <i>January 3, 1990</i>
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6. **APPROVED BY:**

Prapimpan Kosalwat, Ph.D. Staff Toxicologist KBN Engineering and Applied Sciences, Inc.	Signature: <i>P. Kosalwat</i> Date: <i>January 3, 1990</i>
Henry T. Craven Supervisor, EEB/HED USEPA <i>Clyde Houseknecht</i>	Signature: <i>Henry T. Craven</i> Date: <i>6/25/90</i>
7. **CONCLUSIONS:** This study appears scientifically sound and
fulfills the guideline requirements for a 96-hour acute
flow-through study using an estuarine shrimp. The 96-hour
LC50 value for Mysidopsis bahia exposed to technical grade
HWG 1608 was 0.49 mg a.i./L, based on mean measured
concentration. Therefore, technical grade HWG 1608 is
classified as highly toxic to mysid shrimp. The NOEC was
estimated to be < 0.30 mg a.i./L after 96 hours of exposure.
8. **RECOMMENDATIONS:** N/A.

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9. **BACKGROUND:**10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.11. **MATERIALS AND METHODS:**

- A. **Test Animals:** Mysid shrimp (Mysidopsis bahia) were obtained from laboratory cultures maintained at the testing facility. Mysids were fed brine shrimp nauplii two times daily and the soil nematode (Panagrellus redivivus) three times weekly. The mysid culture area received a regulated photoperiod of 16 hours of light and 8 hours of darkness with a light intensity of 70 - 140 footcandles. Commercial aquarium heaters were used to maintain the culture solution temperatures at $25 \pm 1^{\circ}\text{C}$.
- B. **Test System:** The test was conducted using an exposure system consisting of a modified intermittent-flow proportional diluter (Mount and Brungs, 1967), a constant-temperature controlled water bath, and a set of 14 test aquaria. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a self-starting siphon attached to the drain. The flow rate of exposure solutions to each test aquarium was equivalent to approximately 14 volume replacements per aquarium every 24 hours. This system allowed the aquarium volume to fluctuate between 3.1 and 7.0 L and ensured a solution exchange within the organism retention chamber.

Each test aquarium contained two mysid retention chambers. The retention chambers were constructed from glass Petri dishes, 10 centimeters in diameter to which 15 cm high Nitex screen collars were attached. The aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test water temperature at $25 \pm 1^{\circ}\text{C}$. A photoperiod of 16 hours of light and 8 hours of darkness with a light intensity of 30 - 70 footcandles was provided each day.

The dilution water used was filtered natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts. The dilution water was characterized as having a salinity of $31^{\text{‰}}$, a pH range of 7.7 - 7.8 and a dissolved oxygen concentration range of 5.9 - 6.4 mg/L in the dilution water control during the exposure period.

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- C. **Dosage:** 96-hour acute flow-through test.
- D. **Design:** The test was initiated when 20 (\leq 24-hour old) mysid shrimp, ten per replicate (five per retention chamber), were impartially selected and distributed to each of the treatment levels and controls. A control, solvent control, and five nominal technical grade HWG 1608 concentrations of 0.26, 0.51, 1.0, 2.1 and 4.1 mg a.i./L were tested. All treatment levels and the controls were maintained in duplicate. The solvent control solution contained the maximum amount of acetone present in any test concentration (212 μ L/L).

All concentrations were observed for mortality and abnormal effects at test initiation and at every subsequent 24-hour interval during the exposure period. The water quality parameters (dissolved oxygen, pH, salinity and temperature) were measured and recorded once daily in each replicate of the controls and each treatment level. Test solution temperature was also continuously monitored in one replicate of the solvent control solution throughout the study. Analytical determination of technical grade HWG 1608 was performed on all test solutions, control and solvent control at 0, 48 and 96 hours using high pressure liquid chromatographic (HPLC) analysis.

- E. **Statistics:** The mean measured test concentrations and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period by using the computer program developed by Stephan et al. (1977, 1982).
12. **REPORTED RESULTS:** The mean measured test concentrations, the corresponding mortalities and the observations made during the 96-hour test are presented in Table 2 (attached). The mean measured concentrations of technical grade HWG 1608 in exposure solutions during the 96-hour definitive test were 0.30, 0.45, 0.79, 1.6 and 3.4 mg a.i./L. The mean measured concentrations of technical grade HWG 1608 ranged from 76 to 115% of the nominal concentrations.

Following 96 hours of exposure, mortality ranged from 30 to 95% in all treatment levels and was clearly concentration dependent. It should be noted however, that no behavioral effects were observed at the lowest concentration of technical grade HWG 1608 tested (0.30 mg a.i./L). The 24-,

48-, 72- and 96-hour LC50 values (95% confidence interval) for mysid shrimp exposed to mean measured concentrations of technical grade HWG 1608 were calculated to be 2.2 (1.6 - 3.3), 1.0 (0.70 - 1.6), 0.55 (0.38 - 0.74) and 0.46 (0.29 - 0.61) mg a.i./L, respectively.

Based on mortality and behavioral effects, the no-observed effect concentration (NOEC) for mysid shrimp exposed to technical grade HWG 1608 was determined to be <0.30 mg a.i./L, the lowest mean measured concentration tested. The water quality parameters measured during this study remained within acceptable ranges for the survival of the mysid shrimp and were unaffected by the concentrations of technical grade HWG 1608 tested. Continuous temperature monitoring demonstrated that the test solution temperature ranged from 23 - 25°C during the exposure period.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

The study was audited by the QA unit of Springborn Life Sciences, Inc. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP states that natural or reconstituted seawater of 10 to 17 ‰ salinity should be used when testing euryhaline shrimp species. The natural seawater used during the toxicity study had a salinity of 31 ‰.

o The recommended temperature for testing the mysid shrimp is 22 ± 1°C. Continuous temperature monitoring demonstrated that the test solution temperature ranged from 23 - 25°C during the exposure period.

The toxicity report did not provide the following information required by the SEP:

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period

between light and dark was maintained.

B. **Statistical Analysis:** The reviewer used the Toxanal computer program to calculate the LC50 values and the slope of the concentration-response curve. These calculations are attached. The probit method provides a 96-hour LC50 value of 0.49 mg a.i./L mean measured concentration with a 95 percent confidence interval of 0.33 to 0.66 mg a.i./L which is similar to that reported by the author (i.e., 0.46 mg a.i./L with a 95% confidence interval of 0.29 - 0.61 mg a.i./L). The slope of the concentration-response curve was 2.3.

C. **Discussion/Results:** The study results appear to be scientifically valid. The 96-hour LC50 value based on mean measured concentrations was determined to be 0.49 mg a.i./L. Therefore, technical grade HWG 1608 is considered highly toxic to mysid shrimp (Mysidopsis bahia). The no-observed-effect concentration (NOEC) was estimated to be <0.30 mg a.i./L after 96 hours of exposure.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the toxicity results.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 12-29-89.

Shaughnessy No. 128997

Chemical Name Folicur Chemical Class _____ Page _____ of _____

Study/Species/Lab/
Accession _____ Chemical
X a. l.

(or Tebuconazole)

Reviewer/
Date _____ Valid
Stat _____

14-Day Single Dose Oral LD50

LD50 = . mg/kg (95% C.L.) Contr. Mort. (X) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

14-Day Single Dose Oral LD50

LD50 = mg/kg. (95% C.L.) Contr. Mort. (X) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____
Sex = _____

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

48-Hour LC50

LC50 = pp (95% C.L.) Contr. Mort. (X) = _____

Species _____

Slope = # Animals/Level = _____ Sol. Contr. Mort. (X) = _____
Temperature = _____

Lab _____

48-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

96-Hour LC50

LC50 = 0.49 ppm (95% C.L. (probit method)) Contr. Mort. (X) = 0

Species Mysidopsis bahia

Slope = 2.3 # Animals/Level = 20 Sol. Contr. Mort. (X) = 5

Lab Springborn Life Sciences, Inc.

96-Hour Dose Level ppm/(X Mortality)
Temp. = 23-25°C

MRID = 409959-02

0.30 (30) 0.45 (50) 0.79 (75) 1.6 (90) 3.4 (95)

Comments: Based on mean measured concentrations.

96-Hour LC50

LC50 = pp (95% C.L.) Contr. Mort. (X) = _____

Species _____

Slope = # Animals/Level = _____ Sol. Contr. Mort. (X) = _____
Temp. = _____

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

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NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

KIMBERLY RHODES FOLICUR (OR TEBUCONAZOLE) MYSIDOPSIS BAHIA 12-29-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.4	19	18	94.7368	3.814697E-03
1.6	19	17	89.4737	3.643036E-02
.79	19	14	73.6842	3.178405
.45	19	9	47.3684	50
.3	19	5	26.3158	3.178405

THE BINOMIAL TEST SHOWS THAT 0 AND 1.6 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 .4751826

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.2265073	.4843833	.3093197	.6522231

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1740856	1	.8052561

SLOPE = 2.29102
 95 PERCENT CONFIDENCE LIMITS = 1.335125 AND 3.246915

* LC50 = .4931001
 95 PERCENT CONFIDENCE LIMITS = .3252797 AND .6628618

LC10 = .1375932
 95 PERCENT CONFIDENCE LIMITS = 4.217492E-02 AND .2324147

Page 11 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.

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Folicur (or Tebuconazole).
Shaughnessey Number: 128997.
- 2. **TEST MATERIAL:** Technical grade HWG 1608; Batch No. 86 R00 82I; 96.28% active ingredient; an off-white powder.
- 3. **STUDY TYPE:** Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (Crassostrea virginica).
- 4. **CITATION:** Surprenant, D.C. 1988. Acute Toxicity of Technical Grade HWG 1608 to Eastern Oysters (Crassostrea virginica). Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. SLS Report #87-7-2417. SLS Study #274-0187-6130-504. Submitted by Mobay Chemical Corporation, Stilwell, Kansas. MRID No. 409959-03.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date:

6. **APPROVED BY:**

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

Signature:

Date:

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

Signature: *Henry T. Craven*

6/25/98

Date:

Clyde R. Hausenwoirt

Date:

Clyde Hausenwoirt, 4/22/90

- 7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for an oyster shell deposition study. The 96-hour EC50 value for eastern oysters exposed to technical grade HWG 1608 was 2.7 mg a.i./L based on mean measured concentration. Therefore, technical grade HWG 1608 is classified as moderately toxic to Eastern oysters (Crassostrea virginica). The NOEC was 1.7 mg a.i./L..

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

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9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A11. MATERIALS AND METHODS:

- A. Test Animals: Eastern oysters (Crassostrea virginica) were obtained from Aquacultural Research Corporation, Dennis, Massachusetts where they were reared in natural flowing seawater from approximately the same source (Massachusetts Bay) as that used as dilution water during the toxicity test. During the 7 days prior to testing, the salinity range was 29 - 30‰ and the temperature range was 19.5 - 21°C. No mortality was recorded for this period and the mortality for the month of May was <5%. No mortality occurred during the 48 hours prior to testing.

Oysters were transported to the testing laboratory in styrofoam containers, and were outside of water for a maximum of 1.5 hours. During the last 24 hours of acclimation and testing, oysters were fed a supplementary diet of Isochrysis galbana Parke, clone T-ISO and Tetraselmis maculata, such that the algal density in the aquaria was 10^5 cells/mL.

- B. Test System: The test was conducted in a continuous flow serial diluter with a dilution factor of 0.60. The system was a modification of the diluter described by Benoit et al (1982). Each glass aquarium measured 60 x 30 x 30 centimeters (cm) and was equipped with a 10-cm high standpipe which maintained a test solution volume of approximately 18 liters (L).

The flow of test solution to each aquarium was 75 mL/minute, which provided approximately six volume replacements every 24 hours. In addition, the contents of each aquarium were continuously circulated at a flow rate of 1.75 L per minute or about 5 L per oyster per hour. This circulation system aided in evenly distributing the algae fed to the oysters and in mixing the flow of fresh test solution throughout each aquarium. The temperature was maintained by a water bath at $20 \pm 2^\circ\text{C}$. The photoperiod provided 16 hours of light and 8 hours of darkness per day.

The dilution water was natural unfiltered seawater collected from the Cape Cod Canal near Bourne, Massachusetts. The dilution water was characterized as

having a salinity of 30 ‰ and a pH of 7.7 to 8.1 (based on the measurement of control solutions).

- C. **Dosage:** 96-hour acute flow-through test.
- D. **Design:** Twenty-four hours prior to testing, 2-3 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge. Immediately prior to the test initiation, the outer shell was buffed with an emery board to remove any new shell deposition.

The exposure of oysters was initiated by impartially selecting and placing 20 oysters in each test aquarium (40 per treatment). Oysters were spaced equidistant from one another with their valve openings facing toward the flow from the circulator tube. A control, solvent control and five nominal technical grade HWG 1608 concentrations of 1.9, 3.2, 5.4, 9.0 and 15 mg a.i./L were tested. The concentration of acetone in the solvent control was equal to that present in the highest test concentration (0.429 mL acetone/L). During the exposure period, the oysters received supplemental feeding of algae (*Isochrysis galbana* and *Tetraselmis maculata*). One hundred eighty (180) mL of a concentrated algal suspension of 10^7 cells/mL were added to each test aquarium three times daily, resulting in an algal density of approximately 10^5 cells/mL in each aquarium.

Biological observations were made daily during the exposure in order to detect any mortality of oysters and to record any visible abnormalities. After 96 hours of exposure the oysters were removed and the new shell growth was measured microscopically to 0.1 mm using a calibrated micrometer. During the exposure, the pH and dissolved oxygen concentration were measured daily in each aquarium. The salinity and temperature in one replicate of the control was monitored daily. Temperature was also monitored continuously in one test aquarium. Analytical determination of technical grade HWG 1608 was performed on all test solutions, control and solvent control at 0, 48 and 96 hours using high pressure liquid chromatographic (HPLC) analysis.

- E. **Statistics:** A computer program developed at the testing laboratory was utilized to compute four linear regression curves based on least squares. Both untransformed and transformed data were regressed. The regression line which provided the best fit of the

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untransformed or transformed data was selected based on the highest associated coefficient of determination. The regression equation was then applied to calculate the EC50 and its 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1969).

The no-observed-effect concentration (NOEC) was determined by subjecting the biological response (shell growth) data to analysis of variance and Dunnett's test. The highest test concentration causing no significant reduction of shell growth was identified as the NOEC.

12. **REPORTED RESULTS:** The mean measured concentrations of technical grade HWG 1608 in the test solutions during the 96-hour definitive test were 1.7, 2.8, 3.3, 4.3 and 9.3 mg a.i./L. The mean measured concentrations of technical grade HWG 1608 ranged from 47% to 89% of the nominal concentrations. Although the measured concentrations of nominal, exposure levels maintained were generally consistent during the 96 hour study and sufficient to produce a biological response (reduced shell growth).

Mean measured test concentrations of technical grade HWG 1608 and the corresponding mean shell deposition measurements are presented in Table 2 (attached). No significant difference ($P = 0.05$) was indicated between the amount of shell deposition for the control and solvent control oysters when the data were subjected to analysis of variance and Dunnett's test. Therefore, the treatment response was compared to the pooled responses of the two control groups.

During the 96-hour test, 78% mortality occurred among oysters exposed to a mean measured test concentration of 9.3 mg a.i./L, the highest concentration tested. Oysters exposed to treatment levels ≥ 4.3 mg/L exhibited a lack of feces and pseudofeces production throughout the exposure period. The mean shell deposition of oysters exposed to test concentrations ≥ 2.8 mg a.i./L technical grade HWG 1608 was significantly reduced when compared to control shell growth. Oysters exposed to 1.7 mg a.i./L had shell growth which was not significantly different from control shell growth. The 96-hour EC50 and 95% confidence interval calculated by linear regression were 3.0 (1.4-5.6) mg a.i./L. The no-observed effect concentration (NOEC) was 1.7 mg a.i./L. The slope of the dose-response curve was calculated to be 3.43. Based on EPA (1985) criteria, the test material would be classified as moderately toxic.

Water quality was unaffected by test concentrations of technical grade HWG 1608 and was satisfactory for the survival and growth of the test organisms.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

The study was audited by the QA unit of Springborn Life Sciences, Inc. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP and ASTM as follows:

o A flow rate of the test solution to each aquarium was 75 mL/minute was utilized providing a flow of 200 mL/oyster/hour. This rate is only 5% of the rate set forth in test protocols recommended in the Guidelines (approximately 5 liter/oyster/hour). To offset the reduced flow, the test facility supplemented the water with the algae, Isochrysis galbana Parke and Tetraselmis maculata at a density of 1×10^5 cells/mL three times daily, and utilized pumps to recirculate test solutions at a flow rate of 5 L/oyster/hour. It should be noted however, that a flow-through test, as defined by ASTM (1980), consists of a "test solution that flows through the test chamber on a once-through basis throughout the test" and not on a recirculated flow.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour EC50 value. These calculations are attached. The moving average method provided a 96-hour EC50 value of 2.7 mg a.i./L with a 95 percent confidence interval of 2.5 and 3.0 mg a.i./L which is similar to that reported by the author (i.e., 3.0 mg a.i./L with a 95% confidence interval of 1.4 and 5.6 mg a.i./L). Since the raw data on shell

growth were not submitted with the report, statistical comparison of shell growth between the control and each treatment level could not be verified.

C. **Discussion/Results:** The study results appear to be scientifically valid. The 96-hour EC50 value, based on mean measured concentrations of technical grade HWG 1608, was 2.7 mg a.i./L. Therefore, technical grade HWG 1608 is classified as moderately toxic to Eastern oysters (Crassostrea virginica). The NOEC was 1.7 mg a.i./L.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** Although the test procedures deviated from the guidelines, the deviations probably did not significantly affect the toxicity results.

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, 01-02-90.

Shaughnessy No. 128997

Chemical Name Folicur Chemical Class _____ Page _____ of _____

Study/Species/Lab/ Accession Chemical & a.l.

(or Tebuconazole)

Results

Reviewer/ Valid Date Stat

14-Day Single Dose Oral LD50

LD50 = mg/kg (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) = Sex =

Lab _____

14-Day Dose Level mg/kg/(% Mortality)

Acc. _____

Comments:

14-Day Single Dose Oral LD50

LD50 = mg/kg. (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) = Sex =

Lab _____

14-Day Dose Level mg/kg/(% Mortality)

Acc. _____

Comments:

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) = Sex =

Lab _____

8-Day Dose Level ppm/(% Mortality)

Acc. _____

Comments:

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (X) =

Species _____

Slope = # Animals/Level = Age (Days) = Sex =

Lab _____

8-Day Dose Level ppm/(% Mortality)

Acc. _____

Comments:

~~48~~-Hour EC50

Species Crassostrea virginica

Lab Springborn Life Sciences, Inc

Age: MRID# - 409959-03

EC50 = 2.7 ppm (95% C.L.) Reduced shell growth Contr. Mort. (X) = 0

Slope = N/A # Animals/Level = 40 Sol. Contr. Mort. (X) = 0

96 48-hour Dose Level ppm/(% Mortality) Reduced shell growth Temperature = 20 ± 1 °C 01/02/90 CAS

1.7 (14), 2.3 (52), 3.3 (66), 4.3 (72), 9.3 (93)

Comments: Based on mean measured concentration.

96-Hour EC50

Species _____

Lab _____

Acc. _____

LC50 = PP (95% C.L.) Con. Mort. (X) = Sol. Con. Mort. (X) =

Slope = # Animals/Level = Temp. =

96-Hour Dose Level pp /(% Mortality)

Comments:

96-Hour LC50

Species _____

Lab _____

Acc. _____

LC50 = PP (95% C.L.) Con. Mort. (X) = Sol. Con. Mort. (X) =

Slope = # Animals/Level = Temp. =

96-Hour Dose Level pp /(% Mortality)

Comments:

Page 19 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.
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 - The document is not responsive to the request.
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KIMBERLY RHODES FOLICUR (OR TEBUCONAZOLE) CRASSOSTREA VIRGINICA 01-02-9

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.3	100	93	93	0
4.3	100	72	72	0
3.3	100	66	66	0
2.8	100	52	52	0
1.7	100	14	14	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 2.734358

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN G * LC50 95 PERCENT CONFIDENCE LIMITS
 2 6.137299E-02 2.729711 (2.542047 2.953642)

RESULTS CALCULATED USING THE PROBIT METHOD
 ITERATIONS G H GOODNESS OF FIT PROBABILITY
 3 .2767903 2.964345 3.074753E-02

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

SLOPE = 3.459978
 95 PERCENT CONFIDENCE LIMITS = 1.639653 AND 5.280302

LC50 = 2.897905
 95 PERCENT CONFIDENCE LIMITS = 2.114552 AND 3.694627

LC10 = 1.2446
 95 PERCENT CONFIDENCE LIMITS = .4165121 AND 1.811719

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Folicur (or Tebuconazole).
Shaughnessey Number: 128997.
- 2. **TEST MATERIAL:** Technical grade HWG 1608; Lot No. 86R00 82I;
96.28% active ingredient; an off-white colored powder.
- 3. **STUDY TYPE:** Estuarine fish 96-hour acute toxicity test.
Species Tested: Sheepshead minnow (Cyprinodon variegatus).
- 4. **CITATION:** Surprenant, D.C. 1988. Acute Toxicity of
Technical Grade HWG 1608 to Sheepshead Minnow (Cyprinodon
variegatus) Under Flow-Through Conditions. Prepared by
Springborn Life Sciences, Inc., Wareham, Massachusetts. SLS
Report #87-5-2435. SLS Study #274.1186.6127.505. Submitted
by Mobay Corporation, Stilwell, Kansas. MRID No. 409959-04.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Kimberly D. Rhodes*

Date: *January 3, 1990*

6. **APPROVED BY:**

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

Signature: *P. Kosalwat*

Date: *January 3, 1990*

Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*
6/25/98

Date: *Clyde R. Amelnecker June 21, 1990*

Clyde R. Amelnecker

- 7. **CONCLUSIONS:** This study appears scientifically sound and
fulfills the Guideline requirements for a 96-hour acute
flow-through toxicity study using estuarine fish. The 96-
hour LC50, based upon mean measured concentrations, of
technical grade HWG 1608 to sheepshead minnows (Cyprinodon
variegatus) was 5.9 mg a.i./L. Therefore, technical grade
HWG 1608 is classified as moderately toxic to sheepshead
minnows. The NOEC was determined to be <1.2 mg a.i./L after
96 hours of exposure.

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

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Sheepshead minnow (Cyprinodon variegatus) used in this test were obtained from a commercial fish supplier in Massachusetts. The fish were held in a 500-L fiberglass tank containing natural seawater for a minimum of 14 days at 21 - 22°C. The water had a salinity of 32 - 33 ‰, a pH range of 7.4 - 7.5, and a dissolved oxygen concentration range of 89 - 96% of saturation. The fish were fed a dry commercial pelleted food, ad libitum, daily.

No mortality occurred in the test fish population during the 48 hours prior to testing. The sheepshead minnow used for testing had a mean wet weight of 0.11 grams (g) with a range of 0.01 to 0.31 g and a mean length of 15 millimeters (mm) with a range of 9 to 23 mm. The maximum organism loading concentration was 0.073 g of biomass per liter of test solution.

- B. Test System: The test was conducted using an exposure system consisting of a continuous flow serial diluter (Benoit et al., 1982), a constant-temperature controlled water bath, and a set of 14 test aquaria. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a 19.5-cm high standpipe which maintained a constant test water volume of 15 L.

The diluter provided approximately 6.5 volume additions per aquarium every 24 hours. The aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test water temperature at $22 \pm 1^\circ\text{C}$. A photoperiod of 16 hours of light and 8 hours of darkness with a light intensity of 20 - 34 footcandles was provided each day.

The dilution water used was filtered natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts. The dilution water was characterized as having a salinity range of 30 - 32‰ and a pH range of 7.3 - 7.9.

- C. Dosage: 96-hour acute flow-through test.

- D. **Design:** The test was initiated when 10 sheepshead minnows were impartially selected and distributed to each replicate aquarium (20 per concentration). A control, solvent control, and five nominal technical grade HWG 1608 concentrations of 1.3, 2.1, 3.2, 4.9 and 7.5 mg a.i./L were tested.

All treatment levels and the controls were maintained in duplicate. The solvent control solution contained the maximum amount of acetone present in any test concentration (324 uL/L). All concentrations were observed at 24, 48, 72, and 96 hours for mortality and abnormal effects. The water quality parameters (dissolved oxygen, salinity, pH and temperature) were measured once daily in each replicate of each treatment level and the controls throughout the exposure. Analytical determination of technical grade HWG 1608 was performed on all test solutions, control and solvent control at 0, 48 and 96 hours using a high pressure liquid chromatographic (HPLC) analysis.

- E. **Statistics:** The mean measured test concentrations and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period by using the computer program developed by Stephan et al. (1977, 1982).

12. **REPORTED RESULTS:** The mean measured test concentrations, the corresponding cumulative mortalities and the observations made during the 96-hour test are presented in Table 2 (attached). The mean measured concentrations of technical grade HWG 1608 in exposure solutions during the 96-hour definitive test were 1.2, 1.9, 2.8, 4.3 and 6.8 mg a.i./L. The mean measured concentrations of technical grade HWG 1608 in the exposure solutions ranged from 88 to 92% of the nominal concentrations. During the exposure, solutions were clear, colorless and contained no visible sign of insoluble test material (e.g., precipitate, film, on the solution's surface).

Following 96 hours of exposure, 70% mortality was recorded in the highest mean measured concentration of technical grade HWG 1608 tested (6.8 mg a.i./L). Mortality in the remaining treatment levels (4.3 - 1.2 mg a.i./L) ranged from 0 - 10%. Toxicant-related behavior abnormalities were observed in the 4.3-mg a.i./L treatment level. The 24-, 48-, 72- and 96-hour LC50 values (95% confidence interval)

for sheepshead minnow exposed to technical grade HWG 1608 were >6.8, >6.8, 6.5 (>4.3) and 5.9 (5.3 - 7.0) mg a.i./L, respectively. Based on the results of this study, the no-observed effect concentration (NOEC) for sheepshead minnow exposed to technical grade HWG 1608 was determined to be 2.8 mg a.i./L.

The water quality parameters measured during this study remained within acceptable ranges for the survival of the sheepshead minnow and were unaffected by the concentrations of technical grade HWG 1608 tested.

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

No conclusions were made by the author.

The study was audited by the QA unit of Springborn Life Sciences, Inc. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP states that individual fish should weigh between 0.5 and 5 grams. The standard length of the longest fish should not be more than twice the length of the shortest fish. The fish used in this study weighed between 0.01 and 0.31 grams and the total length of the test fish ranged from 9 to 23 millimeters.

o The recommended temperature for testing the sheepshead minnow is $22 \pm 1^{\circ}\text{C}$. The temperature ranged from 19 to 23°C during the exposure period of this toxicity test.

o The SEP states that in the flow-through test, the dissolved oxygen concentration in each chamber must be between 60% and 100% saturation at all times during the study. During this study, the dissolved oxygen concentration was as low as 51% saturation at 96 hours of exposure.

o The SEP states that natural or reconstituted seawater of 10 to 17 ‰ salinity should be used when testing euryhaline fish species. The natural seawater

used during this toxicity study had a salinity range of 30 - 32‰.

o During this test, the salinity was measured only in the dilution water source. The salinity of each exposure solution should have been measured daily.

The toxicity report did not provide the following information required by the SEP:

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the LC50 value. These calculations are attached. The moving average method provides a 96-hour LC50 value of 5.9 mg a.i./L with a 95 percent confidence interval of 5.3 to 7.0 mg a.i./L which is the same as that reported by the author. The author determined the NOEC to be 2.8 mg a.i./L. However, the reviewer feels that this value should be <1.2 mg a.i./L (the lowest concentration tested) since mortality occurred in the two lowest test concentrations.
- C. **Discussion/Results:** The study results appear to be scientifically valid. The 96-hour LC50 value, based upon mean measured concentrations, was determined to be 5.9 mg a.i./L. Therefore, technical grade HWG 1608 is considered moderately toxic to sheepshead minnow (Cyprinodon variegatus). The no-observed effect concentration (NOEC) was determined to be <1.2 mg a.i./L.
- D. **Adequacy of the Study:**
- (1) **Classification:** Core.
 - (2) **Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the toxicity results.
 - (3) **Repairability:** N/A.
15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 12-30-89.

Shaughnessy No. 128997

Chemical Name Folicur Chemical Class _____ Page _____ of _____

(or Tebuconazole)

Study/Species/Lab/
Accession _____ Chemical
_____ & a. l.

Reviewer/
Date _____ Valid
Stat _____

14-Day Single Dose Oral LD50

Results
LD50 = . mg/kg (95% C.L.) Contr. Mort. (X) =
Slope = # Animals/Level = Age (Days) =
Sex =
14-Day Dose Level mg/kg/(% Mortality)
() () () () () ()

Species _____

Lab _____

Acc. _____

Comments:

14-Day Single Dose Oral LD50

LD50 = mg/kg. (95% C.L.) Contr. Mort. (X) =
Slope = # Animals/Level = Age (Days) =
Sex =
14-Day Dose Level mg/kg/(% Mortality)
() () () () () ()

Species _____

Lab _____

Acc. _____

Comments:

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (X) =
Slope = # Animals/Level = Age (Days) =
Sex =
8-Day Dose Level ppm/(% Mortality)
() () () () () ()

Species _____

Lab _____

Acc. _____

Comments:

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (X) =
Slope = # Animals/Level = Age (Days) =
Sex =
8-Day Dose Level ppm/(% Mortality)
() () () () () ()

Species _____

Lab _____

Acc. _____

Comments:

48-Hour LC50

LC50 = pp (95% C.L.) Contr. Mort. (X) =
Sol. Contr. Mort. (X) =
Slope = # Animals/Level = Temperature =
48-Hour Dose Level pp/(% Mortality)
() () () () () ()

Species _____

Lab _____

Acc. _____

Comments:

96-Hour LC50

LC50 = 5.9 ppm (95% C.L.) + moving average method
Con. Mort. (X) = 5
Sol. Con. Mort. (X) = 0
Slope = N/A # Animals/Level = 20 Temp. = 19-23°C
96-Hour Dose Level ppm/(% Mortality)
1.2 (10) 1.9 (5) 2.8 (0) 4.3 (10) 6.8 (70)

Species Cyprinodon variegatus

Lab Springborn Life Sciences, Inc.

Acc. MRID # 409959-04

Comments: Based on mean measured concentrations.

96-Hour LC50

LC50 = ppm (95% C.L.) Con. Mort. (X) =
Sol. Con. Mort. (X) =
Slope = # Animals/Level = Temp. =
96-Hour Dose Level ppm/(% Mortality)
() () () () () ()

Species _____

Lab _____

Acc. _____

Comments:

Page 27 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.

KIMBERLY RHODES FOLICUR (OR TEBUCONAZOLE) CYPRINODON VARIEGATUS 12-30-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
6.8	20	14	70	5.765915
4.3	20	2	10	2.012253E-02
2.8	20	0	0	9.536742E-05
1.9	20	1	5	2.002716E-03
1.2	20	2	10	2.012253E-02

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 5.898282

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN G * LC50 95 PERCENT CONFIDENCE LIMITS
 1 .235757 5.898282 5.27371 6.960131

RESULTS CALCULATED USING THE PROBIT METHOD
 ITERATIONS G H GOODNESS OF FIT PROBABILITY
 6 4.215223 6.298052 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 = 2.80392
 95 PERCENT CONFIDENCE LIMITS = -2.952811 AND 8.560651

LC50 = 6.997418
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.466042
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

DATA EVALUATION RECORD

1. **CHEMICAL:** Folicur (or Tebuconazole).
Shaughnessey No. 128997.
2. **TEST MATERIAL:** HWG 1608 technical; a fungicide; chemical name: alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; 97.5% active ingredient; Batch No. 16012/86; a gray-white powder with lumps.
3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Scenedesmus subspicatus.
4. **CITATION:** Heimbach, F. 1987. Growth Inhibition of Green Algae (Scenedesmus subspicatus) by HWG 1608 (Tech.). Laboratory Project ID No. 94853. Conducted by Bayer AG, Chemical Research Institute of Environmental Biology, Federal Republic of Germany. Submitted by Mobay Corporation. MRID No. 409959-08.
5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D. Staff Toxicologist KBN Engineering and Applied Sciences, Inc.	Signature: P. Kosalwat Date: January 3, 1990
--	---
6. **APPROVED BY:**

Michael L. Whitten, M.S. Wildlife Toxicologist KBN Engineering and Applied Sciences, Inc.	Signature: Michael L. Whitten Date: 1-3-90
Henry T. Craven, M.S. Supervisor, EEB/HED USEPA CLYDE HOUSEKNECHT	Signature: Henry T. Craven Date: 6/25/90 Clyde Houseknecht 6/21/90
7. **CONCLUSIONS:** This study is scientifically sound. However, it does not fulfill the guideline requirements for a Tier-2 growth and reproduction test using a non-target green algal species. Based on cell counts, the 4-day EC25 and EC50 values of HWG 1608 for Scenedesmus subspicatus were 0.88 and 1.45 mg/L nominal concentrations, respectively. The NOEC value was determined to be 0.32 mg ai/L nominal concentration.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

A. Test Species: The test species was a green algal species (Scenedesmus subspicatus, strain SAG 86/81). The source of the algae was not reported. The stock cultures were maintained under 16 hours of light per day at 20°C in an autoclaved nutrient solution in accordance with Bringmann and Kuehn (1980, Water Research, 14:231-241). The stock cultures were transferred into fresh nutrient solution once a week. All nutrient solutions were prepared using deionized filter-sterilized water.

B. Dosage: Four-day growth and reproduction test.

C. Test System and Design: Based on a range-finding test, seven nominal HWG 1608 concentrations of 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10 mg ai/L were selected for the definitive test. The stock solution was prepared in acetone and deionized water.

Test vessels used were 300-ml Erlenmeyer flasks. The test medium (based on 1 L) contained 15 mg NH_4Cl , 12 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 18 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 15 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6 mg KH_2PO_4 , 80 ug $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 100 ug $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 185 ug H_3BO_3 , 415 ug $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3 ug ZnCl_2 , 1.5 ug $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01 ug $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 7 ug $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 50 mg NaHCO_3 .

The test was initiated when a calculated volume of inoculum was added to each flask, yielding an initial concentration of 10,000 cells/ml. The flasks were kept in an incubator at $23 \pm 1^\circ\text{C}$ and 8,000 lux continuous lighting (using fluorescent lamps). At test initiation and every 24 hours, the pH was monitored in the solvent control, and lowest and highest test concentrations. The test temperature was measured only at 96 hours.

The cell count was obtained indirectly via a photometric extinction/turbidity measurement. In order to determine possible cell changes, a random sample from each of the control, highest, middle and lowest concentrations was examined microscopically for abnormalities (such as atypical cell sizes that could

possibly influence the extinction) every 24 hours. The photometric cell count was performed only when no changes that could alter the extinction were found.

- E. **Statistics:** The EC50 for the growth of the biomass and for the algal growth rate were calculated using a probit analysis by the method of "maximum likelihood." The author stated that "for mathematical reasons, the calculation of a 95% confidence interval is not practical for this species and for its corresponding evaluation."
12. **REPORTED RESULTS:** During the 4-day exposure period, the pH values ranged from 7.71 to 8.4. The temperature measured at the end of the test was between 22.3 and 22.6°C. Table 3 (attached) presents cell counts at every 24 hours. No abnormalities were observed. Areas under the growth curves and growth rates of algae in the control, solvent control, and each test concentration are presented in Tables 4 and 5 (attached), respectively.

Figures 2 and 3 (attached) showed concentration-effect curves of HWG 1608 technical for the growth based on the biomass and growth rate, respectively. Based on the biomass, the 3-day and 4-day EC50 values were 1.96 and 1.64 mg ai/L, respectively. Based on the growth rate, the 3-day and 4-day EC50 values were 5.3 and 4.01 mg ai/L, respectively. The slopes for 4-day EC50 were 2.36 and 2.44 based on the biomass and growth rate, respectively. The no-observed-effect concentration (NOEC) was 0.32 mg ai/L for biomass and 1.0 mg ai/L for growth rate, the lowest tested concentration with signs of toxicity was 0.56 mg ai/L for biomass and 1.8 mg ai/L for growth rate.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
A test under the same conditions using a reference standard ($K_2Cr_2O_7$) was conducted. The 4-day EC50 for the biomass (0.44 mg/L) agreed well with the results of a collaborative study which ranged from 0.20 to 0.75 mg/L. The 4-day EC50 for the growth rate (1.2 mg/L) fell slightly above the collaborative study range of 0.60-1.03 mg/L.

A GLP statement was included in the report, indicating that the study was performed in accordance with GLP regulations (TSCA 40 CFR Part 792 of November 29, 1983; FIFRA 40 CFR Part 160, November 29, 1983; OECD C(81) 30 (Final), May 12, 1981, published in the Bundesanzeiger, February 4, 1983).

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

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

- o Selenastrum capricornutum was not tested.
- o Scenedesmus subspicatus is not a recommended species. A justification for using this species was not provided.
- o Source of the test organisms was not reported.
- o The maximum label rate was not provided in the report. Therefore, it could not be determined whether the concentrations tested were less than the maximum label rate as though it were applied directly to the surface of a 15-cm water column.
- o The nutrient medium contained 100 ug/L of $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$. According to Subdivision J guidelines, EDTA should not be used in the experimental medium.
- o The study length was only 4 days. At least 5 days of exposure are recommended for an algal test.

B. Statistical Analysis: The reviewer calculated EC50 and EC25 values based on the cell counts, areas under the growth curves, and the growth rates, using a regression analysis. The EC50 values based on the areas under the growth curves and the growth rates were similar to those calculated by the author.

Analysis of variance and multiple comparison (Tukey's and Bonferroni's) tests were performed to compare cell counts at each treatment level to those of the controls. The results showed that test concentrations of ≥ 1.0 mg ai/L significantly ($p \leq 0.05$) reduced the cell counts of S. subspicatus at the end of the test (day 4). Therefore, the no-observed-effect concentration was 0.56 mg ai/L, based on the cell counts.

- C. **Discussion/Results:** This study is scientifically sound. However, the algae were exposed to the test chemical for only 4 days when a period of 5 days is a minimum requirement for algal species. In addition, the test organism is not a recommended species. The 4-day EC25 and EC50 values of HWG 1608 for S. subspicatus calculated by the reviewer are summarized in the following table:

Parameter	4-day	
	EC25 (mg/L)	EC50 (mg/L)
Cell counts	0.88	1.45
Areas under growth curves	0.91	1.69
Growth rates	2.16	4.15

The EC25 and EC50 derived from the cell counts yielded the most conservative values. Therefore, these values should be used in the risk assessment. The author did not indicate how the NOEC values based on the biomass and growth rates were determined. From the data submitted, only the NOEC value based on the cell counts could be determined. Based on the reduction of cell counts at concentrations of ≥ 1.0 mg ai/L, the NOEC was determined to be 0.56 mg ai/L. However, the lowest value of NOEC was determined by the author to be 0.32 mg ai/L, based on biomass. Therefore, to better protect aquatic life from the effects of HWG 1608, 0.32 mg ai/L should be used as the no-observed-effect level.

D. **Adequacy of the Study:**

- (1) **Classification:** Supplemental.
- (2) **Rationale:** The study length was only 4 days. A minimum period of 5 days is required. In addition, S. subspicatus is not a recommended species.
- (3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, January 3, 1990.

Cell counts

REGRESSION EQUATION:

$$Y = 4.50201 + 3.079591 X$$

COEFFICIENT OF CORRELATION = .9864181

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PROBITS (of % inhibition)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-.4949	3.12	2.97792	.1420796
2	-.2518	3.77	3.726569	4.343128E-02
3	0	4.08	4.50201	-.4220099
4	.2553	5.18	5.28823	-.1082296
5	.5051	6.41	6.057511	.3524885
6	.7482	7.05	6.80616	.2438402
7	1	7.33	7.581601	-.2516012

$$EC_{50} = 1.45 \text{ mg ai/L}$$

$$EC_{25} = 0.88 \text{ mg ai/L}$$

Areas under growth curves

REGRESSION EQUATION:

$$Y = 4.433748 + 2.471425 X$$

COEFFICIENT OF CORRELATION = .9821631

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PROBITS (of % inhibition)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-.2518	3.82	3.811443	8.557081E-03
2	0	4.19	4.433748	-.2437477
3	.2553	5.13	5.064703	.0652976
4	.5051	5.99	5.682065	.3079348
5	.7482	6.41	6.282868	.1271315
6	1	6.64	6.905173	-.2651734

$$EC_{50} = 1.69 \text{ mg ai/L}$$

$$EC_{25} = 0.91 \text{ mg ai/L}$$

Growth rates

REGRESSION EQUATION:

$$Y = 3.541449 + 2.361008 X$$

COEFFICIENT OF CORRELATION = .9820569

ACTUAL VERSUS ESTIMATED VALUES

DATA POINT	X = LOG CONCENTRATION	Y = PROBITS (of % inhibition)	ESTIMATED Y	ERROR
1	-.4949	2.67	2.372986	.2970142
2	-.2518	2.95	2.946947	3.053427E-03
3	0	3.12	3.541449	-.4214487
4	.2553	3.96	4.144214	-.1842136
5	.5051	4.85	4.733994	.1160059
6	.7482	5.52	5.307955	.2120452
7	1	5.88	5.902457	-2.245665E-02

$$EC_{50} = 4.15 \text{ mg ai/L}$$

$$EC_{25} = 2.16 \text{ mg ai/L}$$

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
13200000.000		61363333.380	
23216666.800		7 245000.000	
33093333.200		8 56666.668	
42853333.200		9 30000.000	
52630000.000			

Comparison	Tukey-B*	Bon- ferroni
1 < 2		
1 > 3		
1 > 4		
1 > 5	0.0500	0.0342
1 > 6	0.0100	0.0000
1 > 7	0.0100	0.0000
1 > 8	0.0100	0.0000
1 > 9	0.0100	0.0000
2 > 3		
2 > 4		
2 > 5	0.0500	0.0267
2 > 6	0.0100	0.0000
2 > 7	0.0100	0.0000
2 > 8	0.0100	0.0000
2 > 9	0.0100	0.0000
3 > 4		
3 > 5	0.0500	
3 > 6	0.0100	0.0000
3 > 7	0.0100	0.0000
3 > 8	0.0100	0.0000
3 > 9	0.0100	0.0000
4 > 5		
4 > 6	0.0100	0.0000
4 > 7	0.0100	0.0000
4 > 8	0.0100	0.0000
4 > 9	0.0100	0.0000
5 > 6	0.0100	0.0000
5 > 7	0.0100	0.0000
5 > 8	0.0100	0.0000
5 > 9	0.0100	0.0000
6 > 7	0.0100	0.0000
6 > 8	0.0100	0.0000
6 > 9	0.0100	0.0000
7 > 8		
7 > 9		
8 > 9		

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

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