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

OCT 19 2000

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review of data on the Chronic Toxicity of RH-287 to Mysid (*Americamysis bahia*)

TO: Marshall Swindell, Team 33
Regulatory Management Branch I
Antimicrobials Division (7510C)

FROM: Srinivas Gowda, Biologist/Microbiologist/Chemist Srinivas Gowda 10-12-00
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

THRU: Winston Dang, Team Leader, Team One
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

Norm Cook, Chief N. Cook 10.19.00
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

DP Barcode: D267455
Submission: S582345
PC Codes: 128101
EPA Reg. No.: 707-175
Common Name: RH-287
Case: 189682
Case Type: Amended Registration
CAS#: 65359-81-5
MRID No.: 451531-01
Peer Reviewed by: K. Montague, RASSB
Chemical Name: 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone
Data Submitter: Rohm & Haas Company
Empeirical Formula: $C_{11}H_{17}Cl_2ONS$

INTRODUCTION:

Rohm and Haas Company, has submitted data on chronic toxicity of 4,5-Dichloro-2-n-octyl-3-isothiazolone (RH-287) to the Mysid (*Americamysis bahia*) to meet the U.S. Environmental Protection Agency's Ecological Effects Data Requirements published in Pesticide Assessment Guidelines, Subdivision E, § 72-4 (Esturine Invertibrate Life-Cycle Test) in support of amended registration. The submitted Mysid Chronic Toxicity Study has undergone review by Srinivas Gowda of Antimicrobials Division's Risk Assessment and Science Support Branch and the review reflects EPA's Pesticide Assessment Guidelines requirements and regulations.

BACKGROUND:

RH-287 (4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone), is an active ingredient in several EPA registered Antifouling paint products. The submitted study was conducted to determine the chronic toxicity of RH-287 to Mysid (*Americamysis bahia*) in support of amended registration.

The Mysid Chronic Toxicity study entitled "RH-287: Flow-Through Chronic Toxicity to the Mysid, *Americamysis bahia*," by Timothy J. Ward and Robert L. Boeri, T.R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, Massachusetts 01945, dated June 1, 2000 (MRID No. 451531-01) has been submitted to the Agency to fulfil the amended registration requirements for the chemical, RH-287.

SUMMARY OF DATA:

See attached Data Evaluation Record.

The survival of first generation mysids (the most sensitive parameter measured) at the end of 28-day exposure to RH-287 resulted in a lowest observed effect concentration (LOEC) of 1.24 μg/L, a no observed effect concentration (NOEC) of 0.627 μg/L, and a maximum acceptable toxicant concentration (MATC) of 0.882 μg/L. The LOEC, NOEC, and MATC for the number of offspring produced by first generation mysids were 2.39 μg/L, 1.24 μg/L, and 1.72 μg/L respectively. The LOEC, NOEC, and MATC for other measured parameters (the survival of second generation mysids, the subletha! effects of first and second generation mysids, the length of surviving first and second generation mysids, and the weight of surviving first and second generation mysids) were 4.97 μg/L, 2.39 μg/L, and 3.45 μg/L respectively. The 7-day LC_{50} was greater than 4.97 μg/L. The 14-day LC_{50} was greater than 4.1 μg/L. The 21-day LC_{50} was greater than 3.4 μg/L. The 28-day LC_{50} was greater than 2.5 μg/L. The study is scientifically sound and fulfills the Subdivision E, §72-4 guideline requirements

RASSB's CONCLUSIONS AND RECOMMENDATIONS:

Risk Assessment and Science Support Branch (RASSB) concludes that the submitted Mysid *(Americamysis bahia)* Chronic Toxicity Study is scientifically sound and fulfills the Subdivision E, §72-4 guideline requirements for amended registration. The survival of first generation mysids (the most sensitive parameter measured) at the end of 28-day exposure to RH-287 resulted in a lowest observed effect concentration (LOEC) of 1.24 μg/L, a no observed

effect concentration (NOEC) of 0.627 μg/L, and a maximum acceptable toxicant concentration (MATC) of 0.882 μg/L. The 28-day LC_{50} was greater than 2.5 μg/L. The study can be classified as core for a technical grade active ingredient.

cc: Srinivas Gowda/RASSB/AD

Chemical File(128101)/AD

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Reviewed by: Srinivas Gowda, Microbiologist/Chemist, Team 1 Srinivas Gowda, Date 10-12-00

Secondary Reviewer: Kathryn Montague, Biologist, Team 2 KM, Date 10-17-00

DATA EVALUATION RECORD

STUDY TYPE:	Mysid (*Americamysis bahia*) Chronic Toxicity Study (Guidelines E, § 72-4 72-4/OPPTS 850.1350)
DP BARCODE:	D267455
PC CODE:	128101
SUBMISSION CODE:	S582345
CASE TYPE:	Amended registration
TEST MATERIAL:	4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one
SYNONYMS:	RH-287
CITATION:	"RH-287: flow-through chronic Toxicity to the Mysid *Americamysis bahia*," by Timothy J. Ward and Robert L. Boeri, T.R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, Massachusetts 01945, dated June 1, 2000 (MRID No. 451531-01)
SPONSOR:	Rohm and Haas Company

DATA EVALUATION RECORD

72-4© – ESTUARINE INVERTEBRATE LIFE CYCLE TEST - OPPTS 850.1350

1. CHEMICAL: 4-Chloro-2-n-octyl-3-(2H)-isothiazolone PC Code No. 128101

2. TEST MATERIAL: RH-287 Technical;
tan solid; lot # 0159F005 Purity: 100.3%

3. CITATION

Authors:	T. J. Ward and R. L. Boeri
Title:	RH-287: Flow-Through Chronic Toxicity to the Mysid, *Americamysis bahia*
Study Completion Date:	June 1, 2000
Laboratory:	T.R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, MA 01945
Sponsor:	Rohm and Haas Company 727 Norristown Road Spring House, PA 19477-0904
Laboratory Report ID:	T.R. Wilbury Study No. 1927-RH; Rohm and Haas Report No. 99RC-0197
MRID No.:	451531-01
DP Barcode:	D267455

4. REVIEWED BY: Srinivas Gowda, Biologist
US EPA/OPP/AD/RASSB

Signature: Srinivas Gowda Date: 10-12-00

5. APPROVED BY: Norm Cook, Chief
US EPA/OPP/AD/RASSB

Signature: Norm Cook Date: 10.19.00

6. STUDY PARAMETERS

Age of Test Organism:	< 24 hours post release from brood sac
Definitive Test Duration:	28 days (2/4/00 to 3/3/00)
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. CONCLUSIONS: The study is scientifically sound and fulfills the guideline requirements for a chronic toxicity study and can be classified as core for a technical grade active ingredient.

The survival of first generation mysids (the most sensitive parameter measured) at the end of 28-day exposure to RH-287 resulted in a lowest observed effect concentration (LOEC) of 1.24 μg/L, a no observed effect concentration (NOEC) of 0.627 μg/L, and a maximum acceptable toxicant concentration (MATC) of 0.882 μg/L.

The LOEC, NOEC, and MATC for the number of offspring produced by first generation mysids were 2.39 μg/L, 1.24 μg/L, and 1.72 μg/L respectively.

The LOEC, NOEC, and MATC for other measured parameters (the survival of second generation mysids, the sublethal effects of first and second generation mysids, the length of surviving first and second generation mysids, and the weight of surviving first and second generation mysids) were 4.97 μg/L, 2.39 μg/L, and 3.45 μg/L respectively.

The 7-day LC_{50} was greater than 4.97 μg/L. The 14-day LC_{50} was greater than 4.1 μg/L. The 21-day LC_{50} was greater than 3.4 μg/L. The 28-day LC_{50} was greater than 2.5 μg/L.

Results Synopsis
NOEC: 0.627 μg ai/L (adult survival)
LOEC: 1.24 μg ai/L (adult survival)
MATC: 0.882 μg ai/L (adult survival)

NOEC's for specific effects
Young/Female: 1.24 μg ai/L
Survival: 0.627 μg ai/L (1st generation)
Growth: 2.39 μg ai/L (dry weight and length)

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: None of the guideline deviations were considered major or likely to have biased the results.

C. Repairability: Not applicable

8. GUIDELINE DEVIATIONS

1) One of the ten measurements of the test substance concentration per treatment level (samples taken from two replicates on days 0, 7, 14, 21, and 28) was slightly more than 30% higher than the time-weighted average value for that treatment level. That was the measurement of 0.366 on day zero in the 0.277.µg/L mean measured treatment group. This exceedance of 0.006 µg/L above the mean +30% limit (i.e., 0.360 µg/L). Thus, the upper limit was exceeded in more than 5% of the measurements (Table C.1 in report). Because this exposure group represented the NOAEL for the experiment (i.e., not

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statistically different than controls), this deviation apparently did not affect the results.

2) Several deviations from Good Laboratory Practice were noted in the GLP Compliance Statement, but are unlikely to affect the results of the study:

Not all original chromatograms were dated and initialed on the day of printing;

Analytical sample dilution documentation was not always recorded directly, dated, or initialed, and the dilution of analytical samples on days 20 and 21 can not be verified from the raw data.

The original, signed Protocol Amendment 3 has been misplaced (a certified true copy is in the study file.)

The stability, characterization, verification of the test material identity, and maintenance of records on the test material were considered the responsibility of the Study Sponsor.

3) In their statement of Protocol Deviations (Section XI of the study), the study authors stated that the delivery of test solution to each replicate was not always within 10% of the other four replicates. No data were provided to document the meaning of "not always within 10%." Given the measurements of the active ingredient in the test solutions, however, this is not a serious deviation.

4) The authors noted that the salinity of the dilution water during the range-finding toxicity test was 16 to 17 ppt rather than 17 to 23 ppt.

5) The authors stated that one set of pretest samples was not analyzed within five days of collection or extracted onto Empor disks. This protocol deviation should not affect the study results.

6) The authors noted that one of the nominal concentrations was changed by the final diluter calibration (0.47 μg/L rather than 0.48 μg/L). Therefore, nominal concentrations differed from the ones listed in the protocol. This error has no influence on the study results, which depend on the measured concentrations.

7) The authors noted that offspring gender for 2nd generation mysids could not be determined because offspring were still immature at the end of the toxicity test.

8) The authors noted that pretest samples were analyzed along with samples collected on the first day of the definitive toxicity test rather than prior to testing.

9) The percentage of control females producing young could not be determined. This was acknowledged by the authors and attributed to the fact that the OPPTS test guideline does not allow isolation of individual 1st generation female mysids.

10) There was insufficient documentation to verify the 14 hour light/10 hour dark photoperiod during the 14 days prior to the beginning of the definitive toxicity test, as

acknowledged by the authors.

11) The incidence of pathological or histological effects was not reported.

10. MATERIALS AND METHODS

A. Biological System

Guideline Criteria	Reported Information
Species An estuarine shrimp species, preferably *Americamysis bahia*	*Americamysis bahia*
Duration of the Test A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment	28 days (test was terminated after 7 days past the median time of 1st brood release in the control treatment)
Source (or supplier)	T.R. Wilbury Laboratories in-house culture (originally obtained from Aquatic BioSystems, Inc., Fort Collins, CO)
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1) Parental stock was maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids were reported to be free from disease, injuries, and abnormalities during acclimation.
Parental Acclimation Period At least 14 days	14 days
Chamber Location Treatments should be randomly assigned to test chamber locations	Test vessels were randomly arranged in a water bath.
Brood Stock Test started with mysids: 1) from only one brood stock; or 2) from brood stock which has not obtained sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	Test started with mysids from culture that was maintained for 14 days prior to the start of the test under conditions similar to test conditions.

Guideline Criteria	Reported Information
Distribution Minimum of 40 mysids per concentration, with a minimum of 5 concentrations. No more than 8 individuals per replicate group. Once sex of mysids can be determined, there should be at least one female and one male per replicate vessel. If not, one should be obtained from other replicate vessels of the same concentration.	Eight mysids per replicate test vessel, 5 test vessels per concentration for a total of 40/treatment level. The sex of the mysids was determined by day 16, and it was confirmed that there was at least one male and one female per replicate vessel.
Feeding 1) Mysids should be fed live brine shrimp nauplii at least once daily. 2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	1) Mysids were fed newly hatched *Artemia salina* nauplii three times each day. 2) Mysids were fed at the rate of approximately 150 *Artemia salina* nauplii per mysid per day.
Counts Live adult mysids should be counted daily Live young must be counted and removed daily. Missing or impinged animals should be recorded.	Surviving adult organisms were counted every 24 hours. Young were counted and removed daily, beginning with day 16 (the first day that young were present). Dead organisms were removed when first observed.
Controls A negative control and carrier control (when applicable) are required.	Negative (dilution water) and solvent controls were used.

Comments: Offspring of the 1st generation mysids were counted, and the first eight offspring from each replicate vessel were separated into retention chambers at the same test concentration as the chambers where they originated. If a batch of more than eight offspring was produced, eight were randomly selected. Survival, sublethal effects, length, and the weight (wet and dry) of these 2nd generation mysids were recorded.

B. Physical System

Guideline Criteria	Reported Information
Test Water 1) May be natural (sterilized and filtered) or a commercial mixture. 2) Water must be free of pollutants. 3) During the test, the difference between highest and lowest measured salinities must be less than 10 ‰. Should be measured weekly.	1) Natural seawater collected from Atlantic Ocean near Marblehead, MA, and filtered through 5 and 20 μm filters. 2) The dilution water was measured for various pollutants (e.g., metals, pesticides) twice per year. Measured values and detection limits were provided. No pesticides or PCBs were detected, and the concentrations of inorganics appeared to be well below toxic levels (i.e., below EPA ambient water quality criteria). 3) During the test, the difference between highest and lowest measured salinities was less than 1 ‰. The salinity was measured 455 times throughout the 28 day test period, but the authors did not report the exact frequency for each replicate.
4) Salinity should be between 15 and 30 ‰. 5) pH should be measured at the beginning, end of test and weekly. 6) DO must be measured in each concentration at least once a week. 7) See details in ASTM E-1191.	4) All salinity measurements were reported as 17 ‰. 5) The pH, which ranged from 7.6 to 8.0, was measured at the beginning and end of the test, as well as every two to three days in each replicate during the test. 6) DO, which ranged from 6.6 to 8.0 mg/L (approximately 80 to 95% saturation), was measured every two to three days in each replicate. 7) Details in OPPTS guideline no. 850.1350 consulted.
Test Temperature 1) Measured daily in one chamber and at least 3 times in all chambers. 2) The test temperature should be 25°C ± 2°C	1) Measured every two to three days in each replicate test vessel (> 3 times) and continuously in a control vessel. 2) The mean temperature for all chambers at test termination was 24.8°C. All measured temperature values were within 0.7°C of the mean temperature.
Photoperiod Recommend 16 light /8 dark, but 14 light/ 10 dark is also acceptable.	14 light /10 dark reported in protocol

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Guideline Criteria	Reported Information
Dosing Apparatus 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations should be used. 3) A dilution factor not greater than 0.5 and controls should be used.	1) An Intermittent flow proportional diluter was used. 2) Five toxicant concentrations were used. 3) The dilution factor was approximately 0.5, and controls (solvent and dilution water) were used.
Toxicant Mixing 1) A mixing chamber is recommended but not required. 2) Aeration should not be used for mixing. 3) It must be demonstrated that the test solution is completely mixed before introduction into the test system. 4) Flow splitting accuracy must be within 10%.	1) A mixing chamber was used. 2) Aeration was not used. 3) The mixing system appeared adequate to ensure complete mixing of the test solutions prior to their introduction into the test system; see comments below. 4) Flow splitting accuracy was not always within 10%; see Section 8.
Test Vessels 1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic **Test Chambers** 1) Most common: 300x450x150 mm deep with solution depth of 100 mm 2) Should be covered **Test Compartments** (within chambers) 1) Size: 250 ml beaker with side cutouts covered with nylon mesh or stainless steel screen, or 2) 90 or 140 mm inside diameter glass Petri dish bottoms with collars made of 200 - 250 µm mesh screen.	Test Vessels: All test chambers and compartments were made of glass. All parts of the diluter that contacted the test substance were made of glass or Teflon. Test Chambers: Loosely-covered, 1-liter glass vessels that contained up to 800 mL of test solution. (A depth of approximately 110 mm was maintained.) Test Compartments: There was no further division of the test chambers into test compartments.

Guideline Criteria	Reported Information
Flow Rate 1) Flow rates should provide 5 to 10 volume additions per 24 hour. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period.	1) 50 exchanges per 24 hours. 2) DO was maintained at or above 80% of saturation (technical reviewer estimates that the lowest DO level, 6.6 mg/L, is approximately equivalent to 80% of saturation at 25°C at sea level). 3) Meter systems were calibrated before and after the study and were checked twice daily during test period.
Aeration 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	1) Dilution water was aerated. 2) Test vessels were not aerated.

Comments: To make the stock solutions, the test substance was liquefied in a 50°C water bath, and a 7,000 mg/L stock solution was prepared by combining 0.6981 grams of the liquified test substance with acetone and adjusting the final volume of solvent to 100 ml. This stock solution was stored in a dark freezer. A series of secondary stock solutions were prepared by combining 10 ml of the 7,000 mg/L solution with 990 ml of triethylene glycol. These secondary 70 mg/L stock solutions were added directly to the dilution water by the toxicant injector of a proportional diluter (0.30 ml stock combined with 3,000 ml of water during each diluter cycle) to produce a final solution nominally of 7.0 μg/L. The concentration of RH-287 was measured in these secondary stock solutions on days 0, 7, 14, 21, and 28. The mean measured concentration in the secondary solution was 5.33 μg/L, indicating a 24% loss of the test substance during the initial test solution preparation. This final diluter toxicant solution was mixed by a high shear pump prior to distribution to test vessels.

C. Chemical System

Guideline Criteria	Reported Information
Concentrations 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control, if appropriate. 2) Toxicant concentration must be measured in one tank at each treatment level every week. 3) One concentration must adversely affect a life stage and one concentration must not affect any life stage.	1) Solvent and dilution water controls, plus five nominal concentrations (all replicated): 0.47, 0.91, 1.8, 3.5, 7.0 µg/L. Given the 24% loss of RH-287 in preparation of the secondary stock solution, the corrected nominal concentrations were 0.36, 0.69, 1.4, 2.7, and 5.3 µg/L. 2) Toxicant concentration was measured in samples collected from two replicate test vessels of each treatment level every week. Which two of the five replicates were tested changed from week to week. 3) One concentration adversely affected a life stage and one concentration did not affect any life stage.
4) The measured concentration of the test material of any treatment should be at least 50% of the time-weighted average measured concentration for >10% of the duration of the test. 5) The measured concentration for any treatment level should not be more than 30% higher than the time-weighted average measured concentration for more than 5% of the duration of the test.	4) The measured concentration of the test material of all treatments was not less than 50% of the time-weighted average measured concentration for >10% of the duration of the test. 5) The measured concentrations for all samples for all treatments, except for one sample of one replicate at the treatment level of 0.277 µg/L (mean measured concentration), were less than 30% higher than the time-weighted average measured concentration for that treatment level. A sample from one replicate on day 0 measured 0.366 µg/L. That exceeds the mean (0.277 µg/L) + 30% (0.083 µg/L) limit of 0.360 µg/L by 0.006 µg/L. Thus, the upper limit was exceeded in 10% (i.e., 1/10) of the measurements.
Solvents 1) Should not exceed 0.1 ml/L in a flow-through system. 2) The following solvents are acceptable: triethylene glycol, methanol, acetone, and ethanol.	1) 0.1 ml/L 2) Solvent: acetone:triethylene glycol (1:99)

Comments: A range-finding test was conducted prior to the definitive test. Nominal concentrations for this test were 0 (control and solvent control), 0.70, 1.5, 2.4, 5.0, and 10 µg/L. After 17 days of exposure, there was at least 80% survival at 0 µg/L, 100% survival at 0.70 and 1.5 µg/L, 90% survival at 2.4 and 5.0 µg/L, and 0% survival at 10 µg/L. In the range-finding test, no surviving mysid exhibited sublethal effects.

11. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Guideline Deviations above)
Controls 1) Survival of the 1st generation mysids in the controls (between pairing and test termination) must not be less than 70%. 2) At least 75% of the paired 1st generation females in the controls produced young, or 3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	1) Survival of the 1st generation mysids from day 0 in the controls was 83% for the dilution water control and 80% for the solvent control. 2) Not reported (and not possible to calculate from the reported raw data because several females were included in each test vessel). 3) The average number of young produced per 1st generation female was reported to be 4.7 for the dilution water control and 5.3 for the solvent control.
Data Endpoints Must include: 1) Survival of 1st generation mysids (both male and female) 2) Number of live young produced per female 3) Dry weight of each 1st generation mysid alive at the end of the test (both male and female) 4) Length of each 1st generation mysid alive at the end of the study (both male and female) 5) Incidence of pathological or histological effects 6) Observations of other effects or clinical signs	Data endpoints included: 1) Survival of 1st generation mysids for each test concentration and control 2) Number of live young produced per female after 28 days of exposure 3) Dry weight of each 1st generation mysid alive at the end of the test (both male and female) 4) Length of each 1st generation mysid alive at the end of the study (both male and female) 5) The incidence of pathological or histological effects was not reported. 6) Sublethal effects data for 1st generation mysids

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Guideline Criteria	Reported Information
Raw data included? (Y/N) At a minimum, individual data should be included for: 1) Surviving 1st generation male and female mysids. 2) Number of live young produced per female. 3) Individual length measurements of male and female mysids. 4) Individual dry weight measurements for male and female mysids at the end of the test.	Raw data were included for: 1) Surviving 1st generation male and female mysids: (a) number of surviving mysids (out of 40) in each replicate; and (b) number of surviving female mysids (out of 8) for each replicate vessel. 2) Number of live young produced per female. 3) Individual length measurements of male and female mysids. 4) Individual dry weight measurements for male and female mysids at the end of the test. 5) Individual blotted wet weight of each 1st generation mysid alive at the end of the test (both male and female) 6) Sublethal effects.

A. Effects Data

Toxicant concentration (µg/L)		Number of live young produced per female by day 28	% Survival (28 days)	Mean Total Length (mm)		Mean Dry Weight (mg)	
Corrected Nominal[1]	Mean Measured		♂ & ♀	♂	♀	♂	♀
Control	ND[2]	4.7	83	10.0	9.2	0.56	0.71
Solvent control	ND[2]	5.3	80	11.3	10.7	0.53	0.62
0.36	0.227	4.7	83	11.6	10.3	0.56	0.74
0.69	0.627	5.1	68	11.7	10.7	0.57	0.75
1.4	1.24	4.5	53	11.7	10.1	0.55	0.71
2.7	2.39	1.6	55	10.5	10.8	0.52	0.78
5.3	4.97	0	5	9.2	N/A	0.35	N/A

1 Authors reported a 24% loss of test substance in the secondary stock solution prepared in dilution water by the proportional diluter.

2 None detected at or above the limit of quantitation of 0.0322 µg/L.

Toxicity Observations: Mysids were observed for sublethal effects (i.e., loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) at least three times per week (once every 24 hours once gender could be determined). Sublethal effects were observed in the 1st generation only in replicates of the highest mean measured concentration (4.97 µg/L) beginning at day 17.

B. Statistical Results

Statistical Method: Control and solvent data were compared with a parametric "t-test," and in all cases, except the length of female mysids on the first day of gender determination, no significant differences were observed at the 95% confidence interval. The negative control and solvent control data were pooled prior to subsequent statistical analysis. A chi-square test was used to determine if data were normally distributed. Bartlett's test was used to determine if variances were homogeneous. For all data but the survival of 2nd generation mysids, the data were normally distributed and the variances homogeneous. For all data except the survival of 2nd generation mysids, a one-way analysis of variance (ANOVA) and Bonferroni's test were used to compare treatment data to [pooled] control and solvent control data. Data for the survival of 2nd generation mysids were analyzed using a nonparametric William's test. All calculations were performed using mean measured concentrations of the active ingredient. Data from the highest treatment group were assumed to be different from the pooled control data because no females survived and no offspring were produced. Those data, therefore, were not included in statistical analyses of female survival, production of young per female,

second generation survival, length, or weight data. Male survival occurred in only one replicate of the highest treatment, and therefore data from this treatment could not be included in the length and weight data for statistical analysis. The LC_{50}s were calculated using the binomial/nonlinear interpolation method. The 7-day LC_{50} could not be calculated because survival at all tested concentrations exceeded 50%. Only the endpoints specified by the EPA/OPP pesticide assessment guidelines are listed below.

Endpoint	Method	NOAEC (µg ai/L)	LOAEC (µg ai/L)	MATC (µg ai/L)
Survival	One-way ANOVA and Bonferroni's test	0.627	1.24	0.882
Reproduction (number of young per female)	One-way ANOVA and Bonferroni's test	1.24	2.39	1.72
Dry Weight (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Dry Weight (female)		2.39	4.97	3.45
Length (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Length (female)		2.39	4.97	3.45

Most sensitive endpoint: Survival of 1st generation mysids

Comments: The 28-day LC_{50} (95% confidence interval) calculated by the probit method was 2.5 µg/L (0.627 to 4.97 µg/L). The authors also reported EC_{50} values, and corresponding 95% confidence intervals, for days 7, 14, and 21 using the binomial method. The 7-day LC_{50} was greater than 4.97 µg/L. The 14-day LC_{50} was 4.1 µg/L (2.39 to 4.97 µg/L). The 21-day LC_{50} was 3.4 µg/L (2.39 to 4.97 µg/L).

12. REVIEWER'S STATISTICAL RESULTS

Control and solvent data were compared with the parametric "t-test" and in all cases, except the length of female mysids on the first day of gender determination, no significant differences were observed with $\alpha = 0.05$. A chi-square test and Shapiro-Wilk's test were used to determine if data were not distributed normally. Bartlett's test was used to determine if variances were not homogeneous. For all data but the survival of 2nd generation mysids, the null hypotheses of normally distributed and homogeneous variances could not be rejected at the $\alpha = 0.05$ level. We did not analyze the survival of the 2nd generation mysids because that endpoint is not specified by the EPA OPP pesticide assessment guidelines and because it was not the most sensitive endpoint. For the other data, we used a one-way ANOVA and Bonferroni's test to identify the NOAEL and LOAEL, excluding data from the highest treatment level for the same reasons the authors excluded those data. We did not estimate the 14-day,

21-day, or 28-day LC_{50} values because those are not specified by the EPA OPP pesticide assessment guidelines. We identified the same NOAEL and LOAEL values as did the authors (see table below).

Endpoint	Method	NOAEC (µg ai/L)	LOAEC (µg ai/L)	MATC (µg ai/L)
Survival	One-way ANOVA and Bonferroni's test	0.627	1.24	0.882
Reproduction (number of young per female)	One-way ANOVA and Bonferroni's test	1.24	2.39	1.72
Dry Weight (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Dry Weight (female)		2.39	4.97	3.45
Length (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Length (female)		2.39	4.97	3.45

Most sensitive endpoint: Survival of 1st generation mysids.

Comments: We were not able to calculate the same value for the number of live young produced per female by day 28 as estimated by the study authors, although we tried two methods. The difficulty with the estimate is that the number of females alive each day decreased in some of the replicates in some of the treatment levels. We estimated the total number of female-days per replicate and divided by the number of days to estimate an "average" number of females alive to compare against the total number of offspring produced by day 28. Our "time-weighted" average total number of offspring per female are listed in the table below. We also estimated male survivorship by subtracting from total number of mysids surviving in a given replicate on a given day the number of female mysids surviving in that replicate on that day. Finally, we estimated 1st generation male and female mysid survival using the number alive on day 16 as the starting point (sex could not be determined before day 14). Although our calculations of average number of live young produced per female differed from the authors, the difference is slight and does not change the conclusions of this study. Our analysis of male and female survival after day 16 indicates a NOAEL for both sexes combined of 0.627 µg/L, and survivorship decreased with increasing dose for the females, but not for the males. We cannot determine if the unusual dose-response pattern exhibited by the male mysids is cause for concern with this study.

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Toxicant concentration (µg/L)		Number of live young produced per female by Day 28		Percent Survival by Day 28 (Survivors/Total Number)			
Corrected Nominal[1]	Mean Measured	Estimated by Study Authors	Estimated by Reviewer[3]	♂ & ♀[4] from Day 1	♂ from Day 16	♀ from Day 16	♂ & ♀ from Day 16
Control	ND[2]	4.7	4.9	83 (33/40)	81 (21/26)	92 (12/13)	85 (33/39)
Solvent control	ND[2]	5.3	5.4	80 (32/40)	76 (19/25)	87 (13/15)	80 (32/40)
0.36	0.227	4.7	4.7	83 (33/40)	90 (19/21)	88 (14/16)	89 (33/37)
0.69	0.627	5.1	4.8	68 (27/40)	58 (14/24)	81 (13/16)	43 (27/40)
1.4	1.24	4.5	4.4	53 (21/40)	54 (13/24)	53 (8/15)	54 (21/39)
2.7	2.39	1.6	1.9	55 (22/40)	75 (15/20)	37 (7/19)	56 (22/39)
5.3	4.97	0	0	5 (2/40)	17 (2/12)	0 (0/0)	17 (2/12)

1 Authors reported a 24% loss of test substance in the secondary stock solution prepared in dilution water by the proportional diluter.
2 None detected at or above the limit of quantitation of 0.0322 µg/L.
3 Estimated from daily average number of live young produced per female (i.e., number of young produced in treatment group on a given day/total number of females alive that day).
4 Calculated by subtracting number of females alive (Table A.3 of the report) from total number alive of both sexes (Table A.1 of the report) each day.

13. REVIEWER'S COMMENTS

The study authors reported that no insoluble material was observed in any vessel during the test. Mean measured concentrations among replicates varied by less than 20%. Mean measured concentrations of the test substance ranged from 68% to 71% of the RH-287 nominal concentrations in the four highest concentrations and 60% of the nominal RH-287 concentration in the lowest treatment concentration. After correcting the nominal concentrations for the 24% loss of RH-287 during preparation of the secondary stock solution, the mean measured concentrations of RH-287 in the test vessels ranged from 78 to 93% of nominal. However, concentrations in the test vessels of the two lowest exposure concentrations appeared to decline by approximately 20 to 25% over the 28-day period, falling around 6-7% each week (Table C.1). There is no obvious reason for that decline. Given that the decline is moderate and the concentrations were measured adequately, the decline is not a serious concern.

The study is scientifically sound, fulfills the guideline requirements, and is classified as core for a technical grade active ingredient.

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DP BARCODE: D267455

CASE: 189682 DATA PACKAGE RECORD DATE: 10/25/0(
SUBMISSION: S582345 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 400 DATA-MISC DATA-NOT REQUES
CHEMICALS: 128101 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone 100.0000%
128101 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone 100.0000%
999999 Inert Chemical 0.0000%
128102 4-Chloro-2-n-octyl-3(2H)-isothiazolone 0.0000%

ID#: 000707-00175 ANTI-FOULANT C-9211M
COMPANY: 000707 ROHM & HAAS CO
PRODUCT MANAGER: 33 MARSHALL SWINDELL 703-308-6341 ROOM: CS1 6B
PM TEAM REVIEWER: KAREN LEAVY-MUNK 703-308-6237 ROOM: CS1 6W9
RECEIVED DATE: 06/27/00 DUE OUT DATE: 10/25/00

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 267455 EXPEDITE: N DATE SENT: 07/13/00 DATE RET.: 10/19/00
CHEMICAL: 128101 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone
DP TYPE: 001
CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : AD	07/13/00	10/19/00
BRAN: RASSB	07/25/00	10/19/00
SECT: RASSB1	07/25/00	10/17/00
REVR : SGOWDA	07/25/00	10/12/00
CONTR:	/ /	/ /

ADMIN DUE DATE: 10/31/00
NEGOT DATE: / /
PROJ DATE: / /

* * * DATA REVIEW INSTRUCTIONS * * *

Please review the requested Shrimp data (MRID #45153101).

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

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DATA EVALUATION REPORT

4-CHLORO-2-N-OCTYL-3(2H)-ISOTHIAZOLONE

Study Type: Estuarine Invertebrate Life Cycle (Chronic) Test (*Americamysis bahia*)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer Michael Ragan — *Michael Ragan, B.A.* Date 8/25/00

Independent Reviewer Allison Benjamin — *Allison Benjamin, M.E.M.* Date 8/25/00

Project Manager (QA/QC Manager) Margaret E. McVey — *Margaret McVey, Ph.D.* Date 8/25/00

Contract Number: 68-W6-022
Work Assignment No.: 5-24
EPA Project Manager: Wanda Jakob

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

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DP Barcode: D267455 MRID No.: 451531-01

DATA EVALUATION RECORD
72-4(c) – ESTUARINE INVERTEBRATE LIFE CYCLE TEST - OPPTS 850.1350

1. **CHEMICAL:** 4-Chloro-2-n-octyl-3-(2H)-isothiazolone PC Code No.: 128101

2. **TEST MATERIAL:** RH-287 Technical;
tan solid; lot # 0159F005 Purity: 100.3%

3. **CITATION**

Authors:	T. J. Ward and R. L. Boeri
Title:	RH-287: Flow-Through Chronic Toxicity to the Mysid, *Americamysis bahia*
Study Completion Date:	June 1, 2000
Laboratory:	T.R. Wilbury Laboratories, Inc., 40 Doaks Lane, Marblehead, MA 01945
Sponsor:	Rohm and Haas Company 727 Norristown Road Spring House, PA 19477-0904
Laboratory Report ID:	T.R. Wilbury Study No. 1927-RH; Rohm and Haas Report No. 99RC-0197
MRID No.:	451531-01
DP Barcode:	D267455

4. **REVIEWED BY:** A. Vaughan, Ph.D., Antimicrobial Division

Signature: Date:

5. **APPROVED BY:** W. Jakob, Ph.D., Antimicrobial Division

Signature: Date:

6. **STUDY PARAMETERS**

Age of Test Organism:	< 24 hours post release from brood sac
Definitive Test Duration:	28 days (2/4/00 to 3/3/00)
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. **CONCLUSIONS**

Results Synopsis
NOEC: 0.627 µg ai/L (adult survival)
LOEC: 1.24 µg ai/L (adult survival)
MATC: 0.882 µg ai/L (adult survival)

NOEC's for specific effects
Young/Female: 1.24 μg ai/L
Survival: 0.627 μg ai/L (1st generation)
Growth: 2.39 μg ai/L (dry weight and length)

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: None of the guideline deviations were considered major or likely to have biased the results.

C. Repairability: Not applicable

8. MAJOR GUIDELINE DEVIATIONS

1) One of the ten measurements of the test substance concentration per treatment level (samples taken from two replicates on days 0, 7, 14, 21, and 28) was slightly more than 30% higher than the time-weighted average value for that treatment level. That was the measuremnt of 0.366 on day zero in the 0.277.µg/L mean measured treatment group. This exceedance of 0.006 µg/L above the mean +30% limit (i.e., 0.360 µg/L). Thus, the upper limit was exceeded in more than 5% of the measurements (Table C.1 in report). Because this exposure group represented the NOAEL for the experiment (i.e., not statistically different than controls), this deviation apparently did not affect the results.

Several deviations from Good Laboratory Practice were noted in the GLP Compliance Statement, but are unlikely to affect the results of the study:

1) Not all original chromatograms were dated and initialed on the day of printing;

2) Analytical sample dilution documentation was not always recorded directly, dated, or initialed, and the dilution of analytical samples on days 20 and 21 can not be verified from the raw data.

3) The original, signed Protocol Amendment 3 has been misplaced (a certified true copy is in the study file).

4) The stability, characterization, verification of the test material identity, and maintenance of records on the test material were considered the responsibility of the Study Sponsor.

The other Guideline Deviations listed below are considered minor.

1) In their statement of Protocol Deviations (Section XI of the study), the study authors stated that the delivery of test solution to each replicate was not always within 10% of the other four replicates. No data were provided to document the meaning of "not always

within 10%." Given the measurements of the active ingredient in the test solutions, however, this is not a serious deviation.

2) The authors noted that the salinity of the dilution water during the range-finding toxicity test was 16 to 17 ppt rather than 17 to 23 ppt.

3) The authors stated that one set of pretest samples was not analyzed within five days of collection or extracted onto Empor disks. This protocol deviation should not affect the study results.

4) The authors noted that one of the nominal concentrations was changed by the final diluter calibration (0.47 μg/L rather than 0.48 μg/L). Therefore, nominal concentrations differed from the ones listed in the protocol. This error has no influence on the study results, which depend on the measured concentrations.

5) The authors noted that offspring gender for 2nd generation mysids could not be determined because offspring were still immature at the end of the toxicity test.

6) The authors noted that pretest samples were analyzed along with samples collected on the first day of the definitive toxicity test rather than prior to testing.

7) The percentage of control females producing young could not be determined. This was acknowledged by the authors and attributed to the fact that the OPPTS test guideline does not allow isolation of individual 1st generation female mysids.

8) There was insufficient documentation to verify the 14 hour light/10 hour dark photoperiod during the 14 days prior to the beginning of the definitive toxicity test, as acknowledged by the authors.

9) The incidence of pathological or histological effects was not reported.

10. MATERIALS AND METHODS

A. Biological System

Guideline Criteria	Reported Information
Species An estuarine shrimp species, preferably *Americamysis bahia*	*Americamysis bahia*
Duration of the Test A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment	28 days (test was terminated after 7 days past the median time of 1st brood release in the control treatment)

Guideline Criteria	Reported Information
Source (or supplier)	T.R. Wilbury Laboratories in-house culture (originally obtained from Aquatic BioSystems, Inc., Fort Collins, CO)
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1) Parental stock was maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids were reported to be free from disease, injuries, and abnormalities during acclimation.
Parental Acclimation Period At least 14 days	14 days
Chamber Location Treatments should be randomly assigned to test chamber locations	Test vessels were randomly arranged in a water bath.
Brood Stock Test started with mysids: 1) from only one brood stock; or 2) from brood stock which has not obtained sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	Test started with mysids from culture that was maintained for 14 days prior to the start of the test under conditions similar to test conditions.
Distribution Minimum of 40 mysids per concentration, with a minimum of 5 concentrations. No more than 8 individuals per replicate group. Once sex of mysids can be determined, there should be at least one female and one male per replicate vessel. If not, one should be obtained from other replicate vessels of the same concentration.	Eight mysids per replicate test vessel, 5 test vessels per concentration for a total of 40/treatment level. The sex of the mysids was determined by day 16, and it was confirmed that there was at least one male and one female per replicate vessel.
Feeding 1) Mysids should be fed live brine shrimp nauplii at least once daily. 2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	1) Mysids were fed newly hatched *Artemia salina* nauplii three times each day. 2) Mysids were fed at the rate of approximately 150 *Artemia salina* nauplii per mysid per day.

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Guideline Criteria	Reported Information
Counts Live adult mysids should be counted daily Live young must be counted and removed daily. Missing or impinged animals should be recorded.	Surviving adult organisms were counted every 24 hours. Young were counted and removed daily, beginning with day 16 (the first day that young were present). Dead organisms were removed when first observed.
Controls A negative control and carrier control (when applicable) are required.	Negative (dilution water) and solvent controls were used.

Comments: Offspring of the 1st generation mysids were counted, and the first eight offspring from each replicate vessel were separated into retention chambers at the same test concentration as the chambers where they originated. If a batch of more than eight offspring was produced, eight were randomly selected. Survival, sublethal effects, length, and the weight (wet and dry) of these 2nd generation mysids were recorded.

B. Physical System

Guideline Criteria	Reported Information
Test Water 1) May be natural (sterilized and filtered) or a commercial mixture. 2) Water must be free of pollutants. 3) During the test, the difference between highest and lowest measured salinities must be less than 10 ‰. Should be measured weekly.	1) Natural seawater collected from Atlantic Ocean near Marblehead, MA, and filtered through 5 and 20 μm filters. 2) The dilution water was measured for various pollutants (e.g., metals, pesticides) twice per year. Measured values and detection limits were provided. No pesticides or PCBs were detected, and the concentrations of inorganics appeared to be well below toxic levels (i.e., below EPA ambient water quality criteria). 3) During the test, the difference between highest and lowest measured salinities was less than 1 ‰. The salinity was measured 455 times throughout the 28 day test period, but the authors did not report the exact frequency for each replicate.

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Guideline Criteria	Reported Information
4) Salinity should be between 15 and 30 ‰. 5) pH should be measured at the beginning, end of test and weekly. 6) DO must be measured in each concentration at least once a week. 7) See details in ASTM E-1191.	4) All salinity measurements were reported as 17 ‰. 5) The pH, which ranged from 7.6 to 8.0, was measured at the beginning and end of the test, as well as every two to three days in each replicate during the test. 6) DO, which ranged from 6.6 to 8.0 mg/L (approximately 80 to 95% saturation), was measured every two to three days in each replicate. 7) Details in OPPTS guideline no. 850.1350 consulted.
Test Temperature 1) Measured daily in one chamber and at least 3 times in all chambers. 2) The test temperature should be 25°C ± 2°C	1) Measured every two to three days in each replicate test vessel (> 3 times) and continuously in a control vessel. 2) The mean temperature for all chambers at test termination was 24.8°C. All measured temperature values were within 0.7°C of the mean temperature.
Photoperiod Recommend 16 light /8 dark, but 14 light/ 10 dark is also acceptable.	14 light /10 dark reported in protocol
Dosing Apparatus 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations should be used. 3) A dilution factor not greater than 0.5 and controls should be used.	1) An Intermittent flow proportional diluter was used. 2) Five toxicant concentrations were used. 3) The dilution factor was approximately 0.5, and controls (solvent and dilution water) were used.
Toxicant Mixing 1) A mixing chamber is recommended but not required. 2) Aeration should not be used for mixing. 3) It must be demonstrated that the test solution is completely mixed before introduction into the test system. 4) Flow splitting accuracy must be within 10%.	1) A mixing chamber was used. 2) Aeration was not used. 3) The mixing system appeared adequate to ensure complete mixing of the test solutions prior to their introduction into the test system; see comments below. 4) Flow splitting accuracy was not always within 10%; see Section 8.

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Guideline Criteria	Reported Information
Test Vessels 1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic **Test Chambers** 1) Most common: 300x450x150 mm deep with solution depth of 100 mm 2) Should be covered **Test Compartments** (within chambers) 1) Size: 250 ml beaker with side cutouts covered with nylon mesh or stainless steel screen, or 2) 90 or 140 mm inside diameter glass Petri dish bottoms with collars made of 200 - 250 µm mesh screen.	Test Vessels: All test chambers and compartments were made of glass. All parts of the diluter that contacted the test substance were made of glass or Teflon. Test Chambers: Loosely-covered, 1-liter glass vessels that contained up to 800 mL of test solution. (A depth of approximately 110 mm was maintained.) Test Compartments: There was no further division of the test chambers into test compartments.
Flow Rate 1) Flow rates should provide 5 to 10 volume additions per 24 hour. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period.	1) 50 exchanges per 24 hours. 2) DO was maintained at or above 80% of saturation (technical reviewer estimates that the lowest DO level, 6.6 mg/L, is approximately equivalent to 80% of saturation at 25°C at sea level). 3) Meter systems were calibrated before and after the study and were checked twice daily during test period.
Aeration 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	1) Dilution water was aerated. 2) Test vessels were not aerated.

Comments: To make the stock solutions, the test substance was liquefied in a 50°C water bath, and a 7,000 mg/L stock solution was prepared by combining 0.6981 grams of the liquified test substance with acetone and adjusting the final volume of solvent to 100 ml. This stock solution was stored in a dark freezer. A series of secondary stock solutions were prepared by combining 10 ml of the 7,000 mg/L solution with 990 ml of triethylene glycol. These secondary 70 mg/L stock solutions were added directly to the dilution water by the toxicant injector of a proportional diluter (0.30 ml stock combined with 3,000 ml of water during each diluter cycle) to produce a final solution nominally of 7.0 µg/L. The concentration of RH-287 was measured in these secondary stock solutions on days 0, 7, 14, 21, and 28. The mean measured concentration in the secondary solution was 5.33 µg/L, indicating a 24% loss of the test

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substance during the initial test solution preparation. This final diluter toxicant solution was mixed by a high shear pump prior to distribution to test vessels.

C. Chemical System

Guideline Criteria	Reported Information
Concentrations 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control, if appropriate. 2) Toxicant concentration must be measured in one tank at each treatment level every week. 3) One concentration must adversely affect a life stage and one concentration must not affect any life stage.	1) Solvent and dilution water controls, plus five nominal concentrations (all replicated): 0.47, 0.91, 1.8, 3.5, 7.0 µg/L. Given the 24% loss of RH-287 in preparation of the secondary stock solution, the corrected nominal concentrations were 0.36, 0.69, 1.4, 2.7, and 5.3 µg/L. 2) Toxicant concentration was measured in samples collected from two replicate test vessels of each treatment level every week. Which two of the five replicates were tested changed from week to week. 3) One concentration adversely affected a life stage and one concentration did not affect any life stage.
4) The measured concentration of the test material of any treatment should be at least 50% of the time-weighted average measured concentration for >10% of the duration of the test. 5) The measured concentration for any treatment level should not be more than 30% higher than the time-weighted average measured concentration for more than 5% of the duration of the test.	4) The measured concentration of the test material of all treatments was not less than 50% of the time-weighted average measured concentration for >10% of the duration of the test. 5) The measured concentrations for all samples for all treatments, except for one sample of one replicate at the treatment level of 0.277 µg/L (mean measured concentration), were less than 30% higher than the time-weighted average measured concentration for that treatment level. A sample from one replicate on day 0 measured 0.366 µg/L. That exceeds the mean (0.277 µg/L) + 30% (0.083 µg/L) limit of 0.360 µg/L by 0.006 µg/L. Thus, the upper limit was exceeded in 10% (i.e., 1/10) of the measurements.

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Guideline Criteria	Reported Information
Solvents 1) Should not exceed 0.1 ml/L in a flow-through system. 2) The following solvents are acceptable: triethylene glycol, methanol, acetone, and ethanol.	1) 0.1 ml/L 2) Solvent: acetone:triethylene glycol (1:99)

Comments: A range-finding test was conducted prior to the definitive test. Nominal concentrations for this test were 0 (control and solvent control), 0.70, 1.5, 2.4, 5.0, and 10 µg/L. After 17 days of exposure, there was at least 80% survival at 0 µg/L, 100% survival at 0.70 and 1.5 µg/L, 90% survival at 2.4 and 5.0 µg/L, and 0% survival at 10 µg/L. In the range-finding test, no surviving mysid exhibited sublethal effects.

11. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Guideline Deviations above)
Controls 1) Survival of the 1st generation mysids in the controls (between pairing and test termination) must not be less than 70%. 2) At least 75% of the paired 1st generation females in the controls produced young, or 3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	1) Survival of the 1st generation mysids from day 0 in the controls was 83% for the dilution water control and 80% for the solvent control. 2) Not reported (and not possible to calculate from the reported raw data because several females were included in each test vessel). 3) The average number of young produced per 1st generation female was reported to be 4.7 for the dilution water control and 5.3 for the solvent control.

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Guideline Criteria	Reported Information
Data Endpoints Must include: 1) Survival of 1st generation mysids (both male and female) 2) Number of live young produced per female 3) Dry weight of each 1st generation mysid alive at the end of the test (both male and female) 4) Length of each 1st generation mysid alive at the end of the study (both male and female) 5) Incidence of pathological or histological effects 6) Observations of other effects or clinical signs	Data endpoints included: 1) Survival of 1st generation mysids for each test concentration and control 2) Number of live young produced per female after 28 days of exposure 3) Dry weight of each 1st generation mysid alive at the end of the test (both male and female) 4) Length of each 1st generation mysid alive at the end of the study (both male and female) 5) The incidence of pathological or histological effects was not reported. 6) Sublethal effects data for 1st generation mysids
Raw data included? (Y/N) At a minimum, individual data should be included for: 1) Surviving 1st generation male and female mysids. 2) Number of live young produced per female. 3) Individual length measurements of male and female mysids. 4) Individual dry weight measurements for male and female mysids at the end of the test.	Raw data were included for: 1) Surviving 1st generation male and female mysids: (a) number of surviving mysids (out of 40) in each replicate; and (b) number of surviving female mysids (out of 8) for each replicate vessel. 2) Number of live young produced per female. 3) Individual length measurements of male and female mysids. 4) Individual dry weight measurements for male and female mysids at the end of the test. 5) Individual blotted wet weight of each 1st generation mysid alive at the end of the test (both male and female) 6) Sublethal effects.

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A. Effects Data

Toxicant concentration (µg/L)		Number of live young produced per female by day 28	% Survival (28 days)	Mean Total Length (mm)		Mean Dry Weight (mg)	
Corrected Nominal[1]	Mean Measured		♂ & ♀	♂	♀	♂	♀
Control	ND[2]	4.7	83	10.0	9.2	0.56	0.71
Solvent control	ND[2]	5.3	80	11.3	10.7	0.53	0.62
0.36	0.227	4.7	83	11.6	10.3	0.56	0.74
0.69	0.627	5.1	68	11.7	10.7	0.57	0.75
1.4	1.24	4.5	53	11.7	10.1	0.55	0.71
2.7	2.39	1.6	55	10.5	10.8	0.52	0.78
5.3	4.97	0	5	9.2	N/A	0.35	N/A

1 Authors reported a 24% loss of test substance in the secondary stock solution prepared in dilution water by the proportional diluter.

2 None detected at or above the limit of quantitation of 0.0322 µg/L.

Toxicity Observations: Mysids were observed for sublethal effects (i.e., loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) at least three times per week (once every 24 hours once gender could be determined). Sublethal effects were observed in the 1st generation only in replicates of the highest mean measured concentration (4.97 µg/L) beginning at day 17.

B. Statistical Results

Statistical Method: Control and solvent data were compared with a parametric "t-test," and in all cases, except the length of female mysids on the first day of gender determination, no significant differences were observed at the 95% confidence interval. The negative control and solvent control data were pooled prior to subsequent statistical analysis. A chi-square test was used to determine if data were normally distributed. Bartlett's test was used to determine if variances were homogeneous. For all data but the survival of 2nd generation mysids, the data were normally distributed and the variances homogeneous. For all data except the sruvival of 2nd generation mysids, a one-way analysis of variance (ANOVA) and Bonferroni's test were used to compare treatment data to [pooled] control and solvent control data. Data for the survival of 2nd generation mysids were analyzed using a nonparametric William's test. All calculations were performed using mean measured concentrations of the active ingredient. Data from the highest treatment group were assumed to be different from the pooled control

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data because no females survived and no offspring were produced. Those data, therefore, were not included in statistical analyses of female survival, production of young per female, second generation survival, length, or weight data. Male survival occurred in only one replicate of the highest treatment, and therefore data from this treatment could not be included in the length and weight data for statistical analysis. The LC_{50}s were calculated using the binomial/nonlinear interpolation method. The 7-day LC_{50} could not be calculated because survival at all tested concentrations exceeded 50%. Only the endpoints specified by the EPA/OPP pesticide assessment guidelines are listed below.

Endpoint	Method	NOAEC (µg ai/L)	LOAEC (µg ai/L)	MATC (µg ai/L)
Survival	One-way ANOVA and Bonferroni's test	0.627	1.24	0.882
Reproduction (number of young per female)	One-way ANOVA and Bonferroni's test	1.24	2.39	1.72
Dry Weight (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Dry Weight (female)		2.39	4.97	3.45
Length (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Length (female)		2.39	4.97	3.45

Most sensitive endpoint: Survival of 1st generation mysids

Comments: The 28-day LC_{50} (95% confidence interval) calculated by the probit method was 2.5 µg/L (0.627 to 4.97 µg/L). The authors also reported EC_{50} values, and corresponding 95% confidence intervals, for days 7, 14, and 21 using the binomial method. The 7-day LC_{50} was greater than 4.97 µg/L. The 14-day LC_{50} was 4.1 µg/L (2.39 to 4.97 µg/L). The 21-day LC_{50} was 3.4 µg/L (2.39 to 4.97 µg/L).

12. REVIEWER'S STATISTICAL RESULTS

Control and solvent data were compared with the parametric "t-test" and in all cases, except the length of female mysids on the first day of gender determination, no significant differences were observed with $\alpha = 0.05$. A chi-square test and Shapiro-Wilk's test were used to determine if data were not distributed normally. Bartlett's test was used to determine if variances were not homogeneous. For all data but the survival of 2nd generation mysids, the null hypotheses of normally distributed and homogeneous variances could not be rejected at the $\alpha = 0.05$ level. We did not analyze the survival of the 2nd generation mysids because that endpoint is not specified by the EPA OPP pesticide assessment guidelines and because it was not the most sensitive endpoint. For the other data, we used a one-way ANOVA and Bonferroni's test to

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identify the NOAEL and LOAEL, excluding data from the highest treatment level for the same reasons the authors excluded those data. We did not estimate the 14-day, 21-day, or 28-day LC_{50} values because those are not specified by the EPA OPP pesticide assessment guidelines. We identified the same NOAEL and LOAEL values as did the authors (see table below).

Endpoint	Method	NOAEC (µg ai/L)	LOAEC (µg ai/L)	MATC (µg ai/L)
Survival	One-way ANOVA and Bonferroni's test	0.627	1.24	0.882
Reproduction (number of young per female)	One-way ANOVA and Bonferroni's test	1.24	2.39	1.72
Dry Weight (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Dry Weight (female)		2.39	4.97	3.45
Length (male)	One-way ANOVA and Bonferroni's test	2.39	4.97	3.45
Length (female)		2.39	4.97	3.45

Most sensitive endpoint: Survival of 1st generation mysids.

Comments: We were not able to calculate the same value for the number of live young produced per female by day 28 as estimated by the study authors, although we tried two methods. The difficulty with the estimate is that the number of females alive each day decreased in some of the replicates in some of the treatment levels. We estimated the total number of female-days per replicate and divided by the number of days to estimate an "average" number of females alive to compare against the total number of offspring produced by day 28. Our "time-weighted" average total number of offspring per female are listed in the table below. We also estimated male survivorship by subtracting from total number of mysids surviving in a given replicate on a given day the number of female mysids surviving in that replicate on that day. Finally, we estimated 1st generation male and female mysid survival using the number alive on day 16 as the starting point (sex could not be determined before day 14). Although our calculations of average number of live young produced per female differed from the authors, the difference is slight and does not change the conclusions of this study. Our analysis of male and female survival after day 16 indicates a NOAEL for both sexes combined of 0.627 µg/L, and survivorship decreased with increasing dose for the females, but not for the males. We cannot determine if the unusual dose-response pattern exhibited by the male mysids is cause for concern with this study.

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Toxicant concentration (µg/L)		Number of live young produced per female by Day 28		Percent Survival by Day 28 (Survivors/Total Number)			
Corrected Nominal[1]	Mean Measured	Estimated by Study Authors	Estimated by Reviewer[3]	♂ & ♀[4] from Day 1	♂ from Day 16	♀ from Day 16	♂ & ♀ from Day 16
Control	ND[2]	4.7	4.9	83 (33/40)	81 (21/26)	92 (12/13)	85 (33/39)
Solvent control	ND[2]	5.3	5.4	80 (32/40)	76 (19/25)	87 (13/15)	80 (32/40)
0.36	0.227	4.7	4.7	83 (33/40)	90 (19/21)	88 (14/16)	89 (33/37)
0.69	0.627	5.1	4.8	68 (27/40)	58 (14/24)	81 (13/16)	43 (27/40)
1.4	1.24	4.5	4.4	53 (21/40)	54 (13/24)	53 (8/15)	54 (21/39)
2.7	2.39	1.6	1.9	55 (22/40)	75 (15/20)	37 (7/19)	56 (22/39)
5.3	4.97	0	0	5 (2/40)	17 (2/12)	0 (0/0)	17 (2/12)

1 Authors reported a 24% loss of test substance in the secondary stock solution prepared in dilution water by the proportional diluter.
2 None detected at or above the limit of quantitation of 0.0322 µg/L.
3 Estimated from daily average number of live young produced per female (i.e., number of young produced in treatment group on a given day/total number of females alive that day).
4 Calculated by subtracting number of females alive (Table A.3 of the report) from total number alive of both sexes (Table A.1 of the report) each day.

13. REVIEWER'S COMMENTS

The study authors reported that no insoluble material was observed in any vessel during the test. Mean measured concentrations among replicates varied by less than 20%. Mean measured concentrations of the test substance ranged from 68% to 71% of the RH-287 nominal concentrations in the four highest concentrations and 60% of the nominal RH-287 concentration in the lowest treatment concentration. After correcting the nominal concentrations for the 24% loss of RH-287 during preparation of the secondary stock solution, the mean measured concentrations of RH-287 in the test vessels ranged from 78 to 93% of nominal. However, concentrations in the test vessels of the two lowest exposure concentrations appeared to decline by approximately 20 to 25% over the 28-day period, falling around 6-7% each week (Table C.1). There is no obvious reason for that decline. Given that the decline is moderate and the concentrations were measured adequately, the decline is not a serious concern.

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