US EPA ARCHIVE DOCUMENT
1. **CHEMICAL:** $^3$H-Avermectin B$_1$
   Shaughnessey No. 122804

2. **TEST MATERIAL:** $^3$H-Avermectin B$_1$; Lot # 87-20014525-131,
   [H]L-676,863-164L011, 1022 uCi/mg; $^3$H-avermectin B$_1$ was a
   13.0:1 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; Chemical and radiochemical purities
   of $^3$H-Avermectin B$_1$ was >99%.

3. **STUDY TYPE:** 28-Day Chronic Toxicity Test.
   Species Tested: Mysid Shrimp (*Mysisipsis bahia*).

4. **CITATION:** Suprenant D.C. (1988) Chronic Toxicity of $^3$H-
   Avermectin B$_1$ to Mysid Shrimp (*Mysisipsis bahia*). Prepared
   by Springborn Life Sciences, Wareham, Massachusetts.
   Accession No. 408563-06.

5. **REVIEWED BY:**
   Kimberly D. Rhodes
   Aquatic Toxicologist
   Hunter/ESE, Inc.

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

6. **APPROVED BY:**
   Prapimpan Kosalwat, Ph.D.
   Staff Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

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

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

7. **CONCLUSIONS:** This study appears scientifically sound but
   does not fulfill the Guideline requirements for a 28-day
   chronic toxicity test for estuarine and marine shrimp. The
   MATC of $^3$H-avermectin B$_1$ for mysid shrimp was estimated to
   be $\geq 3.5$ ng/L and $\leq 9.3$ ng/L (Geometric Mean MATC = 5.7
   ng/L).

8. **RECOMMENDATIONS:** N/A

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

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile (≤ 24-hours old) mysids (Mysidopsis bahia) used in this test were cultured and acclimated at the testing facility. Prior to testing, mysids were maintained in natural filtered seawater at conditions compatible with those in the test, i.e., a salinity of approximately 30 ‰, and a temperature of 25 degrees Celsius (°C). Mysids were fed live brine shrimp nauplii supplemented with SelcoR twice daily and Hatchfry EncapsulonR, a high protein supplement, three times weekly. The mysid culture area received a regulated photoperiod of 16-hours light and 8-hours 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 Mount and Brungs (1967) proportional diluter, a temperature controlled water bath, and a set of 14 test aquaria. The test system was designed to provide five concentrations of test material, a dilution water (seawater) control and solvent control. The solvent control solution was maintained at 5.7 μL of acetone per liter of solution which was equal to the solvent concentration in the highest treatment level. Mysid retention chambers, constructed from glass petri dishes and nylon screen (363-μm mesh size opening), were used to maintain the mysids during the initial phase (17 days) of the chronic exposure. Cylindrical glass isolation jars containing two 1.9-cm holes covered with nylon screens were used after day 17. The flow rate of exposure solutions to each test aquarium was approximately equivalent to 7 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: 28-day chronic flow-through test.

D. Design: Selection of ³H-Avermectin B₁ concentrations for the 28-day chronic toxicity test with mysid shrimp was based on preliminary exposures of M. bahia to ³H-Avermectin B₁. The test was initiated when 60 (≤ 24 hours old) mysid shrimp, were randomly distributed to each concentration or control (30 mysids per replicate). A control, solvent control and five nominal ³H-Avermectin B₁ concentrations of 0.5, 1.0, 2.0, 4.0, and
8.0 ng/L were tested. When mysids had reached sexual maturity (day 17), they were redistributed within the test aquaria. Mature male/female pairs within each exposure aquaria were transferred from the retention chambers to ten glass isolation jars. The remaining mysids (after isolation of male/female pairs) were pooled and placed in a clean retention chamber within each aquarium where they were maintained for the duration of the chronic test at appropriate test concentrations. Male mysids from this pool were used to replace dead males from the paired (male/female) isolation jars. Females which died in the isolation jars were not replaced. During the first 16 days of the test, the number of dead organisms and any unusual appearance or behavior were recorded daily. After males and females had been paired on day 17, the number of dead males and females, the number of offspring produced by each individual female, and the appearance and abnormal behavior, if observed, of the adult mysids were recorded daily. At test termination, all mysids were separated into male and female groups for each replicate exposure system and were transferred into aluminum pans and dried for approximately 24 hours at 60°C and then cooled in a desiccator. Individual body weights were recorded separately for each replicate of each concentration and the controls. Salinity and temperature were measured daily in the dilution water control. Dissolved oxygen concentration and pH were measured daily in each replicate of each treatment level and the controls throughout the 28-day exposure. Solution temperature was continuously monitored throughout the study in one replicate of the solvent control. Analytical determination of 3H-Avermectin B1 was performed on all treatment levels at test initiation and once weekly thereafter.

E. Statistics: All control and solvent data for each of the measured endpoints were compared for significant difference by analysis of variance (ANOVA). There were no differences between survival and growth of the two control groups, however a significant difference was detected between the reproductive success of the two control groups. The Chi-Square Goodness of Fit Test (Horning and Weber, 1985) was conducted and compared the observed sample distribution with a normal distribution. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data for each endpoint were analyzed using Bartlett's Test (Horning and Weber, 1985). The performance at each dose level of 3H-avermectin B1 was compared with the performance of the solvent control using the William's Test (Williams, 1971, 1972), the Dunnett's Test (Dunnett, 1955, 1964),
or the Kruskal-Wallis Test (Zar, 1985; Sokal and Rohlf, 1981).

The Maximum Allowable Toxicant Concentration (MATC) was calculated by taking the geometric mean of the limits set by the lowest test concentration that showed a statistically significant effect (Lowest Observed Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant difference from the control (No Observed Effect Concentration, NOEC).

12. **REPORTED RESULTS:** "A summary of the percent survival of adult mysids at the termination of the life cycle test is presented in Table 3 (attached). At test termination mysid survival at the highest mean measured test concentration was 10%, which was significantly less than the survival of the solvent control organisms (90%). Survival among mysids exposed to the remaining lower treatment levels (3.5 to 0.35 ng/L) ranged from 75 to 90% which was statistically comparable to the survival of the solvent control organisms." No concentration-related effects on organism survival were observed in the remaining four mean measured concentrations (3.5, 1.4, 0.76, and 0.35 ng $^3$H-avermectin $B_1$/L).

"Comparison of the organism growth data (i.e. female and male dry weight), determined at test termination, established that the growth of mysids was not adversely affected at test concentrations lower than the level (9.3 ng/L) which adversely affected organism survival. Since the percentage survival of mysids exposed to the highest concentration of $^3$H-avermectin $B_1$/L tested (9.3 ng/L) was significantly affected, the growth data for this concentration was not statistically analyzed."

"Mysid reproduction expressed as cumulative number of offspring per female organism per reproductive day ranged from 0.30 to 0.63 at all treatment levels and was statistically comparable to the number of offspring released by the solvent control mysids (0.55)."

"It was established that the adverse effect on survival was the most sensitive indicator of toxicity of $^3$H-avermectin $B_1$ for mysid shrimp. Based on these data, the MATC of $^3$H-avermectin $B_1$ for mysid shrimp was estimated to be ≤ 9.3 ng/L and ≥ 3.5 ng/L (Geometric Mean MATC = 5.7 ng/L)."

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

The MATC of $^3$H-avermectin $B_1$ for mysid shrimp was estimated to be ≤ 9.3 ng/L and ≥ 3.5 ng/L (Geometric Mean MATC = 5.7 ng/L).
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 ASTM procedure as follows:

- ASTM states that during the test each measured salinity should be between 15 and 30 °/oo. During this study, the salinity ranged from 30 – 32 °/oo.

- ASTM states that chronic toxicity tests with *Mysidopsis bahia* should be conducted at 27°C. During this study, the test temperature ranged from 24 – 26°C.

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

- Measurement of total body length was not performed.

**B. Statistical Analysis:** The solvent control data were used to compare the treatment levels. Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. Differences were determined by ANOVA. The reviewer confirmed statistical significance in reduced mysid survival at a mean measured test concentration of 9.3 ng/L.

**C. Discussion/Results:** The study results appear to be scientifically valid, however, statistical analysis could not be conducted on weight and reproductive success since the raw data were not submitted. Based on survival, the MATC of ^3^H-avermectin B₁ for mysid shrimp was estimated to be ≤ 9.3 ng/L and ≥ 3.5 ng/L (Geometric Mean MATC = 5.7 ng/L).

**D. Adequacy of the Study:**

1. **Classification:** Supplemental

2. **Rationale:** Statistical analyses on weight and reproductive success could not be performed due to the lack of raw data.

3. **Repairability:** Yes, submitt raw data on weight and reproductive success.

15. **COMPLETION OF ONE-LINER:** Yes, 2/8/89.
Page 6 is not included in this copy.
Pages ____ through ____ are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients.
___ Identity of product impurities.
___ Description of the product manufacturing process.
___ Description of quality control procedures.
___ Identity of the source of product ingredients.
___ Sales or other commercial/financial information.
___ A draft product label.
___ The product confidential statement of formula.
___ Information about a pending registration action.
___ FIFRA registration data.
___ The document is a duplicate of page(s) ________.
___ The document is not responsive to the request.

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Accession #: 408563-02

Chronic Toxicity of $^3$H-Avermectin B$_1$ to Mysid Shrimp (*Mysidopsis bahia*).

Arcsin $\sqrt{\text{Percentage}}$ Transformation of Survival

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<th>→</th>
<th>90.0</th>
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<tr>
<td></td>
<td>B</td>
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<td>63.44</td>
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<tr>
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<tr>
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<tr>
<td></td>
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Accession #408563-06 M. bahia survival

Summary Statistics and ANOVA

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<td>59.2</td>
</tr>
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</table>

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

Minimum detectable difference for Dunnett's test = -30.224892
This difference corresponds to -39.40 percent of control

Between groups sum of squares = 4625.750800 with 5 degrees of freedom.
Error mean square = 114.066117 with 6 degrees of freedom.

******************************************************************************
* Warning - the test for equality of variances *
* could not be computed as 1 or more of the *
* variances is zero. *
******************************************************************************