2-28-92

EEB BRANCH REVIEW

100.0 PESTICIDE NAME: RH-287

100.1 Submission Purpose:

Submission of aquatic studies in support of new chemical

100.2 Formulation Information:

96.9% active ingredient, a brown solid

100.3 Studies Submitted for Review:

? —	Sheepshead Minnow Early Life Stage Study	417415-04
ok	Sheepshead Minnow Acute Flow-Through	417415-02
ok	Mysid Shrimp Acute Flow-Through	417415-03
? —	American Oyster Acute Static	417568-01
ok	Daphnia magna Acute Flow-Through	417415-01
? —	Daphnia magna Chronic	417415-05
? —	Plant Study for Selenastum Capricornutum Printz	417568-02
	Die-Away Study in Natural and Synthetic Water	417415-06

102.0 Conclusions:

A. Sheepshead Minnow (Acute)

This study is scientifically sound and fulfills the guideline requirements for an acute flow-through toxicity study with estuarine/marine fish. The 96-hour LC_{50} of RH-287 for Cyprinodon variegatus is 20.5 ug/L, based on mean measured concentrations. RH-287 is classified as very highly toxic to the sheepshead minnow. The NOEC was 7.6 ug/L.

B. Sheepshead Minnow (Fish Early Life Stage)

This study is scientifically sound but does not fulfill the guideline requirements for an early life stage toxicity study. The solvent control mortality exceeded limits set forth in the guidelines. Plus 2 replicates per concentration were used instead of 4 replicates as set forth in the SEP. Based on the effects of RH-287 to Cyprinodon variegatus, the MATC was between 6.0 and 14.0 ug/L mean measured concentrations (geometric mean MATC = 9.2 ug a.i./L

C. Mysid Shrimp (Acute Flow-Through)

This study indicates RH-287 is very highly toxic to mysid shrimp with an LC_{50} of 4.7 ppb based on mean measured concentrations. Even though the solvent control mortality exceeded the 5% limits set in the SEP, this study does fulfill the requirement in support of registration for an estuarine/marine species. The 10% mortality (2 died) in the solvent control will not change the toxicity category for the mysid shrimp in this study. However, it does show that triethylene glycol has an effect on mysid shrimp. The actual 96-hour LC_{50} is 5.6 ppb.

D. Eastern Oyster (48-hour Embryo-Larvae)

These studies are not scientifically sound and do not fulfill the guideline requirements for an oyster embryo-larvae study. RH-287 concentrations decreased during both studies (performed with natural seawater and synthetic seawater) making it difficult to determine an accurate EC_{50}. The synthetic seawater study was unacceptable since greater than 30% mortality was observed in the control. The 48-hour EC_{50} for the natural seawater study, based upon nominal concentrations of RH-287, to eastern oysters Crassostrea virginica was 24 ug/L. Therefore, RH-287 is classified as very highly toxic to eastern oysters.

E. Daphnia magna (48-Hour EC_{50})

This study appears to be scientifically sound and meets guideline requirements for a daphnid acute flow-through toxicity test. The EC_{50} was 5.22 ug/L based on mean measured concentrations; therefore RH-287 is classified as very highly toxic to Daphnia magna. The NOEC was 5.0 ug a.i./L based on mean measured concentrations.

F. Daphnia magna (Chronic)

This study is not scientifically sound and does not fulfill the guideline requirements for a daphnid life cycle test. Adult daphnids in the control produced less than 40 young. Mean measured test concentrations varied greater than 30% of nominal concentrations and were highly variable among days and among replicates on the same day. The EC_{50} of RH-287 to Daphnia magna was 1.2 ug a.i./L, based on mean measured concentrations. The NOEC could not be determined, since sublethal/lethal effects were noted in all concentrations.

G. Selenastrum capricornutum (Freshwater Green Algae)

This study is not scientifically sound, and does not meet the requirements for a Tier 2 study on the growth and reproduction of aquatic plants. Using nominal concentrations, the 120-hour EC_{50} of RH-287 for Selenastrum capricornutum was determined to be 0.0075 mg/L. The absence of the chemical in four of the five test solutions calls into question the methods of this study and invalidates it.

H. Die-Away Studies (Natural and Synthetic Waters)

1. Die-Away Study (Study # 1)

This study indicates the die-away of RH-287 was considerably more rapid with the algae present than without algae present, with no detectable RH-287 after 24 hours in hatchery water (both concentrations), and more than 90% loss in synthetic seawater. There is a die-away of RH-287 in natural water even when filtered to 1.0 um to remove most non-bacterial biota. It is also obvious that RH-287 does not die-away in synthetic water.

There was a 40% recovered from the highest nominal concentration of RH-287 and 25% recovered of RH-287 from the low nominal concentration.

2. Die-Away Study (Study # 2)

This study indicates that all water samples had a typically high pH ranging from 7.5 to 8.1 with no trend in relation to salinity. The oxygen concentration was in every case high.

No sulfide was detected in the freshwater samples from the Poropotank River.
In all water samples from stations with measurable salinity, the amount of sulfide was between 0.0 and 0.7 ug-at/L.

In all water types, the loss of RH-287 was quite rapid. Nearly 100% of RH-287 was loss within 24.5 hours in the water samples collected from station 2. There was no direct relationship between salinity of the water and RH-287 loss.

Half-life from all points ranged from 9.2 hours to 49 hours in water from Poropotank River, VIMS Pier and Lyannhaven station. Half-life from water samples Fox Creek ranged from 3.3 hour to 5.7 hours.

3. Die-Away Study (Study # 3)

This study indicated after sterlization, reaeration, and addition of algae, no significant amount of sulfide was detected in any sample.

When no algae were added, the chlorophyll a content of control and u.v. sterilized water was about 0.7 ug/L, whereas it was reduced to 0.2 and 0.3 ug/L by 0.22 um filtration or autoclaving. In the control water with or without algae, the loss of RH-287 was quite rapid with a total loss within 24 hours.

A shor-term half-life ranged from 7.0 hours (autoclaved water) to 10.7 hours (0.22 um filtered water).

Prior to consideration of the proposed registration of RH-287 as an anti-foulant to be used on boat bottoms the following additional studies are required:

1. Required Freshwater and Esturine/Marine Studies

   a. Invertebrate Life Cycle for Estuarine/Marine 72-4
   b. Invertebrate Life Cycle for Freshwater 72-4
   c. Fish Early Life Cycle for Freshwater Fish 72-4
   d. Fish Early Life Cycle for Estuarine/Marine Fish 72-4
   e. Acute Oyster (72-3)
   f. Lemna gibba (Duckweed) 122-2
   g. Skeletonema costatum (Marine diatom) 122-2
   h. Anabaena flos-aquae (Blue-green algae) 122-2
   i. Selenastrum capricorutum (Freshwater green algae) 122-2
   j. (Unspecified species) Freshwater diatom

   All the above studies must be conducted with the technical grade material.

2. Reserved Studies:

   The following studies are being reserved pending the results of the Environmental Fate studies such as half-life in water, leaching rate, etc.

   a. The fish bioaccumulation study (165-4).
   b. Fish Life-Cycle Test for Sheepshead Minnow (72-5)
   c. Aquatic Plant Growth Study (Tier III) 123-2
   d. Oyster Bioaccumulation Study (165-4) and possible
   e. A Special Testing such as Painted Panel Tests.

Curtis E. Laird 2-10-92
Curtis E. Laird, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Allen W. Vaughan 2.14.92
Allen Vaughan, Acting Head-Section 2
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Douglas Urban 2/28/92
Douglas Urban, Acting Cheif
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

ADDENDUM

14D. Adequacy of Study:

This study should be changed from supplemental to core for the following reasons: This study indicates RH-287 ~~thiodicarb~~ is very highly toxic to mysid shrimp with an LC_{50} of 4.7 ppb based on mean measured concentrations. Even though the solvent control mortality exceeded the 5% limits set in the SEP. This study does fulfill the requirement in support of registration for an estuarine/marine species. There were a 10% mortality (2 died) in the solvent control will not change the toxicity category for the mysid shrimp in this study; however, it does show that triethylene glycol has an effect on mysid shrimp. The actual 96-hour LC_{50} is 5.6 ppb.

Curtis E. Laird 10-17-91

Curtis E. Laird, Fishery biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Henry T. Craven 10/17/91

Henry Craven, Head-Section 4
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

DATA EVALUATION RECORD

1. **CHEMICAL:** ~~RTL. Shaughnessey No. 000707.~~ Sh. No. 128101 RH-287

2. **TEST MATERIAL:** RH-287 Technical; T.D. No. 88-156; Lot No. XI-SS-93; 96.9% active ingredient; a brown solid.

3. **STUDY TYPE:** Marine Shrimp Acute Flow-Through Toxicity Test. Species Tested: Mysid shrimp (Mysidopsis bahia).

4. **CITATION:** Boeri, R.L. and T.J. Ward. 1990. Acute Flow-Through Toxicity of RH-287 to the Mysid, Mysidopsis bahia. EnviroSystems Study No. 8962-RH; Rohm and Haas Report No. 89RC-0305. Study performed by EnviroSystems Division Resource Analysts, Inc., Hampton, New Hampshire. Submitted by Rohm and Haas, Spring House, Pennsylvania. EPA MRID No. 417415-03.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 6/6/91

6. **APPROVED BY:**

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

Signature: Louis M Rifici
Date: 6/6/91

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

Signature: Henry T. Craven
Date: 6/7/91

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute flow-through toxicity study. Solvent control mortality exceeded the limits set in the SEP guidelines. The 96-hour LC_{50} of RH-287 for Mysidopsis bahia was 4.7 µg a.i./L, based on mean measured concentrations. Therefore, RH-287 is classified as very highly toxic to mysid shrimp. The NOEC was 1.6 µg a.i./L (mean measured concentration).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The test organisms were obtained from cultures maintained at the test facility. The juvenile shrimp (3 days old) were acclimated to test conditions for three days prior to test initiation. During the pre-test period, the temperature range was 21.6-22.1°C and dissolved oxygen was maintained above 7.4 mg/L. The shrimp were fed once or twice daily with brine shrimp nauplii.

B. **Test System:** The test system consisted of an intermittent-flow proportional diluter, a temperature controlled water bath, and glass test aquaria (20 X 40 X 25cm). All parts of the diluter in contact with the test solutions were made of glass or teflon. The system was activated 859 times during the test resulting in an average volume exchange of 7.2 times every 24 hours. The total solution volume in the tanks was 15 L. Each aquarium contained two mysid retention chambers which were constructed of a nitex collar cemented to petri dishes. The aquaria were randomly positioned in a circulating water bath. The test photoperiod was 16 hours light/8 hours dark. The test vessels were not aerated.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of a definitive sheepshead minnow test, five nominal concentrations were chosen (2.4, 3.8, 6.2, 9.0, and 15.0 µg a.i./L). In addition, a dilution water control and a solvent control (0.1 ml/L solvent) were used.

D. **Design:** The test material was converted to a liquid by heating it in a water bath for 30 minutes at 50°C. A stock solution was prepared by mixing 1.5480 g of RH-287 and acetone and adjusting to 100 mL. Ten milliliters of this stock solution was then mixed with 990 mL of triethylene glycol (nominal concentration of 150,001 µg a.i./L. The test substance solution was delivered to the test aquaria under flow-through conditions by the diluter.

Twenty mysids were randomly and equally distributed into two replicates of each treatment. At any level the organism loading concentration per liter of test solution was approximately 0.0006 g/L. The mysids were

fed newly-hatched brine shrimp once per day during the test.

Mortality and biological observations were noted every 24 hours. Dead mysids were removed when first observed. The dissolved oxygen, pH, salinity, and temperature were measured daily in all control and treatment replicates that contained live organisms. Temperature in a control replicate was also measured continuously throughout the test period.

Chemical analyses of solution from Day 0 and 4 of the test were performed to verify the actual concentrations.

E. **Statistics:** "Results of the toxicity test were interpreted by standard statistical techniques, when warranted. Moving averages or nonlinear interpolation methods were used to calculate the 24, 48, 72, and 96 hour median lethal concentrations (LC_{50}). All calculations of LC_{50} values and the slope of the dose-response curves were performed by the Study Director using computer methods (Stephan, 19832) and mean measured concentrations of the active ingredient. The no observed effect concentration is defined as the concentration of test substance that allows at least 90% survival of test organisms after 96 hours of exposure."

12. **REPORTED RESULTS:** Mean measured concentrations are presented in Table B.1 (attached). Measured concentrations were fairly consistent between sampling times. Records of water quality parameters are presented in Table A.1 (attached). The mortality and observation data based on mean measured concentrations are presented in Table 3 (attached). Table 4 (attached) summarizes the LC_{50} values. Figure 1 (attached) demonstrates the concentration response curve (slope=5.1) established at the end of the test.

"Ninety-five percent survival occurred in the control exposure. Control mysids had an average weight (blotted dry) of 0.0009 g at the end of the test."

"The 96-hour LC_{50} (and associated 95% confidence limits) is 4.7 µg/L active ingredient (3.4-6.1 µg a.i./L). The estimated no observed effect concentration based on survival is 2.8 µg a.i./L. No sublethal effects were observed in any test vessel through the exposure period."

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions in the report.

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was in accordance with GLP regulations. This statement was signed by the Study Director and representatives of the sponsor. In addition, a quality assurance statement was presented in the report and signed by a quality assurance representative of the performing laboratory.

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, except for the following deviations:

Mysids must be ≤24 hours old at test initiation. The mysids used in this test were 3 days old.

Ten percent mortality was observed in the solvent control. This mortality exceeds the allowable limit set forth in the SEP for flow-through marine invertebrate toxicity tests.

The recommended photoperiod for a shrimp acute toxicity study is 16-hour light/8-hour dark with 15- to 30-minute transitions. No transition period was mentioned in the report.

Mortality of the test organisms prior to test initiation was not reported.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value (using solvent and negative controls) and obtained similar results (Printouts 1 and 2, respectively, attached). The authors' LC_{50} value is lower than the reviewer's value, therefore the author's value is accepted. The conclusions as stated by the author indicate that the NOEC was 2.8 µg a.i./L. The reviewer concludes that the NOEC was 1.6 µg a.i./L, since mortality in this concentration was probably not related to effects of the toxicant.

C. **Discussion/Results:** The mortality results are not clear. Since the solvent control demonstrated 10% mortality, it cannot be determined if mortality demonstrated in the test concentrations were substance-

related, solvent-related, or related to some other aspect of test conditions or handling.

This study is scientifically sound but does not meet the guideline requirements for a acute flow-through toxicity study. The 96-hour LC_{50} of 4.7 µg a.i./L (95% confidence limits 3.4 - 6.1 µg a.i./L) based on mean measured concentrations indicates that RH-287 is very highly toxic to mysid shrimp. The NOEC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental. Core - see Addendum

(2) **Rationale:** Solvent control mortality exceeded limits set forth in the SEP for flow-through acute toxicity tests using a marine invertebrate.

(3) **Repairability:** No. NA

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, June 6, 1991.

Printout 1

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

Rosemary Graham Mora RTL Mysidopsis bahia 05-01-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
13.5	18	18	100	3.814697E-04
7.6	18	18	100	3.814697E-04
5.2	18	2	11.1111	.0656128
2.8	18	0	0	3.814697E-04
1.6	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 5.2 AND 7.6 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 6.01371

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1988371	5.425206	4.160693	7.067765

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	11.22012	28.4115	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 = 5.902982
95 PERCENT CONFIDENCE LIMITS =-13.86991 AND 25.67588

LC50 = 5.584792
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3.402996
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

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

Printout 2

Rosemary Graham Mora RTL Mysidopsis bahia 05-01-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
13.5	19	19	100	1.907348E-04
7.6	19	19	100	1.907348E-04
5.2	19	3	15.7895	.2212524
2.8	19	1	5.2632	3.814697E-03
1.6	19	0	0	1.907348E-04

THE BINOMIAL TEST SHOWS THAT 5.2 AND 7.6 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 5.928001

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.130943	5.020257	4.020351	6.112351

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
10	3.707809	5.866877	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 = 8.766258
95 PERCENT CONFIDENCE LIMITS =-8.113761 AND 25.64628

LC50 = 5.594616
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.007713
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

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

Laird RH-287 96-Hour LC50 for Mysid Shrimp

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
13.5	18	18	100	3.814697E-04
7.6	18	18	100	3.814697E-04
5.2	18	2	11.1111	.0656128
2.8	18	0	0	3.814697E-04
1.6	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 5.2 AND 7.6 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 6.01371

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	11.22012	28.4115	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 = 5.902982
95 PERCENT CONFIDENCE LIMITS =-13.86991 AND 25.67588

LC50 = 5.584792
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3.402996
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

Laird RH-287 96-Hour LC50 for Mysid Shrimp

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
13.5	20	20	100	9.536742E-05
7.6	20	20	100	9.536742E-05
5.2	20	4	20	.5908966
2.8	20	2	10	2.012253E-02
1.6	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 5.2 AND 7.6 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 5.851156

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.2625883	4.694037	3.397296	6.138896

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.099923	6.976896	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 = 5.10953
95 PERCENT CONFIDENCE LIMITS =-2.294747 AND 12.51381

LC50 = 5.082073
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.867383
95 PERCENT CONFIDENCE LIMITS = 0 AND 5.682435

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DATA EVALUATION RECORD

Sh. No. 128101

1. **CHEMICAL:** ~~RTL. Shaughnessey No. 000707.~~ RH-287

2. **TEST MATERIAL:** RTL; RH-287; T.D. No. 88-156; Code/Name No. RH-287, XI-SS-93; 96.9% active ingredient; a brown solid.

3. **STUDY TYPE:** Marine Fish Acute Flow-Through Toxicity Test. Species Tested: Sheepshead minnow (Cyprinodon variegatus).

4. **CITATION:** Ward, T.J. and R.L. Boeri. 1990. Acute Flow Through Toxicity of RH-287 to the Sheepshead Minnow, Cyprinodon variegatus. EnviroSystems Study No. 8961-RH; Rohm and Haas Report No. 89RC-0262. Study performed by EnviroSystems Division Resource Analysts, Inc., Hampton, New Hampshire. Submitted by Rohm and Haas, Spring House, Pennsylvania. MRID No. 417415-02.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 6/4/91

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 6/4/91

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

Signature: Allen W. Vaughan 11.2.91 H.T. Craven
Date: 11/2/91

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute flow-through toxicity study. The 96-hour LC_{50} of RH-287 for Cyprinodon variegatus was 20.5 µg a.i./L, based on mean measured concentrations. Therefore, RH-287 is classified as very highly toxic to the sheepshead minnow. The NOEC was 7.6 µg a.i./L.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

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11. **MATERIALS AND METHODS:**

A. **Test Animals:** Sheepshead minnows (Cyprinodon variegatus) were obtained from a commercial supplier and acclimated for 39 days. Prior to test initiation fish were maintained in dilution water and a flow-through system. The fish were not treated for any disease and were free of apparent sickness. During the final seven days of acclimation the range of temperature was 21 to 21.7°C; dissolved oxygen was maintained above 6.5 mg/L. The fish were fed once or twice daily with brine shrimp nauplii and a commercial fish food. Forty-eight hours prior to test initiation the fish were not fed. Control sheepshead minnows were weighed and measured at test termination.

B. **Test System:** The test system consisted of an intermittent-flow proportional diluter, a temperature controlled water bath, and glass aquaria (20 X 40 X 25 cm). The system was activated 904 times during the test resulting in an average total volume exchange of 7.5 times every 24 hours. The total solution volume in the tanks was 15 L. The aquaria were randomly positioned in a circulating water bath. The test photoperiod was 16 hours light/8 hours dark. The test vessels were not aerated.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of a sheepshead minnow screening test, five nominal concentrations were chosen (9.9, 14.9, 24.8, 39.7, and 62.0 µg a.i./L). In addition, a dilution water control and a solvent control (containing 0.1 mL/L solvent (99% triethylene glycol and 1% acetone)) were used. Chemical analyses of solutions from Day 0 and 4 of the test were performed to verify the actual concentrations.

D. **Design:** The test material was converted to a liquid by heating it in a water bath for 30 minutes at 50°C. A stock solution was prepared by mixing 6.4 g of RH-287 and acetone and adjusting to 100 mL. Ten mL of this stock solution was then mixed with 990 mL of triethylene glycol (nominal concentration of 620 µg a.i./L). The test substance solution was delivered to the test aquaria under flow through conditions by the diluter.

Twenty fish were randomly loaded and equally distributed into two replicates of each treatment. The organism loading concentration per liter of test

solution was approximately 0.44 g/L, based on measurements in the control aquaria.

Mortality, biological observations, and observations of physical characteristics were noted at test initiation and every 24 hours thereafter. The dissolved oxygen, pH, salinity, and temperature were measured daily in all control and treatment replicates which contain live fish. Temperature in test vessel was also measured continuously throughout the test period.

E. **Statistics:** "Probit or nonlinear interpolation methods were used to calculate the 24, 48, 72, and 96 hour median lethal concentrations (LC_{50}s). All calculations of LC_{50} values and the slope of the dose-response curve were performed by the Study Director using computer methods (Stephan, 1983) and mean measured concentrations of the active ingredient. The no observed effect concentration is defined as the concentration of test substance that allows at least 90% survival of test organisms at the end of the test."

12. **REPORTED RESULTS:** Mean measured concentrations are presented in Table B.1 (attached). Records of test parameters are presented in Table A.1 (attached). The mortality and observation data based on mean measured concentrations are presented in Table 3 (attached). Table 4 (attached) summarizes the LC_{50} values.

"Control fish had an average weight (blotted dry) of 0.66 g and a total length of 31.1 mm at the end of the test."

"The dose-response curve for organisms exposed to the test substance for 96 hours is presented in Figure 1 [attached] (slope = 8.0)... The 96-hour LC_{50} (and associated 95% confidence limits) is 20.5 µg/L active ingredient (17.7-23.5 µg a.i./L). The estimated no observed effect concentration based on survival is 11.5 µg a.i./L."

13. **STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions in the report.

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was in accordance with GLP regulations. This statement was signed by the Study Director and representatives of the sponsor. In addition, a quality assurance statement was presented in the report and signed by a quality assurance representative of the performing laboratory.

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, except for the following deviations:

The recommended photoperiod for a sheepshead minnow acute toxicity study is 16-hour light/8-hour dark with a 15- to 30-minute transition. No transition period was mentioned in the report.

Each selected nominal concentration was less than 60% of the next highest concentration. The SEP recommends that each concentration be at least 60% of the next concentration.

Mortality of the test organisms prior to test initiation was not reported.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained similar results (printout, attached). The authors' results are accepted. The authors' results indicate that the NOEC based on the authors' definition (see Section 11E) was 11.5 µg a.i./L. The reviewer concludes that the NOEC was 7.6 µg a.i./L, since 5% mortality was observed at 11.5 µg a.i./L.

C. **Discussion/Results:** Judging from the response of the control organisms, the deviations noted above did not affect the outcome of the study.

This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study. The 96-hour LC_{50} of 20.5 µg a.i./L (95% confidence limits 17.7-23.5 µg a.i./L) based on mean measured concentrations indicates that RH-287 is very highly toxic to sheepshead minnow. The NOEC was 7.6 µg a.i./L.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes; June 4, 1991.

Rosemary Graham Mora RTL Cyprinodon variegatus 05-02-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
70	20	20	100	9.536742E-05
34.5	20	20	100	9.536742E-05
21.2	20	9	45	41.19014
11.5	20	1	5	2.002716E-03
7.6	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 11.5 AND 34.5 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 21.89269

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	5.135007E-02	20.06682	16.86874	23.75562

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.16745	1	.5377313

SLOPE = 7.974941
95 PERCENT CONFIDENCE LIMITS = 4.711543 AND 11.23834

LC50 = 20.54392
95 PERCENT CONFIDENCE LIMITS = 17.66295 AND 23.53906

LC10 = 14.23752
95 PERCENT CONFIDENCE LIMITS = 10.28079 AND 16.75981

****

DATA EVALUATION RECORD

1. CHEMICAL: RH-287

2. TEST MATERIAL: Technical

3. TEST TYPE: Die-Away Sstudy in Natural and Synthetic Waters (duration was not given)

Test Species: Algae (Species unknown)

4. STUDY IDENTIFICATION:

Robert Jr., M.H.; Hale, R.C. (1990) RH-287 Technical: Die-Away Study in Natural and Synthetic Waters; Report No. 90RC-0135; Study No. 8912; Prepared By College of Willam and Mary, Gloucester Point, Virginia 23062; MRID No. 417415-06.

5. REVIEWED BY:

Curtis E. Laird
Fishery Biologist
EEB/EFED

Signature: Curtis E. Laird
Date: _____

6. APPROVED BY:

Allen Vaughan
Supervisory Biologist
EEB/EFED

Signature: Allen W. Vaughan
Date: 11 21 91

7. CONCLUSIONS:

This study indicates RH-287 half-life is < 24 hours in natural water and does not die-away in synthetic water. All water samples had a high pH reading (ranging from 7.5 to 8.1). Also the oxygen concentration was high in every case. The percent of active ingredient and species of algea tested were not given. EEB did not request this study; however, we did review this study and placed a copy in our's files for future use.

8. RECOMMENDATIONS:

If this study was requested by EEB, it would not have meet the guideline requirement in support of registration because the percent of active ingredient, species of algea tested, test duration, etc. were unknown.

9. BACKGROUND:

This study was submitted in support of new chemical registration.

10. DISCUSSION of INDIVIDUAL TEST: N/A

11. MATERIAL TESTED:

A. Test Species: algae

B. Test Design:
Algea was tested in 4 L flasks; pH ranged from 7.5 to 8.1; tested at room temperature.

C. Dose:

10^5 cells/ml of algae per dose level; 2 dosage levels plus controls (triethylene glycol, negative control, 1.0 ug/L and 10.0 ug/L).

D. Statistical Analysis:

Regressing the natural logarithms of measured concentrations above the detection limit against time.

12. Reported Results:

The study author found the half-life of RH-287 to be < 24 hours. Lower the concentration shorter the half-life is in water.

13. STUDY AUTHOR'S CONCLUSION/QA MEASURES:

The Quality Assurance Officer stated that "this study was conducted by their facility accordance to the Good Laboratory Practice set forth in EPA 40 CFR, Part 160."

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

A. Test Procedure:

EEB does not have guidelines in place to determine if the test was conducted following the correct test procedures.

B. STATISTICAL ANALYSIS:

No statistics were performed due to lack of actaul raw data or data with number of samples taken at each site (control vs treatment levels).

C. DISCUSSION/RESULTS:

RH-287 has a half-life of less than 24 hour in both natural and synthetic waters.

D. ADEQUACY OF STUDY:

1. Category: Supplemental

2. Rationale: See section 7 above

3. Reparability: possible if a study of this kind is ever needed

15. Completion of One-liner: No

16. CBI Appendix N/A

DATA EVALUATION RECORD

1. **CHEMICAL:** ~~RTL. Shaughnessey No. 000707.~~ Sh. No. 128101 RH-287

2. **TEST MATERIAL:** RTL; RH-287; T.D. No. 88-156; Code No. RH-287; XI-SS-93; 96.9% active ingredient; a brown solid.

3. **STUDY TYPE:** Freshwater Invertebrate Life-Cycle Test. Species Tested: Daphnia magna.

4. **CITATION:** Ward, T.J. and R.L. Boeri. 1990. Chronic Toxicity of RH-287 to the Daphnid, Daphnia magna. EnviroSystems Study No. 9031-RH. Rohm and Haas Report No. 90RC-0050. Study conducted by EnviroSystems Division, Resource Analysts, Inc., Hampton, New Hampshire. Submitted by Rohm and Haas Company, Spring House, Pennsylvania. MRID No. 417415-05.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 6/7/91

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 6/7/91 Concur Kim 10-1-91

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

Signature: Henry T. Craven
Date: 11/21/91

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for a daphnid life-cycle test. Adult daphnids in the control produced less than 40 young. Mean measured test concentrations varied greater than 30% of nominal concentrations and were highly variable among days and among replicates on the same day. The EC_{50} of RH-287 to Daphnia magna was 1.2 µg a.i./L, based on mean measured concentrations. The NOEC could not be determined, since sublethal/lethal effects were noted in all concentrations.

8. **RECOMMENDATIONS:** N/A

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9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Daphnia magna (<24 hours old) were obtained from populations cultured at the testing facility. The cultures were maintained in dilution water under test conditions prior to test initiation.

B. **Test System:** The flow-through test system was an intermittent flow proportional diluter apparatus. Daphnids were contained in 270-ml glass beakers with Nitex collars containing 270-ml of test solution. Test vessels were randomly distributed in a water bath.

The dilution water was well water collected at Envirosystems, Hampton, New Hampshire. The dilution water had a hardness of 160-176 mg/L.

The diluter delivered five nominal concentrations of test material, a solvent control (0.1 ml/l of a 1:99 mixture of acetone and triethylene glycol), and a dilution water control to each replicate. The diluter delivered water to each vessel at an average rate of approximately 103 volume replacements per day.

The target temperature was 20 $\pm$ 1°C. Sixteen hours of light at an intensity of 10 $Es^{-1}m^{-2}$ were provided each day with a 15 minute transition period between light and dark.

C. **Dosage:** Twenty-one-day, life-cycle, flow-through test. Selected nominal test concentrations were based on results of a range-finding study provided to the laboratory by Rohm and Haas Company. These treatment levels were 1.3, 2.0, 3.2, 4.8, and 8.0 µg a.i./L.

D. **Design:** Four replicates of five RH-287 concentrations (see Section 11.C), solvent control, and dilution water control were included in the test. Ten daphnids were equally distributed to each exposure vessel (i.e., 40 daphnids/concentration).

The daphnids were fed a yeast and trout food suspension and Selenastrum capricornutum at least twice each day.

Adult survival was visually determined initially and every 24 hours thereafter. Offspring production was noted at 1 to 3 day intervals. The offspring were discarded after counting. At test termination dry body weight of all surviving adults was determined after drying the daphnids at 60°C for 72 hours and storing in a desiccator for 24 hours.

Dissolved oxygen concentration (DO), pH, conductivity, and temperature were measured daily in every test vessel which contained live organisms.

Water samples were collected from two of the four replicate vessels of all groups on test days 0, 7, 8, 15, and 21 for determination of RH-287 concentrations.

E. **Statistics:** "Results of the toxicity test were interpreted by standard statistical techniques, when warranted. All statistical analyses were conducted separately with the control and solvent control results, and analysis of reproduction data was also conducted with the mean of the control and solvent control. Shapiro-Wilk's test was used to determine if data were normally distributed, and Bartlett's test was used to determine if variances were homogeneous. Because variances were heteroscedastic a nonparametric ANOVA (Steel's Many-One Rank test, Steel and Torrie, 1960) was used to compare control and treatment means. Dichotomous data were transformed (arc sin square root) prior to statistical analysis."

The MATC was calculated as the geometric mean of the NOEC and LOEC. Probit analysis was used to determine the EC_{50}.

12. **REPORTED RESULTS:** "First generation dilution water control daphnids produced an average of 19 young per surviving female after 21 days of exposure, and the solvent control daphnids produced an average of 44 young per surviving female. The difference resulted from the amount of food available to the two controls. In order to maintain acceptable concentrations of RH-287 in test media a high turn over rate was required. This high rate of test media exchange effectively flushed food form the test vessels and resulted in a corresponding decrease in toxicant concentration because biodegradation is exacerbated by the presence of the algae used as food (Rohm and Haas, 1989). The solvent present in the solvent controls (and to some degree in the treatments) increased the productivity of the

microbial community in the test vessels upon which the daphnids fed, despite the frequent cleaning of the test vessels, and effectively increased the food supply available to the solvent controls, resulting in greater reproduction."

A summary of the biological results are presented in Table 3 (attached). The survival and reproductive rates for the dilution water control did not meet the minimum EPA guideline requirements of 40 offspring/female.

Mean measured concentrations (± standard deviations) were 0.63 (± 0.2), 1.1 (± 0.4), 1.8 (± 0.6), 3.1 (± 0.9), and 5.4 (± 1.4) µg a.i./L (Table A1, attached). Mean measured concentrations ranged from 52% to 68% of nominal values and were generally stable throughout the study.

Average dry weight of control daphnids was 0.28 mg. Loading rate was approximately 0.11 g/l. Mean test temperature was 20.4 ± 0.4°C; mean pH was 8.3 ± 0.1; mean conductivity was 1400 ± 100 µmhos/cm (Table 4, attached).

"The percentage of surviving adults was significantly reduced in comparison to the control and solvent control groups at 1.1, 1.8, 3.1, and 5.4 µg a.i./L. No adults survived to day 9, the beginning of the reproduction period, in 3 of 4 replicates of the 3.1 µg a.i./L group and in any of the replicates at 5.4 µg a.i./L...The total number of young produced was significantly lower than the solvent control at all tested concentrations and significantly lower than the dilution water control at all concentrations except 0.63 µg a.i./L, and not significantly lower than the dilution water control at any test level with adequate survival."

Average dry weights in treatment groups with adequate survival were not significantly different from those of the controls. No sublethal effects were noted during the test.

"Exposure of daphnids to RH-287 resulted in a maximum acceptable toxicant concentration (MATC) of 0.83 µg a.i./L when treatment data are compared to the dilution water control or combined controls (first generation survival), and a MATC of <0.63 µg a.i./L when treatment data are compared to the solvent control (production of young) (Table 5, attached). The 21 day median effective concentration (EC_{50}) is 1.2 µg a.i./L (95% confidence interval = 1.0 to 1.4 µg a.i./L).

"RH-287 was judged not to have a biologically significant effect on reproduction at 0.63 µg a.i./L despite the statistical difference between the number of young produced in the solvent control and the number produced at 0.63 µg a.i./L. The difference in production is believed to be related to the amount of food present the test vessels, which appears to be related to the concentration of solvent."

13. **STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Exposure of daphnids to RH-287 resulted in a NOEL of 0.63 µg a.i./L, a LOEL of 1.1 µg a.i./L, and a MATC of 0.83 µg a.i./L. The 21 day EC_{50} is 1.2 µg a.i./L (95% confidence interval = 1.0 to 1.4 µg a.i./L)."

A GLP compliance statement was included in the report indicating that the data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations except in the case of the failed control reproduction and collection of sample for toxicant concentration on day 15 rather than day 14. This statement was signed by the Study Director and a representative of the test sponsor.

A Quality Assurance Statement was included and signed by a representative of the Quality Assurance Unit of the performing laboratory.

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

A. **Test Procedure:** An SEP for Daphnia chronic flow-through studies is not available at this time, thus ASTM recommended procedures were used in this data validation process. The test procedure was in accordance with ASTM, except for the following deviations:

Adult daphnia in the dilution water control did not produce the number of young required for a chronic toxicity test. The average number of young produced in this control was 19. For a test to be acceptable ASTM recommends 40 young per surviving adult.

The measured concentration of test material in any chamber should be no more than 30% higher of lower than the nominal concentration. In this test all mean measured concentrations were more than 30% lower than nominal values.

The ASTM guidelines state that for each treatment the highest measured concentration divided by the lowest measured concentration must be less than 2. This criteria was not met in any treatment concentration.

Individual weights of the surviving daphnia were not collected, as recommended in the ASTM guidelines.

The pre-test feeding regime was not indicated in the report.

The report did not state how the organisms were assigned to test vessels (i.e. randomly).

The authors did not indicate the selection criteria (i.e., brood number) for organisms used in the test.

B. **Statistical Analysis:** The reviewer used the computer program, Toxstat Version 3.3 for determining the NOEC for survival and reproduction (mean number produced per female and total number produced). The solvent control was used for analyses of reproduction and survival data Transformations which were applied to the survival and reproduction data did not correct the heteroscedasticity of the variances. Steels Many-One Rank test was used for each analysis.

The NOEC for the total number of young produced for each treatment could not be determined since an effect was demonstrated at each treatment level when compared to the solvent control (Printout A, attached). This conclusion differs from the authors'. The NOEC for mean number of young produced per female was 0.63 µg a.i./L (Printout B, attached). The NOEC for number of surviving daphnids was 0.63 µg a.i./L (Printout C, attached). The NOEC for the average dry weight was 3.1 µg a.i./L (Printout D, attached).

The EC_{50} was verified using EPA's Toxanal. The reviewer's value was the same as the value presented by the authors (Printout E, attached).

C. **Discussion/Results:** The poor reproduction demonstrated by the adult daphnids in the dilution water effects the validity of the study.

Concentration levels varied greatly between sampling days and between replicates on the same sampling day. This made it impossible to determine the actual

concentration to which the organisms were exposed during the test period.

This study is not scientifically sound and does not meet the guidelines for a flow-through chronic toxicity test using Daphnia magna. The EC_{50} was 1.2 µg a.i./L. The NOEC could not be determined, since the total number of young in all treatment levels was significantly different from the solvent control.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The number of young produced per surviving female in the control was less than 40. Each concentration level was less than 30% of nominal concentration, and was highly variable among sampling days and among replicates on the same day.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, May 10, 1991.

Printout A

RTL - D. magna Total # Young Produced
File: RTL tot Transform: NO TRANSFORM

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Solvent Control	421.750				
2	0.63 ug a.i./L	180.000	10.00	10.00	4.00	*
3	1.1 ug a.i./L	101.250	10.00	10.00	4.00	*
4	1.8 ug a.i./L	21.000	10.00	10.00	4.00	*
5	3.1 ug a.i./L	16.000	10.00	10.00	4.00	*
6	5.4 ug a.i./L	0.000	10.00	10.00	4.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

RTL - D. magna Mean # of Young Produced Per Female
File: RTL mean Transform: NO TRANSFORMATION

Printout B

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Solvent Control	44.500				
2	0.63 ug a.i./L	24.750	14.00	10.00	4.00	
3	1.1 ug a.i./L	18.500	10.00	10.00	4.00	*
4	1.8 ug a.i./L	7.000	10.00	10.00	4.00	*
5	3.1 ug a.i./L	8.000	10.00	10.00	4.00	*
6	5.4 ug a.i./L	0.000	10.00	10.00	4.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Printout C

RTL - D. magna Survival
File: RTL surv Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Solvent Control	0.950				
2	0.63 ug a.i./L	0.825	14.00	10.00	4.00	
3	1.1 ug a.i./L	0.625	10.00	10.00	4.00	*
4	1.8 ug a.i./L	0.250	10.00	10.00	4.00	*
5	3.1 ug a.i./L	0.050	10.00	10.00	4.00	*
6	5.4 ug a.i./L	0.000	10.00	10.00	4.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Printout D

RTL - D. magna Average Dry Wt.
File: RTL wt Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Solvent Control	0.258				
2	0.63 ug a.i./L	0.290	18.00	10.00	4.00	
3	1.1 ug a.i./L	0.378	21.00	10.00	4.00	
4	1.8 ug a.i./L	0.307	21.00	10.00	4.00	
5	3.1 ug a.i./L	0.063	13.00	10.00	4.00	
6	5.4 ug a.i./L	0.000	10.00	10.00	4.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Triadont D, E

ROSEMARY GRAHAM MORA RTL DAPHNIA MAGNA 05-09-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
5.4	40	40	100	0
3.1	40	38	95	0
1.8	40	30	75	0
1.1	40	15	37.5	0
.63	40	7	17.5	0

THE BINOMIAL TEST SHOWS THAT 1.1 AND 1.8 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.291507

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	6.113976E-02	1.223276	1.037425	1.416778

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	5.999198E-02	1	.7052089

SLOPE = 3.880386
95 PERCENT CONFIDENCE LIMITS = 2.929953 AND 4.830818

LC50 = 1.206828
95 PERCENT CONFIDENCE LIMITS = 1.040828 AND 1.381173

LC10 = .568013
95 PERCENT CONFIDENCE LIMITS = .4135611 AND .69942

****

DATA EVALUATION RECORD

1. **CHEMICAL:** ~~RTL. Shaughnessey No. 000707.~~ Sh. No. 128101 RH-287

2. **TEST MATERIAL:** RTL; RH-287; T.D. No. 88-156; Code/Name No. RH-287, XI-SS-93; 96.9% active ingredient; a brown solid.

3. **STUDY TYPE:** Marine Fish Early Life Stage Toxicity Test. Species Tested: Sheepshead minnow (Cyprinodon variegatus).

4. **CITATION:** Ward, T.J. and R.L. Boeri. 1990. Early Life Stage Toxicity of RH-287 to the Sheepshead Minnow, Cyprinodon variegatus. EnviroSystems Study No. 8913-RH; Rohm and Haas Report No. 89RC-0193. Study performed by EnviroSystems Division, Resource Analysts, Inc., Hampton, New Hampshire. Submitted by Rohm and Haas, Spring House, Pennsylvania. MRID No. 417415-04.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 6/6/91

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 6/6/91

Curtis Laird 10-16

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

Signature: Henry T. Craven
Date: 10/17/91

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an early life stage toxicity study. The solvent control mortality exceeded limits set forth in the guidelines. Based on the effects of RH-287 to Cyprinodon variegatus, the MATC was between 6.0 and 14.0 µg a.i./L mean measured concentrations (geometric mean MATC = 9.2 µg a.i./L).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: Sheepshead minnows (Cyprinodon variegatus) were obtained from a commercial supplier as less than 24 hour old embryos. The embryos were not treated for any disease and were free of apparent sickness.

B. Test System: The test system consisted of an intermittent-flow proportional diluter, a temperature controlled water bath, nitex/glass cages (embryo stage) and glass aquaria (15 X 30 X 20 cm) (larval stage). The system was activated 8,291 times during the test resulting in an average total volume exchange of 15.8 times every 24 hours. The total solution volume in the tanks was 7.5 L. The aquaria were randomly positioned in a circulating water bath. The test photoperiod was 16 hours light/8 hours dark. The test vessels were not aerated.

The dilution water used in the study was collected from the Atlantic Ocean at EnviroSystems, Hampton, New Hampshire, and stored and aerated in polyethylene tanks. The salinity was adjusted to 20 ±1 ppt and the pH was 8.0.

C. Dosage: Thirty-five day early life stage flow-through test. Based on the results of a sheepshead minnow screening test, five nominal concentrations were chosen (0.96, 1.9, 4.0, 8.0, and 16.0 µg a.i./L). In addition, a dilution water control and a solvent control (containing 0.1 mL/L solvent (99% triethylene glycol and 1% acetone)) were used. Chemical analyses of solution from Day 0 and every 7 days thereafter were performed to verify the actual concentrations.

D. Design: The test material was converted to a liquid by heating it in a water bath for 30 minutes at 50°C. A stock solution was prepared by mixing 1.652 g of RH-287 and acetone and adjusting to 100 mL. Ten milliliters of this stock solution was then mixed with 990 mL of triethylene glycol (nominal concentration of 160,079 µg a.i./L). The test substance solution was delivered to the test aquaria under flow through conditions by the diluter.

Eighty embryos were randomly loaded and equally distributed into two replicate cages of each treatment. After hatching was 90% complete, fish were reduced to 20 per replicate and released into test vessels.

Mortality, biological observations, and observations of physical characteristics were noted at test initiation and every 24 hours thereafter. The dissolved oxygen, pH, salinity, and temperature were measured daily in all control and treatment replicates which contained live organisms. The temperature in one test vessel was also measured continuously throughout the test period.

E. **Statistics:** "Data related to the number of embryos hatched and the number of dead embryos was analyzed with both the dilution water control and the solvent control, and no differences occurred as a result of the choice of control. Shapiro-Wilk's test was used to determine if data was normally distributed, and Bartlett's test was used to determine if variances were homogeneous. If variances were homogeneous, a parametric one-way analysis of variance (ANOVA) and if necessary, Dunnett's test was used to compare treatment and control means. If variances were heteroscedastic a nonparametric ANOVA (Kruscal and Wallis' Test, Steel and Torrie, 1960) was used to compare control and treatment means. Dichotomous data was transformed (arc sin square root) prior to statistical analysis."

The NOEC and LOEC values were determined for the study. The geometric mean MATC was calculated as the geometric mean of the NOEC and LOEC.

12. **REPORTED RESULTS:** Mean measured concentrations are presented in Table A.1 (attached). The mortality and observation data based on mean measured concentrations are presented in Table 3 (attached). Mean fish weight and length are presented in Table 4 (attached). Table 6 (attached) summarizes the results of the study.

"At least 80% of the control and solvent control embryos hatched, and control survival at 32 days post hatch was at least 95% in each dilution water control test chamber and one of the two solvent control test chambers. Survival in the second solvent control test chamber was 65% at the end of the test. Because survival in the other solvent control replicate was 100%, mortality in this solvent control replicate was apparently not related to water quality, the solvent, or a malfunction of the test media delivery system. Control and solvent control fish had an average wet weight (blotted dry) of 145.7 mg and an average total length of 18.5 mm at the end of the test. The relative standard deviation of the weights of surviving fish in the control and solvent control test chambers was less than 40%.

Loading rate during the toxicity test was approximately 0.32 g/L at any time and 0.02 g/L/24 hours."

The salinity ranged from 19 - 21 ppt. The temperature ranged from 29.0 - 31°C. The dissolved oxygen concentration was always above 75%.

No sublethal effects were observed, and no treatment-related effects on time to hatch, total length, or total weight were demonstrated.

"The most sensitive measure of toxicity determined by statistical analysis of survival and growth data after 32 days of exposure was the survival of fish exposed to RH-287 at 14.0 µg a.i./L. The percent of embryos hatching and the percent survival at hatch on day 4, although not statistically significant, were considered to be biologically significant at 14.0 µg a.i./L based upon the magnitude of changes seen in comparison to the control groups."

The LOEC was 14.0 µg a.i./L. The NOEC was 6.0 µg a.i./L. The geometric mean MATC was 9.2 µg a.i./L.

13. **STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions in the report.

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was in accordance with GLP regulations. This statement was signed by the Study Director and a representative of the sponsor. In addition, a quality assurance statement was presented in the report and signed by a quality assurance representative of the performing laboratory.

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

A. **Test Procedure:** The test procedures were generally in accordance with guidelines recommended in the SEP and Subdivision E, except for the following deviations:

Survival in one of the solvent controls at test termination was 65%. The SEP states that a test is unacceptable if survival is less than 70% in any control chamber.

The SEP recommends a "minimum" of 20 embryos per replicate with four replicates per concentration be used for the embryo stage of the test. There were only two replicates used per concentration during this study.

The report did not indicate that feeding was terminated at least 24 hours prior to test termination as recommended in the SEP.

The report did not indicate a pretreatment of the natural seawater. The SEP recommends filtration and sterilization of natural seawater.

The authors did not indicate in the report that the dilution apparatus contained a mixing chamber to adequately mix the test material as recommended in the SEP.

During the test period the pH ranged from 7.3 to 8.3. The SEP recommends using seawater of constant quality. The quality is considered to be constant "if monthly pH range is less than 0.8 of a pH unit."

B. **Statistical Analysis:** The reviewer did not analyze growth since the lengths and weights of test treatment organisms were greater than those of the controls. Therefore, the NOEC values for these parameters could not be determined. The computer program Toxstat Version 3.3 was used to calculate the NOEC for survival of embryos and fish. The solvent control was used for analyses of survival data, since there was no significant difference between the dilution water control and the solvent control. All transformations applied to the data failed to correct the heteroscedasticity of the variances, therefore Kruskal-Wallis test was used for the analyses.

The Kruskal-Wallis test demonstrated no significant differences between the test concentrations and the solvent control (printouts, attached). However, it can not be ignored that the percentage fish survival for the 14.0 µg a.i./L concentration (mean survival = 10%) was considerably lower than that of the solvent control (mean survival = 82.5%). Therefore, the reviewer accepts the authors' NOEC of 6.0 µg a.i./L for the survival of fish at 32 days.

C. **Discussion/Results:** The unacceptable solvent control mortality effects the validity of the study.

This study is scientifically sound but does not meet the guideline requirements for an early life stage toxicity study. Based on the apparent effect of RH-287 on the survival of sheepshead minnows at test termination, the MATC for the study was between 6.0

and 14.0 µg a.i./L (geometric mean MATC = 9.2 µg a.i./L).

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Solvent control mortality exceeded limits established in the guidelines.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, June 6, 1991.

Survival of Sheepshead to RH-287
File: rh-287 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Solvent Control	16.500	16.500	16.000
2	0.54 ug/l	18.500	18.500	18.000
3	1.2 ug/l	19.000	19.000	19.500
4	2.9 ug/l	13.000	13.000	11.500
5	6.0 ug/l	14.000	14.000	10.000
6	14.0 ug/l	2.000	2.000	3.000

Calculated H Value = 7.340 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Survival of Sheepshead to RH-287
File: rh-287 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 4 5 1 2 3
6	14.0 ug/l	2.000	2.000	\
4	2.9 ug/l	13.000	13.000	. \
5	6.0 ug/l	14.000	14.000	. . \
1	Solvent Control	16.500	16.500	. . . \
2	0.54 ug/l	18.500	18.500	. . . . \
3	1.2 ug/l	19.000	19.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.574

Survival of Embyos Exposed to RH-287
File: rh-287 e Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Solvent Control	0.850	0.850	20.500
2	Control	0.813	0.813	16.500
3	0.54 ug/l	0.850	0.850	23.000
4	1.2 ug/l	0.850	0.850	20.500
5	2.9 ug/l	0.800	0.800	14.500
6	6.0 ug/l	0.713	0.713	6.500
7	14.0 ug/l	0.650	0.650	3.500

Calculated H Value = 9.641 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Survival of Embyos Exposed to RH-287
File: rh-287 e Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 7 6 5 2 1 3 4
7	14.0 ug/l	0.650	0.650	\
6	6.0 ug/l	0.713	0.713	. \
5	2.9 ug/l	0.800	0.800	. . \
2	Control	0.813	0.813	. . . \
1	Solvent Control	0.850	0.850	. . . . \
3	0.54 ug/l	0.850	0.850	. . . . . \
4	1.2 ug/l	0.850	0.850	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.146

MRID No. 417568-01

ADDENDUM

EEB has the following comments:

14A. Test Procedure:

The second statement was a deviation from the SEP, but it was due to the short half-life of RH-287.

14D(1). A Classification ~~Test Procedure~~:

This classification should be changed from invalid to supplemental because this study does contain some useful information that can be used in a risk assessment.

Curtis E. Laird 10-16-91
Curtis E. Laird, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Henry T. Craven 10/17/91
Henry Craven, Head-Section 4
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

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DATA EVALUATION RECORD

1. **CHEMICAL:** ~~RTL. Shaughnessey Number: 000707.~~ Sh. No. 128101 RH-287

2. **TEST MATERIAL:** RH-287 Technical. Lot No. XI-SS-93; 96.9% active ingredient; a waxy solid, tan in color.

3. **STUDY TYPE:** Mollusc 48-hour Embryo-Larvae Study. Species Tested: Eastern Oysters (Crassostrea virginica).

4. **CITATION:** Roberts, M.R., P.F. De Lisle, M.A. Vogelbein, and R.C. Hale. 1990. Acute Toxicity of RH-287 to the American Oyster (Crassostrea virginica) in Static Natural and Synthetic Estuarine Waters. Prepared by Division of Chemistry and Toxicology, Virginia Institute of Marine Science, Gloucester Point, Virginia. VIMS Study No. 8805. Submitted by Rohm and Haas Company, Spring House, Pennsylvania. EPA MRID No. 417568-01.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 6/6/91

6. **APPROVED BY:**

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

Signature: Louis M Rifici
Date: 6/6/91
Curtis Davis 10-16-91

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

Signature:
Date: Henry T. Craven 10/7/91

7. **CONCLUSIONS:** These studies are not scientifically sound and do not fulfill the guideline requirements for an oyster embryo-larval test. RH-287 concentrations decreased during both studies (performed with natural seawater and synthetic seawater) making it difficult to determine an accurate EC_{50}. The synthetic seawater study was unacceptable since greater than 30% mortality was observed in the control. The 48-hour EC_{50} for the natural seawater study, based upon nominal concentrations of RH-287, to eastern oysters (Crassostrea virginica) was 24 µg/L. Therefore, RH-287 is classified as very highly toxic to Eastern oysters.

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8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Embryos of the Eastern oyster (Crassostrea virginica) were obtained by spawning conditioned adult oysters. Adult oysters were obtained from the Rappahannock River, Virginia, and maintained at the Oyster Hatchery (at Virginia Institute of Marine Science (VIMS)) for 1-2 months at a temperature of 20-23°C at which time spawning was induced or the temperature lowered to 19°C until spawning was desired. Spawning was induced by thermal shock.

Females were induced to spawn by briefly raising the water temperature to 25-30°C, followed by the addition of sperm suspension to the water. Shortly after zygote formation, the zygotes were cleaned and provided to the testing laboratory. The embryos were 2-4 hours old at test initiation.

B. **Test System:** Tests were performed in 1000 mL borosilicate jars. Each test concentration was tested in triplicate and each control in quadruplicate. Test jars were maintained in an incubator set at 25°C.

Two definitive tests were performed using unaged natural estuarine seawater (filtered seawater (1 µm) from the York River, at Gloucester Point, Virginia) and filtered (1 µm)synthetic estuarine seawater (Forty Fathoms Sea Salts® and reagent water).

C. **Dosage:** Mollusc 48-hour embryo-larvae static acute toxicity tests. The nominal test concentrations for both definitive tests, were chosen as 0.56, 1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, and 56.0 µg/L. A dilution water control and a solvent control (20 µL solvent/L) were also used.

The test material was dissolved in acetone to prepare a primary stock. Secondary stocks were prepared in triethylene glycol.

D. **Design:** At the start of the test, a volume of embryo suspension was added to each vessel and the solution was mixed with a standard plunger, resulting in a

density of approximately 10-30 embryos/mL. The period between test solution preparation and test initiation was approximately 30-40 minutes. Replicates A, B, and C were randomly placed on the top, middle and bottom shelves of the incubator, respectively.

At test initiation and termination, each vessel was mixed with a plunger, and four 5-mL aliquots of solution were removed, composited, and fixed with 5% buffered formalin. The number of normal and abnormal larvae were counted (in 20 mL (four 5-mL subsamples combined) from each replicate vessel) in Sedgewick-Rafter slides, under 100X magnification.

The temperature, pH, salinity and dissolved oxygen were measured and recorded daily for one replicate in each test concentration and control. In addition, temperature readings were recorded daily from a min-max thermometer located in a beaker of water on the second shelf of the incubator.

The concentration of test solutions was determined at test initiation and test termination.

E. **Statistics:** "The biological data were analyzed in accordance with the methods described in ASTM Designation E 724-89 [1]. Since there were some anomalies associated with the Day 0 data for both definitive test, the data were analyzed as follows. The mean number of live larvae with completely developed shells was calculated for the control treatments. The percentage of live larvae with completely developed shells in each treatment replicate at the end of the test was calculated as:

$$M' = 100 \cdot X/B$$

where M' = the percentage of live larvae with completely developed shells

X = the number of live larvae with completely developed shells in the test chamber

and B = the mean number of live larvae with completely developed shells in the control treatment.

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The mean number of larvae exhibiting an adverse effect (the percentage of larvae, live or dead, without completely developed shells) was then calculated:

$$E' = 100 - M'$$

where E' = the percentage of organisms which gave an adverse response expressed as a percentage of the average control response."

12. REPORTED RESULTS:

1) Natural Seawater Definitive Test:

"There was an extremely rapid die-away of RH-287 in all treatments used in this test. The initial samples were collected within 0.5 hr after preparation, and extracted as much as 3.5 hours after that or 4 hours after collection. After 48 hours, RH-287 was detectable in only the highest two concentrations. Since it is impossible to define an "average" exposure for the test animals, and since the measured concentrations on Day 0 do not really constitute an estimate of the initial concentrations, the nominal concentrations are taken to be the exposure concentrations in this report" (Table 3, attached).

The numbers of embryos on Day 0 and the number of larvae on Day 2 are presented in Table 5 (attached). Poor counts on Day 0 were made by one of the study technicians. After 48 hours, larvae at 32 µg a.i./L, all larvae at 56 µg a.i./L, and few larvae at 1.8 µg a.i./L were adversely affected.

"The NOEC was estimated to be 18.0 µg/L; i.e. there was no significant difference between the number of live larvae with completely developed shells in 20 mL of water at the 18.0 µg/L concentration and the number in the control treatment (minimum significant difference (MSD) = 69.8 larvae/20 mL).... Since the EC_{50} lies between two adjacent test concentrations and since the responses to these concentrations are effectively 0 and 100%, the best estimate of the EC_{50} is the geometric mean of these concentrations, or 24 µg/L. The 95% confidence limits cannot be calculated, but must lie between 18 and 32 µg/L."

"For this test, the mean (± standard deviation) temperature was 26.1 (± 0.4)°C and the mean salinity was 18.0 (± 0.0) g/kg (Table 6, [attached]). The mean oxygen concentration was 7.9 (± 0.1) mg/L or about 90.7 (± 3.4) percent of saturation. The pH was 7.0 (± 0.3)."

2) Synthetic Seawater Definitive Test:

RH-287 demonstrated rapid die-away in this study. Measurable residues were present after 48 hours in all but the three lowest concentrations (Table 7, attached). The number of embryos on Day 0 and larvae on Day 2 are presented in Table 8 (attached).

"In contrast to the test with a natural estuarine water, there was a more gradual appearance of adverse effect with increasing exposure concentration. In this case, the NOEC was estimated to be 10 µg/L and the LOEC was estimated to be 18 µg/L, a non-0 and non-100% response concentration (MSD = 47.4 larvae/20 mL). At both the 32 and the 56 µg/L exposure concentrations, two of the three replicates contained no live larvae with completely developed shells, while one replicate of each had a small number of live larvae. No explanation can be provided for this anomaly."

The EC_{50} was 12.1 µg/L (95% confidence interval of 7.2-17.1 µg/L). This value is about half that of the first study.

The pH was 7.8 ±0.1; the DO was 7.12 ±0.4 mg/L; temperature was 25.6 ±0.5°C; and the salinity was 16.0 g/kg.

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

A Study Compliance Statement was included in the report indicating that the study was conducted in accordance with FIFRA guidelines.

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:

RH-287 concentrations decreased dramatically (approximately 50 to 100% of nominal concentrations) during the test period.

The SEP states that embryos should be tested within one hour of spawning and after fertilization. This test used embryos 2-4 hours after fertilization.

The temperature for each study was higher (25-26°C) than that recommended in the SEP (20°C).

The study using synthetic seawater demonstrated greater than 30% mortality in the control. The SEP states that a test is not acceptable if the control larvae demonstrate more than 30% mortality.

The SEP states that each designated treatment group should be exposed to a concentration of toxicant that is at least 60% of the next highest concentration. Each designated treatment group for the tests were <60% of the next highest concentration.

The photoperiod employed during the test was not reported. 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 pH for both studies was much lower (7.0) than that recommended in the SEP (7.7-8.0).

Poor counting techniques were indicated in the report and data. This contributes to the uncertainty of the study.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 48-hour EC_{50} value for the natural seawater definitive study (printout, attached). The binomial test method provided a 48-hour EC_{50} value of 23.99 mg/L (no 95 percent confidence interval was defined) which is similar to that reported by the author.

C. **Discussion/Results:** Due to numerous deviations from the guidelines, poor data collection techniques, and highly variable test concentrations, the study results are not scientifically sound. The rapid decrease in concentration of RH-287 makes it difficult to interpret the data collected in these studies and derive an accurate EC_{50}.

Based on nominal concentrations the EC_{50} for the natural seawater definitive study was 24.0 µg a.i./L. This would indicate that RH-287 is very highly toxic to Crassostrea virginica embryos.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Invalid~~. Supplemental.

(2) **Rationale:** Poor scientific technique was used. The RH-287 concentrations decreased rapidly during both test studies. Several deviations from protocol were made.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, May 3, 1991.

Rosemary Graham Mora RTL Crassostrea virginica 05-03-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
56	100	100	100	0
32	100	100	100	0
18	100	0	0	0
10	100	0	0	0
5.6	100	0	0	0
3.2	100	10	10	0
1.8	100	13	13	0
1	100	1	1	0
.56	100	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 23.99999

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.33963	55.55168	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 = 2.272296

95 PERCENT CONFIDENCE LIMITS =-.3577137 AND 4.902305

LC50 = 20.29629

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = . 5.604159

95 PERCENT CONFIDENCE LIMITS = 0 AND 16.32485

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DATA EVALUATION RECORD

1. **CHEMICAL:** ~~RTL. Shaughnessey No. 000707.~~ RH-287

2. **TEST MATERIAL:** RH-287 Technical; Lot No. XI-SS-51; CAS No. 64359-81-5; TD No. 89-025; 96.9% active ingredient; white granules.

3. **STUDY TYPE:** Freshwater Invertebrate Acute Toxicity Test. Species Tested: Daphnia magna.

4. **CITATION:** Burgess, D. 1990. Acute Flow-Through Toxicity of RH-287 Technical to Daphnia magna. Final Report No. 37738. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Rohm and Haas Company, Spring House, PA. EPA MRID No. 417415-01.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 6/5/91

6. **APPROVED BY:**

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

Signature: Louis M. Rifici
Date: 6/6/91
Curtis Lara 11-16-91

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

Signature: Henry T. Craven
Date: 11/21/91

7. **CONCLUSIONS:** This study appears to be scientifically sound and meets the guideline requirements for a daphnid acute flow-through toxicity test. The EC_{50} was 5.22 µg a.i./L (mean measured concentration), therefore RH-287 is classified as very highly toxic to Daphnia magna. The NOEC was 5.0 µg a.i./L (mean measured concentration).

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna (<24 hours old) were obtained from populations cultured at the testing facility (original culture obtained from Columbia National Fisheries Research Laboratory, Missouri). The cultures were maintained in a temperature controlled area at 20 ±2°C on a 16-hour daylight photoperiod with 30-minute dawn and dusk simulations. The daphnids were fed a combination of green algae (Selenastrum capricornutum) supplemented with a Tetramin®/cereal leaves/yeast suspension.

B. Test System: The flow-through test system was a 500-mL proportional diluter apparatus. Seven sets of four 1-L glass beakers were used as test vessels, and were immersed in a temperature-controlled bath set to maintain 20 ±1°C. Each beaker drained through a notched drain covered with a stainless steel screen (50-mesh).

The test material was dissolved in triethylene glycol (TEG) and delivered to the diluter using a syringe dispenser.

The diluter delivered five nominal concentrations of test material, a solvent control, and a dilution water control to quadruplicate test vessels. The diluter continuously delivered water to each vessel (via 14-gauge hypodermic needle) at an average rate of 6.0 volume replacements every 24-hours.

The diluent, a blend of reverse osmosis water and ABC well water, had a total hardness range of 160-180 mg/L as $CaCO_3$.

The photoperiod was the same as that used in culturing with a light intensity of 50-70 footcandles.

C. Dosage: Forty-eight hour acute flow-through test. Nominal test concentrations selected based on results of range-finding studies were .60, 1.2, 2.5, 5.0, and 10.0 µg a.i./L. A dilution water control and a solvent control (0.05 mL TEG/L) were also used.

D. Design: Ten daphnids were randomly assigned and distributed to each of 4 exposure vessels (i.e., 40 daphnids/concentration).

The daphnids were not fed during the 48-hour test period. Observations of survival and abnormal effects were made every 24 hours. Dissolved oxygen concentration (DO), temperature, and pH were measured in the controls, high, medium and low concentrations at 0 and 48 hours of testing. Temperature in the water bath was monitored continuously.

Composite samples were collected from each replicate vessel of all groups at 0 and 48 hours for determination of RH-287 concentrations by gas-liquid chromatography.

E. **Statistics:** Concentration versus effect data (total adverse effects) were analyzed by employing an EC_{50} computer program by Stephan et al (1978). For this data, the EC_{50} value (95-percent confidence interval) was calculated using the binomial test method.

12. **REPORTED RESULTS:** Results of GLC analyses indicated that mean measured concentrations were 0.42, 0.70, 1.5, 3.9, and 7.0 µg a.i./L (Table 2, attached). The measured concentrations were fairly consistent between sample observations. The measured concentrations ranged from 52% to 90% of nominal.

"The analytical results would seem to indicate that the RH-287 Technical test compound degrades in aquatic test water."

The 48-hour EC_{50} value for RH-287, based on total adverse effects, was 5.2 µg a.i./L with a confidence interval of 3.9 and 7.0 µg a.i./L (Table 4, attached).

"The adverse effects of mortality, daphnids on the bottom of the test chambers and erratic movement were observed only in the 7.0 µg a.i./L test concentration (Table 5) [attached]."

The dissolved oxygen concentrations in this study was 8.1-8.5 mg/L representing 91% and 98% saturation at the test temperature range of 19°C to 20°C, respectively. The pH values was 7.8-7.9.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were presented in the report.

A GLP compliance statement was included in the report indicating that the data and report prepared for this study were produced and compiled in accordance with EPA Good

Laboratory Practice Standards. This statement was signed by the Study Director and representatives of the study sponsor.

A Quality Assurance Statement was included and signed by a representative of the Quality Assurance Unit of the preforming laboratory.

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:

The report did not indicate a period of aeration of the control water prior to use. At least seven days is the recommended aging period and dilution water should be intensely aerated prior to use.

A period of acclimation of the test organism to test conditions was not indicated in the report. An acclimation period of ten days is recommended.

The length of time between solution preparation and test initiation was not reported. The SEP recommends 30 minutes between test preparation and test initiation.

First instar test organisms should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

Each concentration was not 60% of the next concentration. The SEP recommends that each concentration be at least 60% of the next higher concentration.

Temperature in at least one test vessel should have been monitored as recommended in the SEP.

B. Statistical Analysis: The reviewer used EPA's Toxanal to verify the authors EC_{50} value and 95% confidence interval (printout, attached). The reviewer's results were similar to those of the author.

C. Discussion/Results: Given the results of the controls, it is probable that the above mentioned deviations did not affect the results of the study.

The study is scientifically sound and meets the requirements for an acute flow-through toxicity test using Daphnia magna. The EC_{50} was 5.22 µg a.i./L (95% confidence interval 3.9 to 7.0 µg a.i./L), which indicates that RTL is very highly toxic to Daphnia magna. The NOEC was 5.0 µg a.i./L.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, April 30, 1991.

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Rosemary Graham Mora RTL Daphnia magna 04-30-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
7	40	40	100	0
3.9	40	0	0	0
1.5	40	0	0	0
.7	40	0	0	0
.42	40	0	0	0

THE BINOMIAL TEST SHOWS THAT 3.9 AND 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 5.224941

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

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DATA EVALUATION RECORD

1. **CHEMICAL:** ~~RTI~~ RH-287 Sh. No. 128101
   Shaughnessey No. ~~000707~~

2. **TEST MATERIAL:** RH-287 technical; Lot No. XI-SS-93; TD No. 88-156; 96.9% active ingredient; a tan solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: Selenastrum capricornutum.

4. **CITATION:** Forbis, A.D. 1990. Acute Toxicity of RH-287 to Selenastrum capricornutum Printz. Final Report No. 37740. Conducted by Analytical Bio-Chemistry Laboratories, Inc., Colombia, MO. Submitted by Rohm and Haas Company, Spring House, PA. EPA MRID No. 417568-02.

5. **REVIEWED BY:**

   Mark A. Mossler, M.S.
   Agronomist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: [signature]
   Date: 6/4/91 [signature]

6. **APPROVED BY:**

   Michael L Whitten, M.S.
   Wildlife Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: [signature]
   Date: 6/4/91

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

   Signature: [signature]
   Date: 10/7/91

7. **CONCLUSIONS:** This study is not scientifically sound, ~~but~~ and does not meet the requirements for a Tier 2 study on the growth and reproduction of aquatic plants. Using nominal concentrations, the 120-hour EC_{50} of RH-287 for Selenastrum capricornutum was 0.032 mg ai/l. The 120-hour NOEC value for Selenastrum capricornutum was determined to be 0.0075 mg ai/l. The absence of the chemical in four of the five test solutions calls into question the methods of this study and invalidates it.

8. **RECOMMENDATIONS:** See Section 14 D.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, Selenastrum capricornutum Printz, came from laboratory stock cultures originally obtained from the University of Texas at Austin. Stock cultures were maintained in synthetic algae nutrient medium. The culture used as inoculum had been transferred to fresh medium 5 days before test initiation.

B. **Test System:** The study was conducted in 250 ml Erlenmeyer flasks containing 100 ml of medium and stoppered with a foam plug. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ± 0.1.

The test vessels were incubated at 24°±1°C under continuous cool-white fluorescent light on a shaker set to 100 rpm. The light intensity was 4000 lux.

C. **Dosage:** Based on the results of a preliminary test, five nominal concentrations (0.0075, 0.015, 0.03, 0.06 and 0.12 mg ai/l) and a control were selected for the test. Test solutions were corrected for percent purity (96.9%).

D. **Test Design:** The highest test concentration was prepared by adding the appropriate amount of solid test material to 2000 ml of sterile, deionized water. Lower concentrations were prepared by proportionally diluting the highest test concentration (0.12 mg ai/l). Three replicate 250 ml flasks were used per concentration and the control. A fourth replicate was used as an analytical sample. An inoculum of Selenastrum capricornutum cells calculated to provide 3.0×10^3 cells/ml was aseptically introduced into each flask. The inoculum volume was 1.00 ml per flask.

Cell counts were performed using a hemacytometer and light microscope every 24 hours. One count per replicate was used on each counting day.

Temperature, pH, and light intensity were monitored daily.

One replicate of each level at 0 and 48 hours was sampled for quantification of RH-287 in solution; each replicate was sampled at 120 hours. The samples were analyzed by gas-liquid chromatography.

E. **Statistics:** Cell counts for the exposure concentrations and the control were analyzed by analysis of variance (ANOVA) with subsequent mean testing using Dunnett's test ($p<0.05$). Cell counts for each replicate were first transformed using the square root of the cell count. The EC_{50} value was determined using each exposure concentration's percent difference from the control. The best of two quadratic regression models was chosen by least squares and visual techniques.

12. **REPORTED RESULTS:** The mean measured concentrations are given in Table 5 (attached). Measured concentrations were between 80-88% of nominal at 0 hours and 57-73% of nominal at 48 hours. By 120 hours, only 50% of the nominal concentration was detected at the highest rate. The remaining samples had no detectable RH-287. The results herein are therefore reported as nominal concentrations.

By the end of 120 hours, the growth of Selenastrum capricornutum was significantly inhibited by exposure to the four highest concentrations of RH-287 (Table 2, attached). The NOEC was therefore 0.0075 mg ai/l. The 120-hour EC_{50} value, calculated based on the percent difference of the mean cell count of exposure concentrations compared to the mean cell count of the control and solvent control was calculated as 0.032 mg/l with a 95% confidence interval of 0.028-0.036 mg ai/l.

The pH of the test solutions ranged between 7.0-7.4 at test initiation and decreased to between 6.0-6.4 by the end of the experiment (120 hours).

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

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

6e1

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:

The number of cells in each flask was 5,000 cells/ml; 3000 cells/ml is recommended.

The synthetic algae medium contained sodium-EDTA. Guideline requirements state that EDTA should not be used in the growth media.

The dissolved oxygen and conductivity of the test solutions were not measured.

B. Statistical Analysis: The reviewer used a computer program to analyze the growth data and to determine the 120-hour EC values using nominal concentrations (attached). The reviewer's results are in general agreement with the author's.

C. Discussion/Results: The measured concentrations varied greatly throughout the course of the study. By the end of the experiment, only one test solution contained any detectable test compound. Therefore, the algae apparently were not exposed to the test material throughout the study period.

RH-287 inhibited Selenastrum capricornutum growth at concentrations as low as 0.015 mg ai/l (nominal concentration). Since no maximum application rate for RH-287 was listed in the report, it is not possible to assess the risk of RH-287 applied to water.

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction study. The 120-hour EC_{50} of RH-287 for Selenastrum capricornutum was 0.032 mg ai/l (95% C.I. = 0.028-0.036 mg ai/l). The NOEC was 0.0075 mg ai/l.

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: Measured concentrations were not consistent throughout the test and the test

chemical was not detected in 4 of 5 test concentrations at 120 hours. Therefore, the algae were not exposed to the test material throughout the study period.

(3) **Repairability:** No. The study should be conducted in a system that maintains the test material concentration.

15. **COMPLETION OF ONE-LINER:** Yes, 04/26/91.

CELL COUNT

Summary Statistics and ANOVA

Transformation = None

Group rate (mg/l)	n	Mean	s.d.	cv%
1 = control	3	2005000.0000	77942.2863	3.9
2 0.0075	3	2223333.3333	100166.5280	4.5
3*0.015	3	1463333.3333	41633.3200	2.8
4*0.03	3	1180000.0000	72111.0255	6.1
5*0.06	3	497333.3333	9237.6043	1.9
6*0.12	3	40366.6667	6351.6402	15.7

NOEC = 0.0075 mg ai/l

*) the mean for this group is significantly less than
the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -126840.186338
This difference corresponds to -6.33 percent of control

Between groups sum of squares =********** with 5 degrees of freedom.

Error mean square = ********** with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .025

M. Mossler RH-287 SELENASTRUM 05-03-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.12	100	98	98	0
.06	100	75	75	0
.03	100	40	40	0
.015	100	25	25	0
.0075	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .03 AND .06 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 3.637345E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.027635E-02	3.243189E-02	2.955276E-02	3.564919E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.2312451	4.385661	4.309297E-03

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

SLOPE = 3.008302
95 PERCENT CONFIDENCE LIMITS = 1.561671 AND 4.454932

LC50 = .0325258
95 PERCENT CONFIDENCE LIMITS = 2.148627E-02 AND 4.972345E-02

LC10 = 1.230446E-02
95 PERCENT CONFIDENCE LIMITS = 4.44679E-03 AND 1.915873E-02

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