



Health and Environmental
Sciences Department

Tert-Amyl Methyl Ether (TAME)— Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*) under Static Renewal Conditions

TSCA Guideline §797.1930

FEBRUARY 1995

TOXICOLOGY REPORT NUMBER 407
CAIS ABSTRACT NO. 42-1520



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ACKNOWLEDGMENTS

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QUALITY ASSURANCE/GLP COMPLIANCE STATEMENT

Study Title: Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to the Mysid Shrimp (*Mysidopsis bahia*) Under Static Renewal Conditions.

Testing Facility: Springborn Laboratories, Inc.

SLI Study Number: 12827.0394.6110.510

This study was reviewed by API Quality Assurance personnel under the direction of API Management on the dates indicated below for compliance with EPA (TSCA) Good Laboratory Practice (GLP) regulations. These studies were conducted in accordance with EPA GLP regulations, with the exceptions* listed below, as well as those listed on the Springborn GLP Compliance Statement.

Copies of reports by API Quality Assurance personnel are available upon written request to the Director of the Health and Environmental Sciences Department of the American Petroleum Institute or his designee.

<u>Date(s) of Inspection/Review</u>	<u>Type of Inspection</u>	<u>Date of Report to Management</u>
8/28/92	Protocol Evaluation	8/28/92
4/25/94	Second Protocol Review	4/25/94
4/27-28/94	In-Life Inspection and Data Audit	5/2/94
6/13/94	Draft Report Audit	6/14/94
10/15/94	Revised Final Draft Report Review	10/15/94
12/21/94	Final Report Review	12/21/94
12/30/94	Amended Final Report Review and Acceptance	12/30/94
<i>Christine Sexsmith</i> Christine Sexsmith Quality Assurance Coordinator	<u>12/30/94</u> Date	

*Test article characterization, other than that received by the manufacturer (98.8% pure TAME) was not performed, storage stability for this test article is not known, and the method of fabrication is maintained with the test article supplier.

Springborn Laboratories, Inc.

Environmental Sciences Division

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AMENDMENT TO THE STUDY FINAL REPORT

Final Report Title: Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Mysid Shrimp
(*Mysidopsis bahia*) Under Static Renewal Conditions

Amendment No. 1 **Amendment Date:** 29 December 1994

SLI Study No. 12827.0394.6110.510

SLI Report No. 94-5-5269

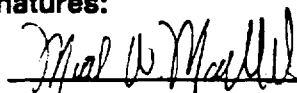
Study Sponsor: American Petroleum Institute

Study Director: Mark W. Machado

Final Report Modifications and / or Additions:

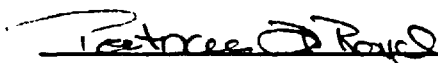
Page 8: The date of Protocol Amendment #1 was changed to the corrected date of the amendment

Page 40-41: The corrected version of Protocol Amendment #1 was included on these pages

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12/29/94
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**TERT-AMYL METHYL ETHER (TAME) -
ACUTE TOXICITY TO MYSID SHRIMP
(*Mysidopsis bahia*) UNDER STATIC
RENEWAL CONDITIONS**

TSCA Guideline § 797.1930

Submitted to:

**American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005**

SLI Report #94-5-5269

SLI Study #12827.0394.6110.510

Study Director: Mark W. Machado

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Environmental Sciences Division
790 Main Street
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29 December 1994

AMENDED FINAL REPORT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*) Under Static Renewal Conditions" were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 792) with the following exception: routine water and food contaminant screening analyses for pesticides, PCBs and toxic metals. These analyses were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, Pennsylvania. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Storage stability, characterization and verification of the test substance identity and maintenance of these records on the test substance are the responsibility of the Study Sponsor. Total organic carbon analyses for filtered seawater conducted by Galbraith Laboratories, Knoxville, Tennessee, utilized standard U.S. EPA procedures, but were not conducted in accordance with Good Laboratory Practice procedures. At the termination of the testing program, all remaining test substance will be sent to the Study Sponsor. Archival of a sample of the test substance is the responsibility of the Study Sponsor.

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 12/29/94

Mark W. Machado Date
Study Director

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SUMMARY

The purpose of this study was to estimate the acute toxicity (LC50) of Tert-Amyl Methyl Ether (TAME) to mysid shrimp (*Mysidopsis bahia*) under static renewal conditions. The LC50 is defined as the concentration of the test substance in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. Twenty organisms (ten per replicate) were exposed in duplicate test vessels to each of six concentrations of TAME and a dilution water control for 96-hours. During the test, nominal concentrations of 1.6, 4.0, 7.3, 15, 30 and 60 mg A.I./L were maintained by renewing solutions at 24-, 48- and 72-hours of exposure. Each replicate solution was sampled and analyzed for TAME concentration at 0-hour (test initiation) and 96-hours (test termination) of exposure. Due to the variability between replicates and sampling intervals, the analytical results obtained for the lowest treatment level will not be reported. Based on the results of these analyses, the mean measured exposure concentrations were defined as 5.0, 9.5, 19, 35 and 65 mg A.I./L. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and every 24 hours thereafter until the test was terminated. Throughout the exposure period, treatment level solutions were observed to be clear and colorless and contained no visible sign of undissolved test substance (e.g., precipitate).

At test termination (96-hours), 100% mortality was observed among mysids exposed to the highest mean measured concentration tested (65 mg A.I./L). Mortality of 60 and 95% was observed among mysids exposed to the 19 and 35 mg A.I./L treatment levels, respectively, while mortality of 20 and 10% was observed among mysids exposed to the 5.0 and 9.5 mg A.I./L treatment levels, respectively. Sublethal effects (e.g., lethargy, darkened pigmentation) were observed among all of the surviving mysids exposed to the 19 and 35 mg A.I./L treatment level and among several of the surviving mysids exposed to the 5.0 and 9.5 mg A.I./L treatment levels. Mortality of 5% was observed in the lowest treatment level tested (i.e., 1.6 mg A.I./L, nominal) with two of the surviving mysids observed to be lethargic. The LC50 values, 95% confidence intervals and No-Observed-Effect Concentration (NOEC) established during this study are summarized in the following table.

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TEST RESULTS

LC50 (mg A.I./L) ^{a,b}				No-Observed- Effect Concentration Through 96 Hours (mg A.I./L) ^a
24-Hour ^c	48-Hour ^c	72-Hour ^d	96-Hour ^e	
> 65	> 65	18 (13 - 23)	14 (10 - 19)	< 5.0

^a Based on mean measured concentrations of TAME (as active ingredient).

^b Corresponding 95% confidence interval is presented in parentheses.

^c LC50 value empirically estimated as being greater than the highest mean measured concentration tested.

^d LC50 value and 95% confidence interval calculated by probit analysis.

^e LC50 value and 95% confidence interval calculated by moving average angle analysis.

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1.0 INTRODUCTION

The purpose of this study was to estimate the acute toxicity (LC50) of TAME to mysid shrimp (*Mysidopsis bahia*) under static renewal test conditions. The LC50 is defined as the concentration of the test substance in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from release of the test substance into aquatic environments. The study was initiated on 25 April 1994, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the 96-hour definitive test was conducted from 3 to 7 May 1994 at the Environmental Sciences Division of Springborn Laboratories, Inc. (SLI), located in Wareham, Massachusetts. All original raw data and a copy of the final report will be stored with the Study Sponsor. A final report for this study was issued to American Petroleum Institute dated 19 December 1994. This amended final report, 28 December 1994, incorporates changes made as presented in Final Report Amendment #1.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used during this acute toxicity study followed those described in the Springborn protocol entitled "TAME: Acute Toxicity to Mysids (*Mysidopsis bahia*) Under Static-Renewal Conditions, Following TSCA Guideline 797.1930", Springborn Laboratories Protocol #:042594/TSCA/510/TAME (dated 25 April 1994) and Protocol Amendment #1 (dated 17 May 1994) (Appendix I). The methods described in this protocol generally follow the standard procedures described in the U.S. EPA Toxic Substance Control Act (TSCA) Test Guidelines § 797.1930 (U.S. EPA, 1985). Where applicable, Springborn Laboratories, Inc. Standard Operating Procedures (SOP) were followed during the conduct of the study.

2.2 Test Substance

Two samples of Tert-Amyl Methyl Ether (TAME) (CAS # 994-05-8), a clear liquid, were received from Experimental Pathology Labs, Inc., Herndon, Virginia. The first sample,

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Lot # 02814BZ, was received at SLI on 17 August 1992 and was used to prepare analytical standards during the method validation/recovery study and to prepare Quality Control samples during the definitive exposure. The sample was identified by Aldrich Chemical to contain 98.8% active ingredient; A.I. (Certificate of Analysis, Appendix II). The second sample, Lot # 07905KZ, was received at SLI on 2 November 1992 and was used to prepare exposure solutions during the preliminary and definitive exposures. The sample was identified as reagent grade by Aldrich Chemical and contained 98.7% active ingredient (Certificate of Analysis, Appendix II). Upon receipt at SLI, the samples of test substance were stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Test concentrations are expressed as milligrams of test substance (as active ingredient) per liter of test solution and are reported as mg A.I./L.

At the request of the Study Sponsor, mass spectral analysis was conducted on the initial batch of TAME received at program initiation, and the additional batches received throughout the course of the program. The purpose of the mass spectral analysis evaluation was to determine test material integrity throughout the duration of the program. Initial evaluation of test material (i.e., lot # 02814BZ) was conducted on 3 December 1992. Following completion of this flow-through acute toxicity test with mysids, spectral analysis was conducted on 20 July 1994 on each of the remaining two lots (lot # 02814BZ and lot # 07905KZ). The spectral analysis conducted on 20 July 1994 on the two remaining lots in comparison to the initial spectral analyses of lot # 02814BZ established that negligible change in test material composition had occurred during storage at Springborn Laboratories, Inc. (i.e., approximately 24 months).

2.3 Test Organisms

The mysid shrimp (*Mysidopsis bahia*) was selected as the test species since it is a recommended (U.S. EPA, 1975) species and commonly used warm water marine invertebrate in static acute toxicity tests. The mysid shrimp used during this study (SLI Lot #94A28) were produced by broodstock originally obtained from Aquatic Biosystems, Inc., a commercial supplier in Ft. Collins, Colorado, and held at Springborn in a 500-L fiberglass tank under a photoperiod of 16 hours of light and 8 hours of darkness. A closed loop recirculating filtration system provided natural seawater to the holding tank. The seawater was characterized as having a

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salinity ranging from 19 to 21 ‰ and a pH of 7.6. Test organisms were maintained under these conditions for a minimum of 14 days prior to testing. The temperature in the holding tank was 24 °C during this 14-day period. Juvenile mysids, $\leq$ 24 hours old, were collected using a variation of the method described by Reitsema and Neff (1980). The mysids were fed live brine shrimp, *Artemia salina*, nauplii twice daily (Daily Record of Mysid Culture Conditions). Representative samples of the food source were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix III). Food sources were considered to be of acceptable quality since the total concentration of pesticide measured was less than 0.3 mg/kg (ASTM, 1985).

2.4 Test Dilution Water

The dilution water used during this study was from the same source as the water which flowed into the tank used to hold the mysid shrimp. The dilution water was collected from the Cape Cod Canal, Bourne, Massachusetts with a pump (fiberglass reinforced thermoplastic housing) and a polyvinyl chloride (PVC) pipe and was then transported to the laboratory in a 3400-L fiberglass tank. In the laboratory, the seawater was passed through a series of polypropylene core filters (20- and 5-micron) and then recirculated within an epoxy-lined concrete reservoir prior to use. The seawater was pumped to the laboratory under constant pressure through PVC pipe and a polypropylene heat exchanger system. The seawater used during this study had a salinity range of 31 to 33 ‰ and a pH range of 7.9 to 8.0. The salinity of the seawater used in the test was adjusted to 20 ± 3 ‰ by diluting with freshwater. The freshwater is characterized as soft and is a combination of on-site well water and Town of Wareham well water. Representative samples of the seawater as well as the freshwater used to adjust the salinity were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix IV). None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with U.S. EPA and ASTM (1985) standard practices. In addition, representative samples of the seawater as well as the freshwater used were analyzed monthly for total organic carbon (TOC) concentration. These analyses established that the TOC concentration of the seawater and freshwater ranged from 0.84 to 2.9 mg/L and 0.43 to 0.79 mg/L, respectively, for the months of November 1993 to April 1994 (TOC and TSS Master Log, Vol. 1). Several species of mysid shrimp are maintained in water from the same source as the

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dilution waters utilized during this study and have successfully survived and reproduced over several culture generations. The performance of the mysid cultures, in combination with the previously mentioned analyses, confirms the acceptability of this dilution water for use during the conduct of bioassays.

2.5 Test Conditions

The test system was designed to provide six concentrations of TAME and a dilution water control to duplicate test vessels. Test vessels were labeled to identify the nominal test substance concentration and designated replicate. Test vessels were impartially placed in a temperature controlled waterbath designed to maintain solution temperatures at 25 ± 2 °C. A photoperiod of 14 hours light and 10 hours darkness provided light with an intensity of 30 footcandles at the surface of the test solutions was provided by Dura-Test Vita-Lite[®] fluorescent bulbs. Sudden transitions from light to dark and vice versa were avoided.

2.6 Test Concentrations

Selection of nominal TAME concentrations for the 96-hour definitive toxicity test with *Mysidopsis bahia* was based on toxicity information developed at Springborn through preliminary testing. The nominal concentrations chosen were 1.6, 4.0, 7.3, 15, 30 and 60 mg A.I./L.

2.7 Exposure Solution Preparation

The toxicity test was conducted in glass mason jars which contained a total solution volume of 940 mL. The test solution in each vessel had a depth of 16 cm and a surface area of 28.3 cm². Duplicate test vessels were established for each treatment level and the control. Control vessels were established which contained the same dilution water as the exposure concentrations but contained no test substance. Replicate treatment level solutions were prepared individually by volumetric addition of the appropriate amount of test substance directly to the test vessels which were previously filled to 90% capacity with dilution water. The test vessels were then completely filled to the top with dilution water, leaving no headspace, covered with a metal screw-top lid with a Teflon[®] liner to minimize evaporation and loss of test substance due to volatilization and inverted several times ensuring adequate mixing. Test solutions were

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renewed at the 24-, 48- and 72-hour interval. A duplicate set of exposure vessels was established to prepare fresh renewal solutions. Solutions were renewed by carefully siphoning the aged solution out of the test vessel using an inverted glass powder funnel and a length of silastic tubing. The open end of the funnel was covered with 363 micron Nitex® screen attached with silicone adhesive to prevent loss of mysids. Approximately 90% of the aged solution was removed, leaving an adequate amount of solution remaining for surviving mysids. Freshly prepared solution was added to the test vessel in the same manner by siphoning into the test vessel using the funnel.

3.0 TEST PROCEDURES

3.1 Stability and Aqueous Solubility Trials

Prior to initiation of this study, a stability and aqueous solubility evaluation of TAME was conducted at Springborn Laboratories, Inc. The evaluation was conducted in a closed system designed to minimize headspace at a nominal concentration of 600 mg A.I./L and in the presence of test organisms (sheepshead minnow). The test solution was analyzed for TAME concentrations at 0, 24, 48 and 96 hours of exposure.

The average measured concentration of TAME at 0, 24, 48 and 96 hours of the exposure period was 755, 491, 484 and 343 mg A.I./L, respectively. Although the 0-hour recoveries were somewhat higher than anticipated, the results established that under the maintained test conditions, the test material was relatively stable during the initial 48-hours of the exposure period. These results also suggest that the water solubility under these conditions approximate 500 to 600 mg A.I./L. In addition, dissolved oxygen concentration measured during this test indicated that in the presence of test organisms (sheepshead minnow), the dissolved oxygen concentration fell below 60% of saturation following 48 hours.

The above results established that TAME was generally stable for a period of 48 hours under the maintained test conditions and support the premise that it is appropriate to conduct the tests with TAME under static renewal conditions. Since dissolved oxygen concentrations fell below 60% of saturation at 48 hours of exposure, the mysid study was conducted with 24-hour

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renewals to avoid the potential for low dissolved oxygen concentrations during the in-life portion of the study.

3.2 Test Initiation

The test was initiated when ten mysid shrimp ($\leq$ 24 hours old, 20 per treatment level and the control) were impartially selected and distributed to each replicate vessel. Mysids were added two at a time to each replicate aquarium until each aquarium contained 10 mysids. Mysids were fed brine shrimp nauplii (*Artemia salina*) once daily during the exposure period.

3.3 Test Monitoring

Biological observations of the exposed mysid shrimp and observations of the physical characteristics of the test solutions were recorded at test initiation and at each subsequent 24-hour interval until test termination (96 hours). Mortalities were recorded and removed from each test vessel every 24 hours during the exposure period.

3.4 Water Quality Measurements

Dissolved oxygen concentration, pH, salinity and temperature were measured once daily in both replicates of each treatment level and the control. At each renewal interval (i.e., 24, 48 and 72 hours) water quality measurements were taken from both the newly prepared and old test solutions. Salinity was measured with an ATAGO refractometer. The pH was measured with a Jenco Model 601A pH meter and combination electrode; the dissolved oxygen concentration was measured with a Yellow Springs Instrument (YSI) Model #57 dissolved oxygen meter and probe and the daily temperature was measured with a Brooklyn alcohol thermometer. Light intensity was measured with a General Electric type 214 light meter. Continuous temperature monitoring of the surrounding water in the waterbath was also performed using a Fisher Scientific Min/Max thermometer.

3.5 Analytical Measurements

During the definitive exposure period, water samples were removed from each replicate solution of each treatment level and the control at 0 and 96 hours for the analysis of TAME

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concentration. Samples analyzed at the 0-hour sampling interval were removed from the freshly prepared test solutions. Samples analyzed at the 96 hour sampling interval were removed from the aged (24 hour old) exposure solutions. Each exposure solution sample was collected from the approximate midpoint of the test vessel with a volumetric pipet. In addition, three Quality Control (QC) samples were prepared at each sampling interval and remained with the exposure solution samples throughout the analytical process. These QC samples were prepared in dilution water at concentrations of TAME similar to the exposure concentration range. Results of the analyses of the QC samples were used to judge the precision and quality control maintained during the analysis of exposure solution samples. All samples were analyzed for TAME using a gas chromatographic (GC) procedure according to the methodology described in Appendix V. A method validation study conducted at SLI prior to the initiation of the definitive test, established an average recovery of $104 \pm 11\%$ for filtered seawater. Conditions and procedures used throughout the analysis of exposure solution samples and QC samples during this study were similar to those described in Appendix V.

4.0 STATISTICS

The mean measured concentrations tested (based on 0- and 96-hour analyses) and the corresponding biological response (mortality) derived from the definitive toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence limits at each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test substance in dilution water lethal to 50% of the test organism population at the stated exposure interval. If $\leq 50\%$ mortality was observed in any of the concentrations tested, the LC50 value was estimated to be greater than the highest treatment level tested and no statistical analyses were performed. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Stephan, 1982, personal communication) was used to calculate the LC50 values and 95% confidence limits.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence limits calculated by

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binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a mortality of between 0 and 100% of the test organism population. The selection of reported LC50 values and 95% confidence limits was based upon an examination of the data base and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect (mortality) relationship, the number of concentrations causing partial responses, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence limit was selected. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there were no toxicant related mortalities or physical and behavioral abnormalities (e.g., lethargy) with respect to the control organisms.

5.0 RESULTS

Copies of excerpted raw data on the exposure conditions (e.g. water quality, test substance concentration analyses) and the concentration-effect response are presented in Appendix VI.

5.1 Preliminary Test

Prior to initiating the definitive study, several preliminary range-finding tests were conducted at SLI. An initial preliminary study was conducted in which mysid shrimp were exposed under static renewal conditions with zero headspace at nominal TAME concentrations of 0.81, 11, 110 and 500 mg A.I./L and a dilution water control. This study was conducted to evaluate the mysid shrimp age class which is most sensitive to exposure to the test substance. Following 96 hours of exposure, mortality of 0, 10, 40 and 100% was observed among the $\leq$ 24 hour old mysid age class exposed to the 0.81, 11, 110 and 500 mg A.I./L concentrations, respectively. Mortalities ranging from 0 to 10% were observed among the 5 to 6 day old mysid age class exposed to the same nominal concentrations. Based on these results, it was determined that the $\leq$ 24 hour old mysids were the most sensitive age class and would be used in the definitive study.

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An additional preliminary exposure was conducted prior to initiating the definitive study. Mysid shrimp were exposed under static renewal conditions (zero headspace) to nominal TAME concentrations of 0.81, 2.4, 5.7, 13, 32, 81 and 200 mg A.I./L and a dilution water control. Following 48 hours of exposure, 100% mortality was observed in the highest concentration tested (200 mg A.I./L). At test termination (72 hours), mortality of 50 and 100% was observed among organisms exposed to the 32 and 81 mg A.I./L treatment levels, respectively, while mortality ranging from 20 to 40% was observed among mysids exposed to the remaining concentrations tested (0.81 to 13 mg A.I./L). Sublethal effects (e.g., lethargy, darkened pigmentation) were observed among surviving organisms exposed to all of the concentrations tested except for the lowest concentration (0.81 mg A.I./L). Based on these results, nominal concentrations of 1.6, 4.0, 7.3, 15, 30 and 60 mg A.I./L were selected for the definitive test.

Based on the results of preliminary exposures, an initial definitive test was initiated by exposing mysids to nominal TAME concentrations ranging from 16 to 500 mg/L. Results of the analysis of the exposure solutions at 0 hour established measured concentrations which were only approximately 25% of the nominal fortified levels. Additional investigations determined that additional mixing of the solutions prior to addition of the test organisms significantly increased solubility of the test article and increased recoveries. Based on the results of these investigations, the initial definitive test was terminated at 72 hours. Since only approximately 25% of the added material was recovered in the exposure solutions during this initial definitive test, the concentration response observed during this study may have been influenced by undissolved material and therefore, may not accurately define the toxicity of TAME based on the available measured concentrations (i.e., approximately 25% of nominal). Subsequently, preliminary testing was performed using the modified solution preparation procedure (i.e., additional mixing) to establish the exposure concentrations for subsequent definitive testing.

5.2 Definitive Test

5.2.1 Evaluation of Test Conditions - Results of the water quality parameters (pH, dissolved oxygen concentration, temperature, and salinity) measured during the definitive toxicity test are presented in Table 1. All water quality parameters measured were unaffected by the

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concentration of TAME and remained within acceptable ranges for the survival of mysid shrimp. Daily measurement of the temperature in the test solutions established that the exposure solution temperatures ranged from 24 to 25 °C throughout the test period. Continuous temperature monitoring of the surrounding water in the waterbath established that the exposure solution temperatures ranged from 24 to 26 °C throughout the test period.

5.2.2 Analytical Results - The results of the analyses of the exposure solutions for TAME concentration during the exposure period are presented in Table 2. Analysis performed at 0 hour for the 1.6 mg A.I./L (nominal) treatment level resulted in measured concentrations which were variable and inconsistent with percent recoveries established at other treatment levels. An additional set of samples from the 1.6 mg A.I./L treatment level were removed and analyzed after the 24-hour interval of this study in an attempt to reevaluate exposure conditions. Results of these analyses along with the samples analyzed at 96 hours demonstrated considerable variability as compared to the 0-hour analysis for this treatment level. Due to resulting variability between replicate exposure solutions and sampling intervals, a mean measured concentration will not be calculated for this treatment level; all conclusions will be based on the nominal concentration. Mean measured concentrations for the remaining treatment levels averaged 121% of nominal and defined the concentrations as 5.0, 9.5, 19, 35 and 65 mg A.I./L. Figure 1 presents the relationship of the nominal to mean measured concentrations. Analysis of the Quality Control (QC) samples at each sampling interval (0 and 96 hours) resulted in measured concentrations which were consistent with the predetermined recovery range (Appendix V) and averaged 98% (N = 6) of the nominal fortified levels (1.5 to 60.0 mg A.I./L). Based on the results of these analyses, it was determined that the appropriate quality control was maintained during the analyses of the exposure solutions.

5.2.3 Biological Results - The mean measured concentrations tested, the corresponding cumulative percent mortality and the observations made during the definitive exposure are presented in Table 3. At test termination (96-hours), 100% mortality was observed among mysids exposed to the highest mean measured concentration tested (65 mg A.I./L). Mortality of 60 and 95% was observed among mysids exposed to the 19 and 35 mg A.I./L

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treatment levels, respectively, while mortality of 20 to 10% was observed among mysids exposed to the 5.0 to 9.5 mg A.I./L treatment levels, respectively. Sublethal effects (e.g., lethargy, darkened pigmentation) were observed among all of the surviving mysids exposed to the 19 and 35 mg A.I./L treatment level, among several of the surviving mysids exposed to the 5.0 and 9.5 mg A.I./L treatment levels. Mortality of 5% was observed in the lowest treatment level tested (1.6 mg A.I./L, nominal) with two of the surviving mysids observed to be lethargic. Figure 2 presents the 96-hour concentration-response (mortality) curve established for this study. Due to the lack of analytical data to define the exposure concentration in the lowest (nominal) treatment level tested (1.6 mg A.I./L), the NOEC is conservatively estimated as < 5.0 mg A.I./L. Data obtained for the lowest treatment level were also excluded from the calculation of the LC50 value as well.

Table 4 presents the LC50 values, corresponding 95% confidence intervals and the No-Observed-Effect Concentration (NOEC). Based on mean measured concentrations, the 96-hour LC50 value for mysid shrimp exposed to TAME was calculated by moving average angle analysis to be 14 mg A.I./L (95% confidence interval 10 to 19 mg A.I./L).

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PROTOCOL DEVIATIONS

1. The study protocol states that the calculated LC50 will be based on measured concentrations of the six test substance concentrations established during the study.

In this study, six test substance concentrations were established for the definitive study and samples were taken in all replicate test solutions and the negative control at test initiation and termination for analysis of test substance concentration. Results obtained for the lowest treatment level, however, were omitted from the calculation of the LC50 due to the noted variability in measured concentrations, preventing an accurate assessment of the exposure conditions. Since the biological responses at measured concentrations ≤ 9.5 mg/L were very similar, calculation of the LC50 will be unaffected by the elimination of the low treatment level (i.e., 1.6 mg/L, nominal) established in this study.

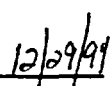
2. The study protocol states that dissolved oxygen concentration will not be allowed to exceed 105% of saturation at any time during the study. In this study, dissolved oxygen concentration exceeded 10% in several test vessels at test initiation, however, the highest dissolved oxygen concentration was 109%.

Reason: The dilution water used in this study are stored in bulk quantity in large holding tanks. It is suspected that the dilution water was maintained and held at temperatures slightly cooler than of test temperature and when warmed to test conditions yielded dissolved oxygen concentrations greater than 100% of saturation.

It is our opinion that these deviations did not affect the results of this study.

SPRINGBORN LABORATORIES, INC.


Mark W. Machado
Study Director


Date

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