Accession No. 408563-05

DATA EVALUATION RECORD

2/23/1989

1. **CHEMICAL:** ^{3}H-Avermectin B_1
Shaughnessey No. 122804

2. **TEST MATERIAL:** ^{3}H-Avermectin B_1; Lot #L-676,863-164L012; ^{3}H-avermectin B_1 was an 11.8:1 mixture of avermectin B_{1a} and avermectin B_{1b}, with the tritium label (at the 5-position) present only in the avermectin B_{1a} fraction.

3. **STUDY TYPE:** Estuarine Organism 96-hour Flow-Through Toxicity Test. Species Tested: Mysid Shrimp (Mysidopsis bahia)

4. **CITATION:** Suprenant D.C. (1988) Acute Toxicity of ^{3}H-Avermectin B_1 to Mysid Shrimp (Mysidopsis bahia) of Different Ages Under Flow-Through Conditions. Prepared by Springborn Life Sciences, Wareham, Massachusetts. Submitted by Merck and Company, Inc., Rahway, New Jersey. Accession No. 408563-05.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Aquatic Toxicologist
Hunter/ESE, Inc.

Signature: Kimberly D. Rhodes
Date: 2/8/89

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 2/23/89

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

Signature: Daniel Rieder 4/14/89
Date: Henry T. Craven

7. **CONCLUSIONS:** This study appears scientifically sound, and fulfills the Guideline requirements for a 96-hour flow-through acute toxicity study for estuarine and marine shrimp. The 96-hour LC50 values, based upon mean measured concentrations of ^{3}H-Avermectin B_1, of each age group of Mysidopsis bahia (≤ 1, 4, 10 and 21 days old) exposed were 20, 24, 32, and 33 ng/L, respectively. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to the mysid. The NOEC value for all age groups tested, was 1.3 ng/L.

ng/L = parts per trillion

8. **RECOMMENDATIONS:** N/A

CORE ?

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Each specific age group of mysids (i.e., ≤ 1-, 4-, 10-, and 21-day-old) used in this toxicity test were cultured and acclimated at the testing facility. Prior to testing, mysids were maintained in natural filtered seawater under recirculating conditions. Mysids were fed brine shrimp nauplii two times daily and Hatchfry Encapsulon^{R} three times weekly. The mysid culture area received a regulated photoperiod of 16-hour light and 8-hour darkness. Commercial aquarium heaters were used to maintain the culture solution temperatures at 25 ± 1°C.

B. Test System: The test was conducted using an exposure system consisting of a modified continuous-flow (Benoit, 1982) proportional diluter, a temperature controlled water bath, and a set of 16 test aquaria. The test system was designed to provide six concentrations of test material, a dilution water (seawater) control and solvent control. The solvent control solution contained the maximum amount of acetone present in any test concentration (19 uL/L). All treatment levels and the controls were maintained in duplicate. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a self-starting siphon attached to a system drain. Four mysid retention chambers, constructed from glass petri dishes and nylon screen (363-um mesh size opening), were positioned in each aquarium. This system allowed the aquarium volume to fluctuate between 3.1 and 7.0 L. The flow rate of exposure solutions to each test aquaria was equivalent to 11 volume additions per 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test solution temperature at 25 ± 1°C.

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

D. Design: Selection of ^{3}H-Avermectin B_1 concentrations for the 96-hour acute toxicity test with mysid shrimp was based on preliminary exposures of M. bahia to ^{3}H-Avermectin B_1. The test was initiated when 20 (≤ 1-, 4-, 10-, and 21-day old) mysid shrimp, were randomly distributed to each concentration or control (10 mysids per replicate). A control, solvent control and six nominal ^{3}H-Avermectin B_1 concentrations of 2.6, 6.4, 16,

40, 100, and 250 ng/L were tested. All concentrations were observed once every 24 hours for mortality and abnormal effects. The water quality parameters (dissolved oxygen, pH, salinity, and temperature) were measured and recorded daily for each replicate of the control solutions and each treatment level. Test solution temperature was continuously monitored in one replicate of the solvent control solution throughout the study. Analytical determination of ^{3}H-Avermectin B_1 was performed on all treatment levels at 0 and 96 hours using radiometric analysis.

E. Statistics: The mean measured concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period. LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities. If at least one test concentration caused mortality of greater than or equal to 50 % of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

12. REPORTED RESULTS: "The mean measured test concentrations, the corresponding mortalities and the observations made during the 96-hour test are presented in Table 3 (attached)." Analytical determination of test concentrations resulted in mean measured concentrations of 1.3, 4.3, 11, 21, 52, and 98 ng/L. The mean measured concentrations were 39 to 69% of the nominal concentrations. "After 96 hours, 100% mortality was observed among all age groups (i.e., $\leq$ 1-, 4-, 10-, and 21-days old) of mysids exposed to the highest mean measured concentration (98 ng/L) of ^{3}H-avermectin B_1 tested. Some mortality was recorded at the next three treatment levels for most age groups and no mortality (all age groups) was observed in the lowest test concentration, 1.3 ng/L. The control mortality for all test organisms was $\leq$ 5% for the duration of the 96-hour test." The 96-hour LC50 values, based on mean measured concentrations, for each age group of mysids ($\leq$ 1-, 4-, 10-, and 21-day-old) exposed are 20, 23, 26, and 26 ng/L, respectively. "The 96-hour LC50 values established that the test organisms of various age groups are similar in sensitivity after 96 hours of exposure to ^{3}H-avermectin B_1. At the earlier time intervals (i.e., 48 and 72 hours), however, the LC50 values suggest that the younger organisms may be more sensitive to the test material. The No Observed Effect Concentration (NOEC) for all age groups of mysid shrimp exposed to ^{3}H-avermectin B_1 was 1.3 ng/L. Based on criteria established by U.S. EPA (1985), ^{3}H-avermectin B_1

would be classified as very highly toxic to each of the age groups of mysid shrimp tested."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC50 values, based on mean measured concentrations, for each age group of mysids (≤ 1-, 4-, 10-, and 21-day-old) exposed were estimated to be 20, 23, 26, and 26 ng/L, respectively. The No Observed Effect Concentration (NOEC) through 96 hours was 1.3 ng/L for each age group of mysids exposed.

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

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

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

o The SEP states that natural or reconstituted seawater of 10 to 17 °/oo salinity should be used when testing euryhaline shrimp species. The natural seawater used during the toxicity study had a salinity of 30 - 31 °/oo.

o The SEP states that most shrimp are to be tested at 22°C and the actual measured temperature should not deviate more than 1°C during the test. During this study, the test temperature ranged from 24 - 26°C.

o Test concentrations were prepared in a 40 percent dilution series; the SEP states that each concentration should be at least 60 percent of the next highest concentration.

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

o The active ingredient of the test substance was not reported. However, the information obtained from the EEB indicated that the active ingredient was 99%.

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

B. Statistical Analysis: The reviewer used the Toxanal computer program to calculate the LC50 values. These calculations are attached. The 96-hour LC50 value for mysids ≤ 24-hours old was calculated by the moving average method to be 20 ng/L with a 95 percent confidence interval of 15 to 27 ng/L which is the same as reported by the author. The 96-hour LC50 value for 4-day old mysids was calculated by the moving average method to be 24 ng/L with a 95 percent confidence interval of 17 to 33 ng/L which is similar to that reported by the author. The 96-hour LC50 value for 10-day old mysids was calculated by the moving average method to be 32 ng/L with a 95 percent confidence interval of 23 to 49 ng/L which is higher than that reported by the author. The 96-hour LC50 value for 21-day old mysids was calculated by the moving average method to be 33 ng/L with a 95 percent confidence interval of 24 to 48 ng/L which is higher than that reported by the author.

C. Discussion/Results: The study results appear to be scientifically valid. The 96-hour LC50 values, based upon mean measured concentrations, for each age group of mysids (≤ 1-, 4-, 10-, and 21-days old) exposed were estimated to be 20, 24, 32, and 33 ng/L, respectively. The No Observed Effect Concentration (NOEC) through 96 hours was 1.3 ng/L for each age group of mysids exposed. Therefore, ^{3}H-Avermectin B_1 is classified as very highly toxic to each of the age groups of the mysid, Mysidopsis bahia tested.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, 2/8/89.

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_____ The product confidential statement of formula.

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^{3}H-Avermectin B.
96-Hour LC-50 ≤ 1 Day old mysids

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KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	7.536742E-05
52	20	18	90	2.012253E-02
21	20	4	20	.5908966
11	20	4	20	.5908966
4.3	20	3	15	.1288414
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 52 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 30.51339

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	4.506168E-02	19.92877	14.93263	27.32767

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6763403	3.531909	5.898761E-03

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

SLOPE = 2.416815
95 PERCENT CONFIDENCE LIMITS = .4792286 AND 4.354403

LC50 = 22.01686
95 PERCENT CONFIDENCE LIMITS = 5.6996 AND 84.4261

LC10 = 6.565539
95 PERCENT CONFIDENCE LIMITS = 1.811056E-02 AND 13.0399

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^{3}H. Avermectin B$_1$

96-Hour LC50 4-day old mysids

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

KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-09

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	13	65	13.1588
21	20	4	20	.5908966
11	20	4	20	.5908966
4.3	20	4	20	.5908966
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 98 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 38.81503

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	4.714533E-02	23.51604	17.471	33.10598

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.698222	3.327818	9.850562E-03

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

SLOPE = 1.864056
95 PERCENT CONFIDENCE LIMITS = .3064568 AND 3.421655

LC50 = 26.62147
95 PERCENT CONFIDENCE LIMITS = 7.045132 AND 196.597

LC10 = 5.545259
95 PERCENT CONFIDENCE LIMITS = 2.901212E-03 AND 14.5741

3H-Avermectin B1
96-Hour LC50 10-day old mysids

KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS BAHIA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	13	65	13.1588
21	20	3	15	.1288414
11	20	2	10	2.012253E-02
4.3	20	4	20	.5908966
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 98 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 40.19604

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	9.753808E-02	32.41923	23.46331	48.5746

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.047357	4.862193	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 = 1.971362
95 PERCENT CONFIDENCE LIMITS =-4.613889E-02 AND 3.958864

LC50 = 29.80213
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 6.761184
95 PERCENT CONFIDENCE LIMITS = 0 AND 19.6772

3H-Avermectin B1

96-Hour LC50 21-day old mysids

KIMBERLY RHODES 3H-AVERMECTIN B1 MYSIDOPSIS SANTA 02-06-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
98	20	20	100	9.536742E-05
52	20	15	75	2.069473
21	20	5	25	2.069473
11	20	0	0	9.536742E-05
4.3	20	3	15	.1288414
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 52 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 33.04542

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0864313	32.939	24.27973	48.11614

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.081974	5.330023	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 = 2.439478

95 PERCENT CONFIDENCE LIMITS = -9.801793E-02 AND 4.976974

LC50 = 28.07993

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 8.468232

95 PERCENT CONFIDENCE LIMITS = 0 AND 22.06503

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