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Chemical Category
TERT-AMYL METHYL ETHER (TAME)

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December 30, 1994

Dear FYI Coordinator:

In accordance with API's policy of providing the federal government with copies of research designed to determine whether any chemical substance or mixture manufactured, processed or distributed by API member companies may cause a risk of injury to health or the environment, we are enclosing a copy of the following amended final report:

(Identification no: FYI-94-001056) Tert-Amyl Methyl Ether (TAME): Acute Toxicity to Mysid Shrimp (Mysidopsis bahia). Final Report.

Please note that this information is provided in accordance with the full disclosure policy of API and does not constitute a formal submission as required by a test rule. This document does not contain confidential information. If you have any questions, please communicate with me.

Sincerely,

Robert T. Drew, Ph.D.

An equal opportunity employer

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

TSCA Guideline § 797.1930

Confidential Bus Info

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

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571-1075

29 December 1994

AMENDED FINAL REPORT

11/30/94

American Petroleum Institute
Health and Environmental Sciences Department
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.

Date(s) of Inspection/Review	Type of Inspection	Date of Report to Management
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

Christine Sexsmith
Quality Assurance Coordinator

12/30/94
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.

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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. 94-5-5289

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

Approval Signatures:

Mark W. Machado — 12/29/94
Mark W. Machado — Date
Study Director

Patricia D. Royal — 12/29/94
Patricia D. Royal — Date
Manager, Regulatory Affairs and
Quality Assurance Unit

Springborn Laboratories

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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.

SPRINGBORN LABORATORIES, INC.

Mark W. Machado 12/5/94

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.

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.

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,

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

salinity ranging from 19 to 21 °/oo 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, ≤ 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 °/oo and a pH range of 7.9 to 8.0. The salinity of the seawater used in the test was adjusted to 20 ± 3 °/oo 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

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 cm^2. 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

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

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 (≤ 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

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 ± 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 ≤ 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

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 ≤ 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 ≤ 24 hour old mysids were the most sensitive age class and would be used in the definitive study.

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

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

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).

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 that 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 — Date 12/9/94
Study Director

QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for **"Tert-Amyl Methyl Ether (TAME) - Acute Toxicity To Mysid Shrimp (*Mysidopsis bahia*) Under Static Conditions"** were inspected by the Quality Assurance Unit (QAU) at Springborn Laboratories Inc., Environmental Sciences Division to determine adherence with the study protocol and laboratory standard operating procedures. In addition, inspection of certain phases of the in-life portion of the study was performed. Dates of study inspections, dates reported to the Study Director and to Management are listed below.

Based on these inspections, it was determined that this report accurately reflects the raw data collected during this study.

Inspection Date	Reported to Study Director	Reported to Management
4/27/93	4/27/94	5/6/94
5/5/94	5/5/94	5/6/94
5/11/94	5/11/94	5/20/94
5/12/94	5/12/94	5/20/94
5/16+17/94	5/17/94	5/20/94
5/18/94	5/18/94	5/20/94
6/2/94	6/2/94	6/3/94
9/26+27/94	9/27/94	10/7/94
9/30/94	9/30/94	10/7/94
10/5/94	10/5/94	10/7/94
12/19/94	12/19/94	12/19/94
12/28/94	12/28/94	12/28/94

SPRINGBORN LABORATORIES, INC.

Patricia D. Royal — Date
Manager, Regulatory Affairs
and Quality Assurance Unit

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TABLES

0 0 2 3

Table 1. The water quality parameters measured during the 96-hour static renewal exposure of mysid shrimp (*Mysidopsis bahia*) to TAME.

Nominal Concentration (mg A.I./L)	0 Hour		24 Hour[a]		48 Hour[a]		72 Hour[a]		96 Hour	
	A	B	A	B	A	B	A	B	A	B
					pH					
Control	8.0	8.0	8.0/7.9	7.9/7.9	8.0/8.0	8.0/8.0	8.0/7.9	8.0/7.9	8.0	8.0
1.8	8.0	8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0	8.0
4.0	8.0	8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0	8.0
7.3	8.0	8.0	8.0/7.9	8.0/7.9	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0	8.0
15	8.0	8.0	8.0/8.0	7.9/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	7.9	7.9
30	8.0	8.0	7.9/8.0	7.9/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	7.9	8.0
60	8.0	8.0	7.9/8.0	7.9/8.0	8.0/8.0	8.0/8.0	8.0/8.0	8.0/8.0	7.9	7.9
					Dissolved Oxygen, mg/L (% Saturation)					
Control	7.6 (104)	7.7 (105)	7.1/7.7 (97)/(105)	7.1/7.7 (97)/(105)	7.2/7.6 (99)/(103)	7.2/7.7 (99)/(104)	7.0/7.8 (96)/(105)	7.1/7.8 (97)/(105)	7.2 (99)	7.0 (96)
1.8	7.7 (105)	7.8 (106)	7.0/7.6 (96)/(104)	7.0/7.6 (96)/(104)	7.0/7.4 (96)/(100)	7.1/7.6 (97)/(103)	7.1/7.6 (97)/(103)	7.1/7.8 (97)/(105)	6.8 (93)	7.1 (97)
4.0	7.8 (107)	7.6 (104)	7.2/7.6 (99)/(104)	7.2/7.7 (99)/(105)	7.1/7.5 (97)/(101)	7.1/7.5 (97)/(101)	7.0/7.7 (96)/(104)	7.0/7.7 (96)/(104)	7.0 (96)	7.0 (96)
7.3	7.8 (107)	7.7 (105)	7.2/7.7 (99)/(105)	7.2/7.7 (99)/(105)	7.2/7.4 (99)/(100)	7.1/7.5 (97)/(101)	7.1/7.8 (97)/(105)	7.1/7.8 (97)/(105)	7.1 (97)	7.0 (96)
15	7.7 (105)	7.8 (107)	7.2/7.7 (99)/(105)	7.2/7.6 (99)/(104)	7.1/7.5 (97)/(101)	7.2/7.4 (99)/(100)	7.0/7.8 (96)/(105)	7.2/7.7 (99)/(104)	7.1 (97)	7.2 (99)
30	7.8 (107)	7.8 (107)	7.3/7.6 (100)/(104)	7.3/7.6 (100)/(104)	7.2/7.4 (99)/(100)	7.1/7.4 (97)/(100)	7.1/7.7 (97)/(104)	7.2/7.7 (99)/(104)	7.1 (97)	7.0 (96)
60	7.8 (107)	7.8 (107)	7.3/7.7 (100)/(105)	7.3/7.6 (100)/(104)	7.1/7.4 (97)/(100)	7.2/7.5 (99)/(101)	7.2/7.7 (99)/(104)	7.0/7.8 (96)/(105)	7.1 (97)	7.0 (96)
					Temperature (°C)[b]					
		25		25/25		25/24		25/24		25
					Salinity (‰)[b]					
		22		22/22		22/22		22/22		22

[a] Measurements at renewal intervals (i.e., 24, 48 and 72 hours) are presented as aged/freshly prepared solutions.

[b] Values presented represent the range of daily temperature (Brooklyn Alcohol Thermometer) and salinity measured in all test concentrations and the control at the stated observation interval. Continuous temperature monitoring (Fisher Scientific Min/Max thermometer) established a temperature range of 24 - 26 °C in the surrounding water bath, throughout the exposure period.

Table 2. **Concentrations of TAME measured in the replicate (A,B) test solutions during the 96-hour static renewal exposure of mysid shrimp (*Mysidopsis bahia*).**

Nominal Concentration (mg A.I./L)	0-Hour Measured Concentration[a] (mg A.I./L)		96- Hour Measured Concentration[b] (mg A.I./L)		Mean Measured Concentration[c] (mg A.I./L)
	A	B	A	B	
Control	< 0.50	< 0.50	< 0.13	< 0.13	
1.6	< 0.50	1.3[d]	4.3	3.7	–[e]
4.0	3.9	5.0	5.8	5.4	5.0 (0.8)
7.3	9.5	7.7	10	10	9.5 (1.3)
15	17	17	20	23	19 (3)
30	29	30	39	41	35 (6)
60	65	60	70	66	65 (4)
QC #1[f]	1.21 (1.50)[g]		1.58 (1.50)		
QC #2	32.4 (30.0)		28.6 (30.0)		
QC #3	62.2 (60.0)		58.9 (60.0)		

[a] Samples analyzed represent the freshly prepared exposure solutions.
[b] Samples analyzed represent the aged (24 hour old) exposure solutions.
[c] Mean measured concentrations were calculated using the actual unrounded analytical results and not the rounded (two significant figures) values presented in this table. Standard deviation is shown in parentheses.
[d] Exposure solutions were resampled at the 24-hour interval in order to reevaluate exposure conditions for this treatment level. Resulting measured concentrations were 0.54 and 1.54 mg A.I./L on the A and B replicates, respectively.
[e] Due to the 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 stated nominal concentration.
[f] QC = Quality Control sample.
[g] Value in parentheses represents the nominal fortified concentration for the corresponding QC sample.

Table 3. Mean measured concentrations tested, corresponding cumulative percent mortality and observations made during the 96-hour static acute exposure of mysid shrimp (*Mysidopsis bahia*) to TAME.

Mean Measured Concentration (mg A.I./L)	Cumulative Mortality (%)								
	24-Hour			48-Hour			72-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean
Control	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
1.6[a]	0 (0)	0 (0)	0	0 (0)	0 (0)	0[de]	0 (0)	0 (0)	0[de]
5.0	0 (0)	0 (0)	0	30 (3)	0 (0)	15[fg]	30 (3)	0 (0)	15[fg]
9.5	0 (0)	0 (0)	0	10 (1)	0 (0)	5[h]	10 (1)	10 (1)	10[h]
19	0 (0)	0 (0)	0	20 (2)	30 (3)	25[h]	60 (6)	50 (5)	55[b]
35	0 (0)	0 (0)	0[b]	30 (3)	40 (4)	35[b]	80 (8)	90 (9)	85[b]
65	0 (0)	20 (2)	10[c]	40 (4)	30 (3)	35[i]	90 (9)	90 (9)	90[j]

Mean Measured Concentration (mg A.I./L)	Cumulative Mortality (%)		
	96-Hour		
	A	B	Mean
Control	0 (0)	0 (0)	0
1.6[a]	10 (1)	0 (0)	5[e]
5.0	30 (3)	10 (1)	20[ef]
9.5	10 (1)	10 (1)	10[h]
19	70 (7)	50 (5)	60[b]
35	90 (9)	100 (10)	95[b]
65	100 (10)	100 (10)	100

[a] Mean measured concentration not available for this treatment level (1.6 mg A.I./L, nominal).
[b] All of the surviving mysids were observed to be lethargic.
[c] Several of the surviving mysids were observed to be lethargic and exhibited darkened pigmentation.
[d] One of the surviving mysids exhibited partial loss of equilibrium.
[e] Two of the surviving mysids were observed to be lethargic.
[f] Two of the surviving mysids exhibited darkened pigmentation.
[g] Several of the surviving mysids were observed to be lethargic.
[h] Several of the surviving mysids exhibited darkened pigmentation.
[i] All of the surviving mysids were observed to be lethargic and exhibited darkened pigmentation.
[j] All of the surviving mysids exhibited partial loss of equilibrium.

Table 4. **The LC50 values, corresponding 95% confidence intervals and the No-Observed-Effect Concentration (NOEC) for the 96-hour static renewal toxicity test exposing mysid shrimp (*Mysidopsis bahia*) to TAME.**

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.

FIGURES

Figure 1. **Relationship between mean measured concentrations (analysis at 0- and 96-hours) and the nominal treatment levels established during the 96-hour static renewal toxicity test exposing mysid shrimp (*Mysidopsis bahia*) to TAME.**

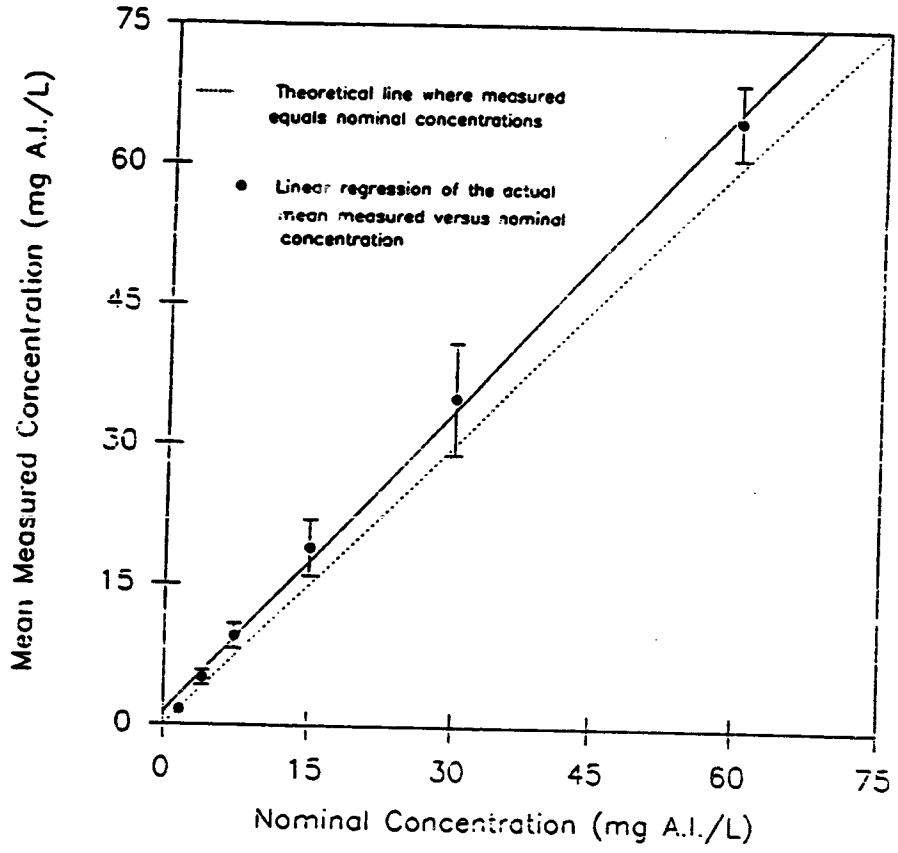
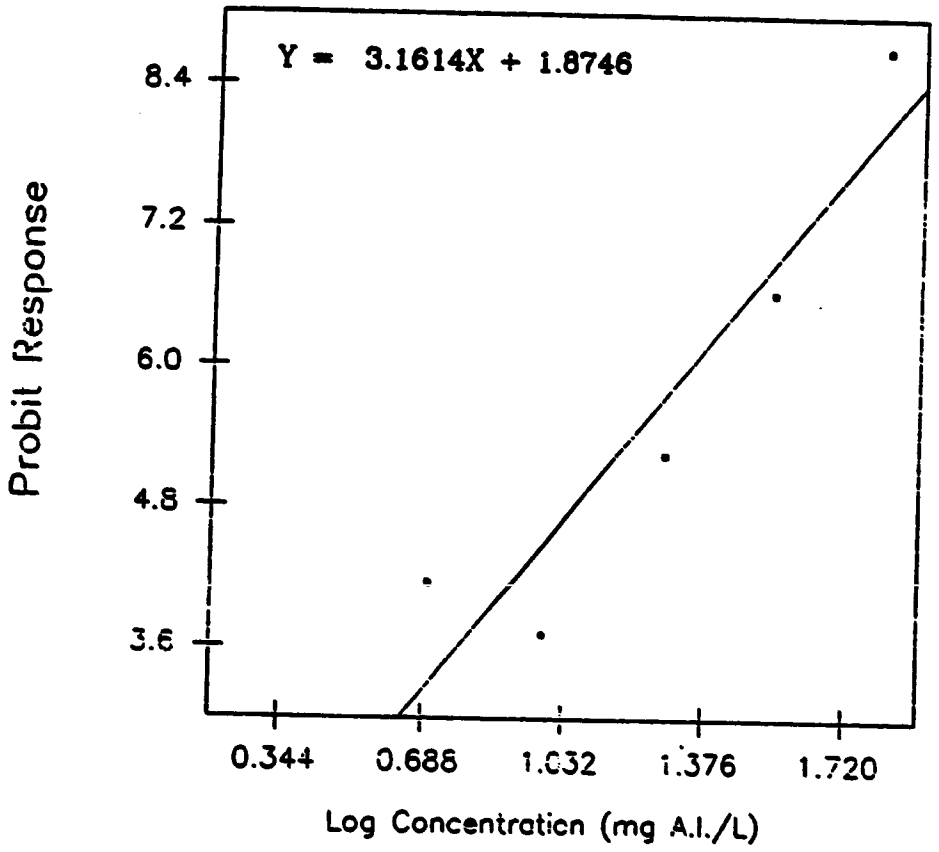


Figure 2. The 96-hour concentration-response (mortality) curve for the static renewal toxicity test exposing mysid shrimp (*Mysidopsis bahia*) to TAME.



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This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

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December 30, 1994

Dear FYI Coordinator:

In accordance with API's policy of providing the federal government with copies of research designed to determine whether any chemical substance or mixture manufactured, processed or distributed by API member companies may cause a risk of injury to health or the environment, we are enclosing a copy of the following final report:

(Identification no: FYI-94-001047) Tert-Amyl Methyl Ether (TAME): Acute Toxicity to Daphnia (Daphnia magna). Final Report.

Please note that this information is provided in accordance with the full disclosure policy of API and does not constitute a formal submission as required by a test rule. This document does not contain confidential information. If you have any questions, please communicate with me.

Sincerely,

Robert T. Drew, Ph.D.

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TERT-AMYL METHYL ETHER (TAME) - ACUTE TOXICITY TO DAPHNIDS (*Daphnia magna*) UNDER FLOW-THROUGH CONDITIONS

TSCA GUIDELINE § 797.1300

Submitted to:

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

SLI Report # 92-12-4545

SLI Study # 12827.0692.6102.115

Study Director: Arthur E. Putt

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19 December 1994

FINAL REPORT

American Petroleum Institute
Health and Environmental Sciences Department

QUALITY ASSURANCE/GLP COMPLIANCE STATEMENT

Study Title: Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Daphnids under Flow-Through Conditions

Testing Facility: Springborn Laboratories, Inc.

SLI Study Number: 12827.0692.6102.115

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.

Date(s) of Inspection/Review	Type of Inspection	Date of Report to Management
8/28/92	Protocol Evaluation	8/28/92
2/16-17/93	Draft Report Audit	2/19/93
	Re-issued	4/29/94
10/15/94	Revised Final Draft Report Acceptance	10/15/94
12/20/94	Final Report Acceptance	12/20/94

Christine Sexsmith
Christine Sexsmith
Quality Assurance Coordinator

12/20/94
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.

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

The data and report for **"Tert-Amyl Methyl Ether (TAME) - Acute Toxicity To Daphnids (*Daphnia magna*) Under Flow-Through Conditions"** were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 792) with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs and metals are conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, PA. 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 material identity and maintenance of these records on the test material are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test material will be sent to the Study Sponsor. Maintenance of a sample of the test material is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

Arthur E. Putt 12/19/94

Arthur E. Putt Date
Study Director

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SUMMARY

The purpose of this study was to estimate the acute toxicity (EC50) of Tert-Amyl Methyl Ether (TAME) to *Daphnia magna* under flow-through conditions. The EC50 is defined as the concentration of test material estimated to cause immobilization among 50% of a test population at the stated exposure interval. Twenty organisms (ten per replicate) were exposed in duplicate test vessels to five concentrations of TAME and a dilution water control for 48 hours. During the test, nominal concentrations of 690, 410, 250, 150 and 89 mg A.I./L were maintained in the exposure vessels by introducing approximately 6.0 test chamber volumes per day of newly prepared test solution via intermittent-flow proportional diluter apparatus. Each replicate solution was sampled and analyzed for TAME concentration at 0-hour (initiation) and 48-hours (termination) of the exposure period. Based on the results of these analyses, the mean measured exposure concentrations were defined as 120, 83, 55, 26 and 15 mg A.I./L. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation, 3, 6, 24 and 48 hours. Throughout the exposure period, no visible signs of undissolved test material (e.g., precipitate) were observed in either the diluter system or in the exposure solutions.

At test termination (48-hours), immobilization of 90% was observed among daphnids exposed to the highest mean measured concentration tested (120 mg A.I./L). In addition, sublethal effects (e.g., lethargy) were observed among all of the mobile daphnids exposed to this treatment level. No immobilization or sublethal effects were observed among daphnids exposed to the remaining concentrations tested (83, 55, 26 and 15 mg A.I./L). The EC50 values and the corresponding 95% confidence intervals determined throughout the exposure period are summarized in the following table. The No-Observed-Effect Concentration (NOEC) through 48-hours of exposure was established to be 83 mg A.I./L.

TEST RESULTS

EC50 (mg A.I./L)[a,b]				No-Observed-Effect Concentration Through 48 Hours (mg A.I./L)[a]
3-Hour[c]	6-Hour[c]	24-Hour[c]	48-Hour[d]	
> 120	> 120	> 120	100 (83 - 120)	83

a Based on mean measured concentrations of TAME (as active ingredient).
b Corresponding 95% confidence interval is presented in parentheses.
c EC50 value empirically estimated to be greater than the highest concentration tested.
d EC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.

1.0 INTRODUCTION

The purpose of this study was to estimate the acute toxicity (EC50) of Tert-Amyl Methyl Ether (TAME) to daphnids (*Daphnia magna*) under flow-through conditions. The EC50 is defined as the concentration of test material in dilution water which causes immobilization 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 the release of the test substance into aquatic environments. The study was initiated on 26 October 1992, 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 48-hour definitive toxicity test was conducted from 10 - 12 December 1992 at Springborn Laboratories, Inc. (SLI), Environmental Sciences Division, Wareham, Massachusetts. All original raw data and the final report produced for this study are stored at SLI.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this acute toxicity study followed those described in the SLI protocol entitled "Protocol for Conducting a Flow-Through Acute Toxicity Test with *Daphnia magna* Following TSCA §797.1300", SLI Protocol #: 091192/TSCA 797.1300 DM-FA and Protocol Amendment # 1 dated 15 December 1992 (Appendix I). The methods described in this protocol generally follow the standard procedures described in the EPA/OTS guidelines for testing the effects of chemicals on daphnids and meet the TSCA guidelines as specified in the appropriate Registration Standard. This protocol is intended to meet premanufacture notice ("PMN") registration requirements.

2.2 Test Material

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, Lot # 02814BZ, was received at SLI on 17 August 1992 and was used to prepare exposure solutions during the preliminary exposure, 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 Company 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 definitive exposures. The sample was identified by Aldrich Chemical Company to contain 98.7% active ingredient A.I. (Certificate of Analysis, Appendix II). Upon receipt at SLI, the samples of test material were stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Test concentrations are expressed as milligrams of test material (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 the flow-through acute toxicity test with mysids (SLI Report # 94-5-5269), 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 *Daphnia magna* used in this toxicity test were obtained from laboratory cultures maintained at Springborn Laboratories, Inc., Wareham, Massachusetts. The culture water was prepared by fortifying well water based on the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column and a carbon filter. Two weeks prior to test initiation, this water had total hardness and total alkalinity as calcium carbonate ($CaCO_3$) of 160 mg/L and 110 mg/L, respectively, a pH of 8.1, a specific conductivity range of 400 to 500 µmhos/cm and a dissolved oxygen concentration of greater than 60% of saturation. The daphnid culture area received a regulated photoperiod of 16 hours of light and 8 hours of

darkness. Light intensity in the culture area ranged from 32 - 48 footcandles (Invertebrate Culture Log, Vol. VIII). A waterbath in the culture area was used to maintain the culture solution temperature at 20 ± 2 °C. Daphnids were fed a combination of a trout food suspension and a unicellular green algae (*Ankistrodesmus falcatus*) once daily. The food solution contained 5.0 mg/mL trout food and approximately 4 X 10^7 cells/mL of algae. 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 pesticides 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 culture water described above and had a total hardness and total alkalinity ($CaCO_3$) of 160 and 110 mg/L, respectively, a pH range of 8.1 - 8.2 and a specific conductivity of 500 μmhos/cm (IWO Log Book, Vol. 13). During holding and prior to use, the dilution water was continuously aerated. Representative samples of the dilution water source 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 ASTM standard practice (ASTM, 1985). In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentrations. The results of these analyses demonstrated that the TOC concentration of the dilution water source ranged from 0.97 - 2.2 mg/L for the months of June - November 1992 (TOC and TSS master log, Volume I). Daphnid cultures are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over several generations. This, in combination with the previously mentioned analyses, confirms the acceptability of this dilution water for bioassays.

2.5 Test Conditions

The toxicity test was conducted using an exposure system consisting of an intermittent-flow proportional diluter (Mount and Brungs, 1967) and a set of 12 exposure vessels. The test system was designed to provide five concentrations of the test material and a dilution water

control. Exposure vessels were maintained in an area illuminated with Duro-Test® Cool-White and Duro-Test® Vitalite fluorescent lights at an intensity of 45 - 70 footcandles. The photoperiod was the same as that of the culture area. Sudden transitions from light to dark and vice versa were avoided. The test was conducted in a temperature controlled room and waterbath which were designed to maintain test solution temperatures at 20 ± 2 °C. Two replicate vessels were established for each treatment level and the control. Exposure vessels were labeled to identify the nominal test material concentration and designated replicate.

2.6 Test Concentrations

Selection of nominal TAME concentrations for the 48-hour definitive flow-through toxicity test with daphnids was based on toxicity information developed at SLI through preliminary testing.

2.7 Exposure Solution Preparation

Prior to test initiation, a 50 mL Glenco® gas-tight syringe in conjunction with a Sage syringe pump (Model # 355) was calibrated to deliver 0.416 mL/cycle of the test material (760 mg/mL) directly to the diluter system's chemical mixing chamber, which also received 0.458 L of dilution water per cycle. The mixing chamber was positioned over a magnetic stirrer which continuously mixed the contents of the mixing chamber and aided in the solubilization of the test material. The solution contained in the mixing chamber constituted the highest nominal treatment level (690 mg A.I./L) and was subsequently diluted (60% dilution factor) to provide the remaining nominal test concentrations (410, 250, 150 and 89 mg A.I./L).

During each cycle of the diluter system, approximately 50 mL of exposure solution was delivered to each replicate test vessel. The system cycled approximately 216 times each day. The diluter system was calibrated prior to test initiation by measuring delivery volumes of toxicant and dilution water. During the study, visual checks of the diluter system and analysis of the exposure solutions for TAME concentration were used to verify proper operation of the diluter system. The exposure system was in proper operation 9 days prior to test initiation to allow equilibration of the test material in the diluter apparatus and exposure vessels. Four glass

capillary tubes with an approximate length of five centimeter (cm) and a diameter of 1-millimeter (mm) (inside diameter) were inserted through silicone stoppers in the mixing/splitting chambers of the diluter and into the test solution delivery tubes. This tubing served to restrict the flow of the test solutions, minimizing potentially stressful turbulence in the exposure vessels and provided equal distribution of the solutions to the replicate vessels. Each glass test vessel maintained a constant solution volume of 1.8 L and a solution depth of approximately 13 cm. Each replicate vessel received approximately 6.0 solution volume replacements per day. The function of the diluter system (e.g., flow rates, stock consumption) was monitored daily and a visual check was made twice daily to verify proper performance during the study period.

3.0 TEST PROCEDURES

3.1 Test Initiation

The test was initiated when 10 daphnids (≤ 24 hours old) were impartially selected and introduced to each replicate exposure vessel (20 per treatment level and the control).

3.2 Test Monitoring

The number of immobilized daphnids observed in each replicate test vessel was recorded at 3-, 6-, 24- and 48-hours during the exposure period. Daphnids were determined immobile if, after gentle prodding, no movement except for minor appendages was observed (i.e., absence of movement within the solution's water column). Biological observations (e.g., abnormal behavior or appearance of the test organisms) and observations of the physical characteristics of the test solutions (e.g. precipitate, film on the surface of the test solution) were also made and recorded at test initiation and at 3-, 6-, 24- and 48-hours of exposure. Daphnids were not fed during the 48 hour definitive exposure.

3.3 Water Quality Measurements

Dissolved oxygen concentration, temperature and pH were measured once daily in both replicate vessels of each treatment level and the control throughout the exposure period. Total hardness, total alkalinity and specific conductance were measured at test initiation in one replicate vessel of each treatment level and control solution. Total hardness concentrations

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presented in this report were measured by the EDTA titrimetric method and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA et al., 1985) using a Jenco Model 601A pH meter and combination electrode. Specific conductivity was measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe; the pH was measured with a Jenco Model 601A pH meter and combination electrode; the dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe; and the daily solution temperature was measured with a Fisher alcohol thermometer. Continuous temperature monitoring was performed in one replicate (B) of the 630 mg A.I./L test solution (nominal) using the Omega Data Acquisition System (ODAS). Light intensity was measured with a General Electric type 214 light meter.

3.4 Analytical Measurements

Both replicate solutions of the high, middle, and low treatment levels and the control were sampled and analyzed for TAME concentration prior to the start of the definitive exposure. Results of these pretest analyses were used to judge whether sufficient quantities of TAME were being delivered to the test vessels and the appropriate test concentrations were being maintained in order to initiate the definitive exposure. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the control at 0- and 48-hours of exposure for analysis of TAME concentration. 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 samples of the exposure solutions throughout the analytical process. Results of the analyses of the QC samples were used to judge the precision and the quality control maintained during the analysis of exposure solution samples. All samples were analyzed for TAME using a gas chromatography (GC) procedure according to the methodology described in Appendix V. A method validation recovery study, conducted at SLI prior to the initiation of the definitive test, established an average recovery of TAME of 102 ± 10% from hard reconstituted water.

4.0 STATISTICS

The mean measured concentrations tested (based on 0- and 48-hour analyses) and the corresponding effect (immobilization) data derived from the definitive toxicity test were used to estimate the median lethal concentration (EC50) and 95% confidence interval for each 24-hour interval of the exposure period. The EC50 is defined as the concentration of the test material in dilution water which caused immobilization of 50% of the test animals population at the stated time interval. When no concentration caused ≥ 50% immobilization of the test population, then the EC50 value was empirically estimated as being greater than the highest mean measured concentration tested. If at least one test concentration caused immobilization of greater than or equal to 50% of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the EC50 values and 95% confidence interval.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a immobilization of between 0 and 100% of the test organism population. The selection of reported EC50 values and 95% confidence intervals 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 (immobilization) relationship, the number of concentrations causing partial responses, and the span of responses bracketing the EC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The No-Observed-Effect Concentration (NOEC) during the 48 hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there were no toxicant related immobilization or physical and behavioral abnormalities, (e.g., lethargy, flared carapace), with respect to the control organisms.

5.0 RESULTS

5.1 Preliminary Test

Prior to initiating the definitive study, a 48-hour preliminary range-finding test was conducted at SLI. During this 48-hour preliminary test, daphnids were exposed under flow-

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through conditions to nominal concentrations of TAME ranging from 690 - 89 mg A.I./L. All test vessels during this preliminary exposure were uncovered. At test termination (48-hours), 100% immobilization was observed among daphnids exposed to the highest nominal concentration tested (690 mg A.I./L). Immobilization of 10% was observed among mobile daphnids exposed to the 410 mg A.I./L, nominal treatment level. Sublethal effects (i.e., lethargy) were observed among all of the mobile daphnids exposed to the 410 mg A.I./L nominal treatment level and one mobile daphnid in the 250 mg A.I./L treatment level. No immobilization or sublethal effects were observed among daphnids exposed to the remaining nominal concentrations tested (150 and 89 mg A.I./L). Based on these results, nominal concentrations of 690, 410, 250, 150 and 89 mg A.I./L were selected for the definitive exposure.

5.2 Definitive Test

Results for the water quality parameters (pH, dissolved oxygen and temperature) measured at 0-, 24- and 48-hours of the definitive exposure are summarized in Table 1. Total hardness, total alkalinity and specific conductance measured at test initiation are presented in Table 2. Throughout the exposure period, the water quality parameters measured were unaffected by the concentrations of TAME tested and remained within acceptable ranges for the survival of *Daphnia magna*. Daily and continuous (replicate B of the 690 mg A.I./L treatment level, nominal) temperature monitoring of the test solutions established that the test solution temperature ranged from 18 - 20 °C throughout the exposure period.

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly during the pretest period and throughout the 48-hour study. Analyses of the solutions during the pretest period established that the concentration of TAME in the exposure solutions was consistent between replicate solutions and that the delivery apparatus maintained the expected concentration gradient (approximately 60% dilution factor). Analyses of the pretest samples resulted in measured concentrations which averaged 19% of nominal. Throughout the exposure period, no visible signs of undissolved test material (e.g., precipitate) was observed in either the diluter system or in the exposure solutions.

The results of the analysis of the exposure solutions for TAME concentration during the in-life portion of the definitive exposure are presented in Table 3. Throughout the exposure period, analytical measurements between replicate solutions and sampling intervals were consistent and established the expected concentration gradient of test material (i.e., 60% dilutions). Mean measured concentrations averaged 19% of the nominal concentrations and defined the treatment levels as 120, 83, 55, 28 and 15 mg A.I./L. The relatively low recovery obtained for the tested treatment levels (mean = 19%) is believed due to the volatile nature of the test material. However, the mean measured concentrations established were sufficient to produce a biological response (immobilization). Coefficients of variation averaged 11% for all mean measured concentrations. Analysis of the Quality Control samples resulted in measured concentrations which were consistent with the predetermined recovery range (Appendix V) and averaged 100% of the nominal fortified levels (695 - 49.7 mg A.I./L). Based on the results of these analyses, it was established that the appropriate quality control was maintained during the analyses of the exposure solutions.

The relationship between the nominal treatment levels and the mean measured concentrations established by the diluter apparatus during this study is illustrated in Figure 1.

The mean measured concentrations tested, the corresponding percent of immobilized daphnids and observations recorded during the 48-hour test are presented in Table 4. At test termination (48-hours), immobilization of 90% was observed among daphnids exposed to the highest mean measured concentration tested (120 mg A.I./L). In addition, sublethal effects (e.g., lethargy) were observed among all of the mobile daphnids exposed to this treatment level. No immobilization or sublethal effects were observed among daphnids exposed to the remaining concentrations tested (83, 55, 28 and 15 mg A.I./L). The 48-hour concentration-response (immobilization) curve established for this study is presented in Figure 2. The slope of this curve was calculated to be 3.3683. Table 5 summarizes the 3-, 6-, 24- and 48-hour EC50 values, corresponding 95% confidence intervals and presents the No-Observed-Effect Concentration (NOEC) through 48 hours. The 48-hour EC50 value was estimated by nonlinear interpolation to be 100 mg A.I./L with a 95% confidence interval calculated by binomial probability to be 83 - 120 mg A.I./L. The NOEC established for this study was determined to be 83 mg A.I./L.

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PROTOCOL DEVIATION

The study protocol states that the calibration of the diluter system is checked prior to test initiation and at test termination. For this study, the diluter calibration check at test termination was inadvertently missed. The diluter calibration was confirmed to be functioning properly by the consistency between measured concentrations at 0 and 48 hours.

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

SPRINGBORN LABORATORIES, INC.

12/19/94

Arthur E. Putt Date
Study Director

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QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity To Daphnids (*Daphnia magna*) Under Flow-Through Conditions" were inspected by the Springborn Laboratories, Inc., Environmental Sciences Division, Quality Assurance Unit (QAU) to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. Dates of study inspections, Dates reported to Study Director and to Management are listed below.

It is the opinion of the QAU that this report accurately reflects the raw data collected during this study.

Inspection Date	Reported to Study Director	Reported to Management
12/12/92	12/13/92	12/18/92
12/17/92	12/17/92	12/18/92
12/18/92	12/18/92	12/18/92
12/29/92	12/29/92	1/4/93
12/30/92	12/30/92	1/4/93
9/26+27/94	9/26/94	10/7/94
9/29/94	9/29/94	10/7/94
12/19/94	12/19/94	12/19/94

SPRINGBORN LABORATORIES, INC.

12/19/94

Patricia D. Royal
Regulatory Affairs
and Quality Assurance Unit

Date

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TABLES

Table 1. **The water quality parameters (i.e., pH, dissolved oxygen, temperature) measured in replicate exposure solutions during the 48-hour flow-through toxicity test exposing daphnids (*Daphnia magna*) to TAME.**

Nominal Concentration (mg A.I./L)	0-Hour		24-Hour		48-Hour	
	A	B	A	B	A	B
			pH			
690	8.2	8.2	8.1	8.1	8.0	8.0
410	8.2	8.2	8.1	8.1	8.0	8.0
250	8.2	8.2	8.0	8.0	8.0	8.0
150	8.2	8.2	8.0	8.0	8.0	8.0
89	8.2	8.2	8.0	8.0	8.0	8.0
Control	8.2	8.2	8.0	8.0	8.0	8.0
			Dissolved Oxygen, mg/L (% Saturation)			
690	9.2 (101)	9.2 (101)	9.1 (98)	9.0 (97)	8.8 (95)	8.9 (96)
410	9.1 (100)	9.1 (100)	9.0 (97)	9.0 (97)	8.8 (95)	8.8 (95)
250	9.2 (101)	9.1 (100)	8.8 (95)	8.7 (94)	8.8 (95)	8.8 (95)
150	9.2 (101)	9.2 (101)	9.0 (97)	9.1 (98)	8.8 (95)	8.8 (95)
89	9.2 (101)	9.2 (101)	9.1 (98)	9.1 (98)	9.0 (97)	8.9 (96)
Control	9.2 (101)	9.1 (100)	9.1 (98)	9.1 (98)	8.8 (95)	8.9 (96)
			Temperature(°C)[a]			
	20	20	19	19	19	19

[a] Values presented represent daily temperature (Fisher Alcohol Thermometer) measured in all test concentrations and the control at the stated observation interval. Continuous temperature monitoring established a temperature range of 18 - 20 °C throughout the exposure period.

Table 2. **Total hardness, total alkalinity and specific conductance determined at the initiation (0-hour) of the 48-hour flow-through exposure of daphnids (*Daphnia magna*) to TAME.**

Nominal Concentration (mg A.I./L)	Total Hardness[a] (mg/L $CaCO_3$)	Total Alkalinity[a] (mg/L $CaCO_3$)	Specific Conductance[a] (μmhos/cm)
690	190	110	500
410	180	110	500
250	180	110	500
150	180	110	500
89	170	120	500
Control	170	120	500

[a] Measurement performed in replicate A of each exposure level and the control.

Table 3. **Concentrations of TAME measured in replicate (A,B) test solutions during the 48-hour flow-through exposure of daphnids (*Daphnia magna*).**

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L)				Mean Measured Concentration[a] (mg A.I./L)
	0-Hour		48-Hour		
	A	B	A	B	
690	120	120	110	120	120 (7.1)
410	90	86	78	80	83 (5.6)
250	55	62	53	51	55 (4.6)
150	27	29	28	28	28 (0.74)
89	18	21	8.9	14	15 (5.1)
Control	< 0.40	< 0.40	< 0.40	< 0.40	
QC #1[b]	784 (695)[c]		570 (695)		
QC #2	295 (248)		211 (248)		
QC #3	57.9 (49.7)		43.3 (49.7)		

[a] Mean measured concentrations are presented with the standard deviation in parentheses and were calculated using the unrounded analytical value and not the rounded (two significant figures) values presented in this table.

[b] QC = Quality Control sample.

[c] Value in parentheses represents the nominal fortified concentration for the corresponding QC sample.

Table 4. Mean measured concentrations tested, corresponding cumulative percent of immobilized daphnids (*Daphnia magna*) and observations made during the 48-hour flow-through exposure to TAME.

Mean Measured Concentration (mg A.I./L)	Cumulative Percent Immobilized Organisms											
	3-Hour			6-Hour			24-Hour			48-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
120	0	0	0	0	0	0	10	20	15[a]	100	80	90[b]
83	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	10	0	5

[a] All of the mobile daphnids were observed to be lethargic.
[b] All of the mobile daphnids were observed to be lethargic and on the bottom of the test vessel.

Table 5. **The EC50 values (95% confidence interval) and No-Observed-Effect Concentration (NOEC) for the 48-hour flow-through exposure of daphnids (*Daphnia magna*) to TAME.**

EC50 (mg A.I./L)[a,b]				No-Observed-Effect Concentration Through 48 Hours (mg A.I./L)[a]
3-Hour[c]	6-Hour[c]	24-Hour[c]	48-Hour[d]	
> 120	> 120	> 120	100 (83 - 120)	83

[a] Based on mean measured concentrations of TAME (as active ingredient).
[b] Corresponding 95% confidence interval is presented in parentheses.
[c] EC50 value empirically estimated as greater than the highest concentration tested.
[d] EC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.

FIGURES

Figure 1. Graphical illustration of the relationship between the mean measured concentrations (analyses at 0- and 3-hours) and the nominal treatment levels during the 48-hour flow-through exposure of daphnids (*Daphnia magna*) to TAME.

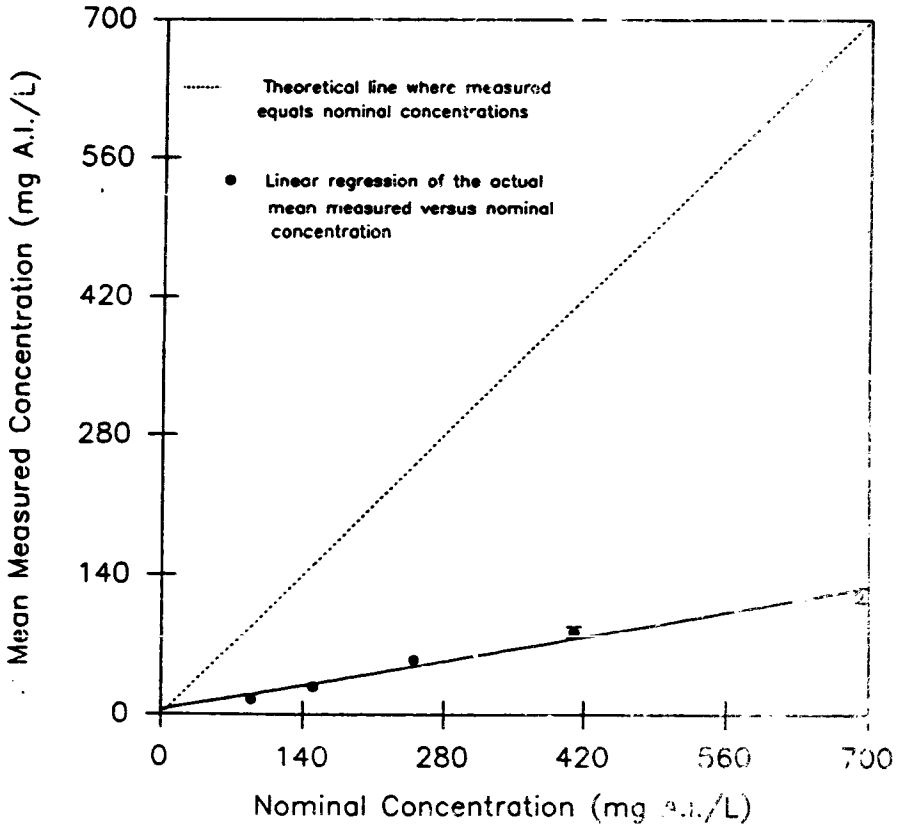
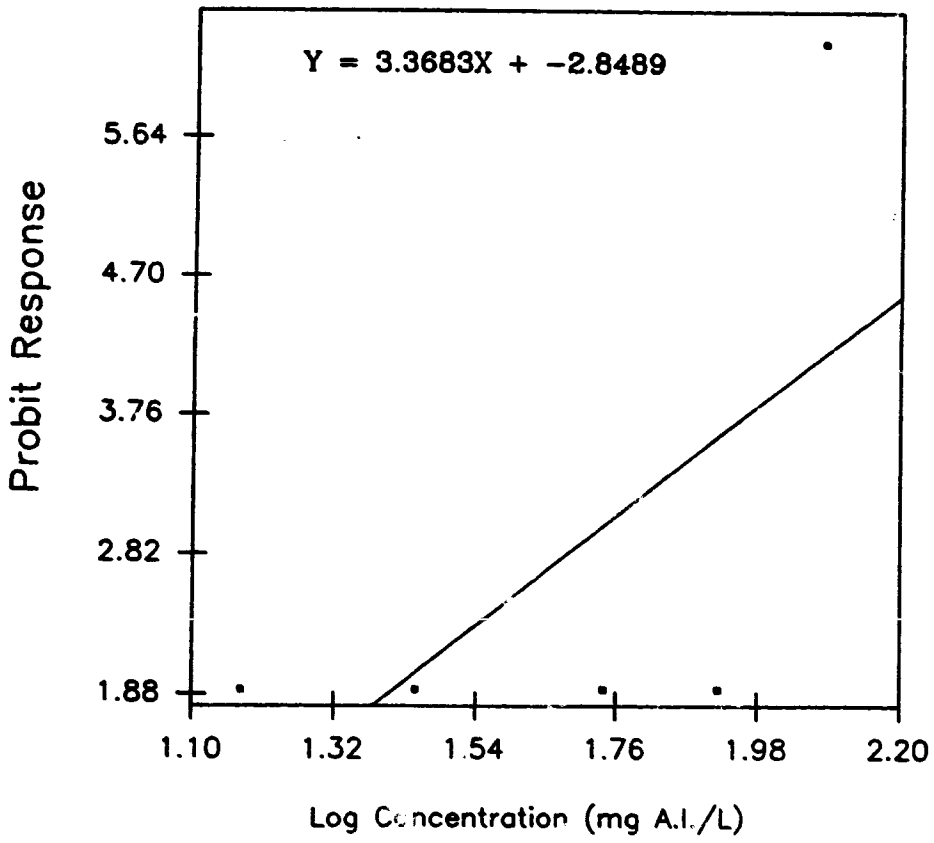


Figure 2. **The 48-hour concentration-response (immobilization) curve for daphnids (*Daphnia magna*) exposed to TAME.**



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This final report has been signed in accordance with SLI SOP No. 4.3.07(1).

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Dear FYI Coordinator:

In accordance with API's policy of providing the federal government with copies of research designed to determine whether any chemical substance or mixture manufactured, processed or distributed by API member companies may cause a risk of injury to health or the environment, we are enclosing a copy of the following final report:

(Identification no: FYI-94-001052) Tert-Amyl Methyl Ether (TAME): Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss). Final Report.

Please note that this information is provided in accordance with the full disclosure policy of API and does not constitute a formal submission as required by a test rule. This document does not contain confidential information. If you have any questions, please communicate with me.

Sincerely,

Robert T. Drew, Ph.D.

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TERT-AMYL METHYL ETHER (TAME) - ACUTE TOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*) UNDER FLOW-THROUGH CONDITIONS

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TSCA GUIDELINE § 797.1400

Submitted to:

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

SLI Report # 93-3-4682

Study # 12827.0692.6104.108

Study Director: Mark W. Machado

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
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19 December 1994

FINAL REPORT

American Petroleum Institute
Health and Environmental Sciences Department

QUALITY ASSURANCE/GLP COMPLIANCE STATEMENT

Study Title: Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Rainbow Trout *(Oncorhynchus mykiss)* Under Flow-Through Conditions

Testing Facility: Springborn Laboratories, Inc.

SLI Study Number: 12827.0692.6104.108

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.

Date(s) of Inspection/Review	Type of Inspection	Date of Report to Management
8/28/92	Protocol Evaluation	8/28/92
5/5/93	Draft Report Audit	5/5/93
4/27-28/94	Draft Report Audit	4/29/94
10/15/94	Revised Final Draft Report Acceptance	10/15/94
12/20/94	Final Report Acceptance	12/20/94

Christine Sexsmith
Quality Assurance Coordinator

12/20/94
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.

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

The data and report presented for **"Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions"** were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 792) with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs and metals are conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, PA. 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. At the termination of the testing program, all remaining test material will be sent to the Study Sponsor. Archival of a sample of the test material is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

Mark W. Machado 01/19/94

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 rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. The LC50 is defined as the concentration of the test material 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 aquaria to each of five concentrations of TAME and a dilution water control for 96-hours. During the test, nominal concentrations of 950, 570, 340, 210 and 120 mg A.I./L were maintained by introducing approximately 6.5 aquarium volumes per day of newly prepared test solution via a modified constant-flow serial diluter apparatus (Bencit, 1982). Each replicate solution was sampled and analyzed for TAME concentration at 0-hour (test initiation) and 96-hours (test termination) of exposure. Based on the results of these analyses, the mean measured exposure concentrations were defined as 640, 360, 310, 150 and 78 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 material (e.g., precipitate).

Following 72-hours of exposure, 100% mortality was observed among fish exposed to the highest mean measured concentration tested (640 mg A.I./L). At test termination (96-hours), mortality of 30% was observed among fish exposed to the 360 mg A.I./L treatment level. In addition, sublethal effects (e.g., loss of equilibrium, darkened pigmentation) were observed among all of the surviving fish exposed to this treatment level. No mortality or sublethal effects were observed among fish exposed to the remaining concentrations tested (310, 150 and 78 mg A.I./L). The LC50 values and the 95% confidence intervals determined throughout the exposure period are summarized in the following table. The No-Observed-Effect Concentration (NOEC) established during this study was 310 mg A.I./L.

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TEST RESULTS

LC50 (mg A.I./L)[ab]				No-Observed-Effect Concentration Through 96 Hours (mg A.I./L)[a]
24-Hour[c]	48-Hour[c]	72-Hour[d]	96-Hour[de]	
600 (580 - 620)	600 (570 - 620)	580 (560 - 640)	580 (310 - 640)	310

[a] Based on mean measured concentrations of TAME (as active ingredient).
[b] Corresponding 95% confidence interval is presented in parentheses.
[c] LC50 value and 95% confidence interval calculated by probit analysis.
[d] LC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.
[e] Since the 96-hour LC50 was not less than 50% of the 48-hour value, the study was not extended beyond 96-hours to determine the incipient LC50.

1.0 INTRODUCTION

The purpose of this study was to determine the acute toxicity of Tert-Amyl Methyl Ether (TAME) to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. The LC50 is defined as the concentration of the test material 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 the potential acute hazards resulting from release of the test material into aquatic environments. The study was initiated on 14 October 1992, 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 flow-through toxicity test was conducted from 27 February - 3 March 1993 at Springborn Laboratories, Inc. (SLI), Environmental Sciences Division, Wareham, Massachusetts. All original raw data and the final report produced for this study are stored at SLI.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this acute toxicity test followed those described in the SLI protocol entitled "Protocol for Conducting a Flow-Through Acute Toxicity Test with Rainbow Trout following TSCA 797.1400", SLI Protocol #:091192/TSCA 797.1400 and Protocol Amendments #1 and #2 dated 10 and 24 March 1993, respectively (Appendix I). The methods described in this protocol generally follow the standard procedures outlined in the EPA/OTS guideline (§ 797.1400) for acute toxicity testing with fish (U.S. EPA, 1985, amended 1987).

2.2 Test Material

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, 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 by Aldrich Chemical to contain 98.7% active ingredient A.I. (Certificate of Analysis, Appendix II). Upon receipt at SLI, the samples of test material were stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Test concentrations are expressed as milligrams of test material (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 the flow-through acute toxicity test with mysids (SLI Report # 94-5-5269), 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

Rainbow trout (*Oncorhynchus mykiss*) were selected as the test species since it is a recommended species (U.S. EPA, 1985) and a commonly used cold water fish in flow-through freshwater fish toxicity tests. The rainbow trout (SLI lot #93B3) used during the study were obtained from Spring Creek Trout Hatchery, a commercial supplier located in Lewistown, Montana. Prior to testing, these fish were held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. The well water which flowed into this holding tank was characterized as having total hardness and total alkalinity ranges as calcium carbonate ($CaCO_3$) of 25 - 27 mg/L and 20 - 22 mg/L, respectively, and a specific conductance of 110 µmhos/cm (Gravity Feed Tank Water Quality Log). Other parameters monitored in the holding tank were pH with a range of 6.8 - 6.9, dissolved oxygen concentration with a range of 80 - 87% of saturation

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and the flow rate with 8.2 - 11 tank volume replacements/day (Weekly Record of Fish Holding Characteristics). Fish used during the definitive exposure were maintained under similar conditions for a minimum of 14 days prior to testing. The temperature in the holding tank ranged from 11 - 12 °C during this 14-day period. All fish were fed a dry commercial pelleted food, *ad libitum*, daily except during the 48 hours prior to, and during the definitive test. 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 pesticides was less than 0.3 mg/kg. Mortality of 0.20% was observed in the test fish population during the two days prior to testing (Daily Record of Fish Holding Conditions). The rainbow trout used during this study were all of the same year class and a representative sample (N = 30) had a mean (range) wet weight and total length of 0.47 (0.28 - 0.75) grams and 37 (30 - 48) millimeters, respectively (Fish Lengths and Weights Log).

2.4 Test Dilution Water

The dilution water used during this study was from the same source as the water which flowed into the fish holding tank and was characterized as having total hardness and total alkalinity (as $CaCO_3$) ranges of 20 - 22 and 25 - 27 mg/L, respectively, a pH range of 6.9 - 7.0 and a specific conductance of 110 µmhos/cm (Gravity Feed Tank Water Quality Log). Representative samples of the dilution water source 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 ASTM standard practice. In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentration. The results of these analyses demonstrated that the TOC concentration of the dilution water ranged from 0.82 - 1.3 mg/L for the months of September 1992 - February 1993 (TOC and TSS Master Log, Vol 1). Several species of daphnids (a representative freshwater organism generally recognized to be sensitive to chemical challenges) are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over several generations. This, in combination with the previously mentioned analyses, confirms the acceptability of this dilution water for bioassays.

2.5 Test Conditions

The toxicity test was conducted using an exposure system consisting of a constant-flow serial diluter (Benoit, 1982), with a 60% dilution factor, a temperature-controlled water bath and a set of 12 exposure aquaria. The test system was designed to provide five concentrations of the test material and a dilution water control. Calibration of the diluter system was confirmed prior to test initiation and again subsequent to test termination. All treatment levels and the controls were maintained in duplicate. Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a 14.5 cm high standpipe which maintained a constant test solution volume of 11 L. The flow of exposure solutions to each test aquarium was approximately 50 mL/min, which provided approximately 6.5 volume replacements per aquarium every 24 hours. Test aquaria were labeled to identify the nominal test concentration and designated replicate. Test vessels were not covered during the exposure period. Test vessels were impartially positioned in a water bath containing circulating water cooled by a Frigid Unit® chiller designed to maintain the test solution temperatures at 12 ± 1° C. Test solutions were not aerated. A photoperiod of 16 hours light and 8 hours dark provided light with an intensity of 30 - 80 footcandles at the test solution surface throughout the study period. Sudden transitions from light to dark and vice versa were avoided. Lighting was provided by Duro-Test® Vitalite fluorescent bulbs.

2.6 Test Concentrations

Selection of nominal TAME concentrations for the 96-hour flow-through definitive toxicity test with rainbow trout was based on toxicity information developed at SLI through preliminary testing.

2.7 Test Solution Preparation and Delivery

During the exposure period, the test material was delivered directly to each replicate test aquarium for each treatment level. The concentration of test material that was delivered to each aquarium was determined to be 759.99 mg A.I./mL based on the test material's density (0.770 g/mL) and percent active ingredient (98.7% A.I.). A series of Sage syringe pumps in conjunction with Glenco® gas-tight syringes were calibrated to deliver the appropriate amount of test material (759.99 mg A.I./mL) directly into the delivery tube for each replicate aquarium. Each

individual delivery tube also received 0.050 L/min of dilution water. Delivery of the test material and dilution water at this point in the system aided in the mixing and solubilization of the test material. Proportional dilution (60%) across the range of nominal concentrations established (i.e., 950, 570, 340, 210 and 120 mg A.I./L) was accomplished by adjusting the size of syringes used (i.e., 20 - 50 mL) and the flow of each pump (0.0625, 0.0375, 0.0225, 0.0135 and 0.0081 mL/min).

The diluter system was calibrated prior to test initiation and at test termination by measuring delivery volumes of toxicant and dilution water. The function of the diluter system (e.g., flow rates, stock consumption) was monitored daily and a visual check was performed twice daily. In addition, analysis of the exposure solutions for TAME concentration was also used to verify proper operation of the diluter system. The exposure system was in proper operation for several days prior to test initiation to allow equilibration of the test material in the diluter apparatus and exposure vessels.

3.0 TEST PROCEDURES

3.1 Test Initiation

The test was initiated when rainbow trout were impartially selected and distributed two at a time to each replicate aquarium until each replicate contained 10 fish (20 fish per treatment level and the control). At any given time during the exposure period, the maximum organism loading concentration was 0.065 g of biomass per liter of flowing test solution per day.

3.2 Test Monitoring

The diluter was visually inspected at least twice daily during the definitive exposure period. Biological observations of the exposed rainbow trout and observations of the physical characteristics of the test solutions (e.g., precipitate, film on the solution's surface) were made at test initiation and at each subsequent 24-hour interval until test termination (96-hours). Effects for this study were based on death, defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding). Mortalities were recorded and removed from each aquarium every 24 hours during the exposure period.

3.3 Water Quality Measurements

Dissolved oxygen concentration, temperature and pH were measured once daily in each replicate of each treatment level and the controls throughout the exposure period. The pH was measured using a Jenco Model 601A pH meter and combination electrode; the dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe and the daily solution temperature was measured with a Brooklyn alcohol thermometer. Light intensity was measured with a General Electric Model 214 light meter. Test solution temperature was continuously monitored in one replicate (B) of the dilution water control solution using a Brooklyn Min/Max thermometer.

3.4 Analytical Measurements

Both replicate solutions of the high, middle and low treatment levels and the control were sampled and analyzed for TAME concentration prior to the start of the definitive exposure. Results of these pretest analyses were used to judge whether sufficient quantities of test material were being delivered and maintained in the exposure aquaria to initiate the definitive test. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls at 0- and 96-hours of exposure for analysis of TAME concentration. Each exposure solution sample was collected from the approximate midpoint of the aquarium 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 TAME concentrations 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 TAME of 102 ± 10% from hard reconstituted water.

4.0 STATISTICS

The mean measured concentrations (0- and 96-hour analysis) and the corresponding mortality data derived from the definitive test were used to estimate the median lethal concentration (LC50) and 95% confidence interval at each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test material in dilution water lethal to 50% of the test animal population at the stated exposure interval. 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 interval. Additionally, the data was evaluated to estimate an incipient LC50. The incipient LC50 is defined as the concentration that is lethal to 50% of a test population when exposure to the test substance is continued until the mean increase in mortality does not exceed 10% in any concentration over a 24-hour period. Since the 96-hour LC50 was not less than 50% of the estimated 48-hour LC50, the duration of the exposure was not continued to determine the incipient LC50 value (U.S. EPA, 1987).

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence interval calculated by 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 interval 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 relationship (mortality), 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 interval 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, loss of equilibrium, darkened pigmentation), with respect to the control organisms.

5.0 RESULTS

5.1 Preliminary Testing

Prior to initiating the definitive study, several preliminary range-finding studies were conducted at SLI. During these preliminary studies, the test material was delivered to the test aquaria using conventional delivery methods in a Benoit-style diluter (i.e., mixing chamber, chemical cells). Test vessels were not covered during any of the exposure. In three separate tests, rainbow trout were exposed to nominal concentrations of TAME ranging from 570 - 15, 1100 - 89 and 1700 - 130 mg A.I./L. After 120 hours of exposure, no toxicant related mortality or sublethal effects were observed among fish exposed to 570 - 15 mg A.I./L treatment levels. Following 72 hours of exposure at treatment levels of 1100 - 89 mg A.I./L, 10% was observed among fish exposed to the highest treatment level. Surviving fish at this treatment exposure concentration (1100 mg A.I./L) were described as exhibiting darkened pigmentation and complete loss of equilibrium. During the third exposure, mortality of 80% was observed among fish exposed to the 1700 mg A.I./L treatment level following 24-hours of exposure. No mortality or sublethal effects were noted among organisms exposed to the remaining test concentrations.

Following the completion of the initial preliminary investigations, the diluter system and toxicant delivery method were modified in an attempt to compensate for the volatile nature of the test material and to maximize the measured to nominal test material concentration in solution. This modification consisted of eliminating the mixing chamber and chemical cells of the Benoit-style diluter and injecting the material directly into the delivery tubing for each replicate aquarium. Using this delivery method, rainbow trout were exposed to a single concentration of 950 mg A.I./L. Following 24-hours exposure, 100% mortality was observed among fish in the treatment level tested. Based on the results of these preliminary tests, it was determined that the definitive study would be conducted utilizing the non-conventional delivery of the test material to the exposure aquaria. Nominal concentrations chosen for the definitive study were 950, 570, 340, 210 and 120 mg A.I./L.

5.2 Definitive Test

The water quality parameters (pH, dissolved oxygen concentration and temperature) measured during the definitive study are presented in Table 1. All water quality parameters measured were unaffected by the concentrations of TAME tested and remained within acceptable ranges for the survival of rainbow trout. Daily monitoring of the test solutions established a temperature range of 11 - 12 °C throughout the exposure period. Continuous temperature monitoring of one replicate (B) of the control solution established that the test solution temperature ranged from 11 - 13 °C throughout the exposure period.

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the 96-hour study. Analyses of the exposure solutions during the pretest period established that the concentrations of TAME in the exposure solutions were generally consistent between replicate samples and that the delivery apparatus maintained the expected concentration gradient (approximately 60% dilution factor). Analyses of the pretest samples resulted in measured concentrations which averaged 65% of nominal. Throughout the exposure period, no visible signs of undissolved test material (e.g., precipitate) was observed in either the diluter system or in the exposure solutions.

The results of the analysis of the exposure solutions for TAME concentration during the in-life portion of the definitive exposure are presented in Table 2. Throughout the exposure period, analytical measurements between replicate solutions were generally consistent and established the expected concentration gradient (60%). Mean measured concentrations averaged 79% of the nominal concentrations and defined the treatment levels as 640, 560, 310, 150 and 78 mg A.I./L. Coefficients of variation averaged 12% for all mean measured concentrations. Analysis of the Quality Control (QC) samples during the definitive study resulted in measured concentrations which were consistent with the predetermined recovery range established during the method validation/recovery study (Appendix V) and averaged 110% of the nominal fortified concentration range (950 - 120 mg/L). Based on the results of these analyses, it was established that the appropriate quality control was maintained during the analyses of the exposure solutions.

The relationship between the nominal treatment levels and the mean measured concentrations established by the diluter apparatus during this study is illustrated in Figure 1.

The mean measured concentrations tested, the corresponding percent mortalities and the observations made during the definitive study are presented in Table 3. Following 72-hours of exposure, 100% mortality was observed among fish exposed to the highest mean measured concentration tested (640 mg A.I./L). At test termination (96-hours), mortality of 30% was observed among fish exposed to the 560 mg A.I./L treatment level. In addition, sublethal effects (e.g., loss of equilibrium, darkened pigmentation) were observed among all of the surviving fish exposed to this treatment level. No mortality or sublethal effects were observed among fish exposed to the remaining concentrations tested (310, 150 and 78 mg A.I./L). Based on these data, it was established that the effects observed during this study were clearly concentration-dependent. Figure 2 presents the 96-hour concentration-response (mortality) curve established for this study. The slope of this curve was calculated to be 5.7417. Table 4 summarizes the 24-, 48-, 72- and 96-hour LC50's and the 95% corresponding confidence interval. The 96-hour LC50 value for rainbow trout exposed to TAME was estimated by nonlinear interpolation to be 580 mg A.I./L (95% confidence intervals calculated by binomial probability of 310 - 640 mg A.I./L). The No-Observed-Effect Concentration (NOEC) for rainbow trout exposed to TAME was determined to be 310 mg A.I./L. An incipient LC50 was not estimated since the 96-hour LC50 value was not less than 50% of the 48-hour LC50 value. Copies of raw data used to establish the maintained exposure conditions (e.g., water quality, test material concentration analyses) and the concentration-effect response used to determine the reported LC50 and NOEC values for this study are presented in Appendix VI.

PROTOCOL DEVIATION

1. The study protocol states that dissolved oxygen concentration exceeds 90% of saturation at the initiation of the test. For this study, dissolved oxygen concentration ranged from 89 - 91% at test initiation.

2. The study protocol states that the dilution water used during this study had a total hardness range of 25 to 40 mg/L (as $CaCO_3$). During this study, the total hardness of the dilution water ranged from 20 to 22 mg/L ($CaCO_3$).

It is our opinion that these deviations did not effect the results or interpretation of this study.

SPRINGBORN LABORATORIES, INC.

Mark W. Machado
Study Director

12/19/94
Date

QUALITY ASSURANCE UNIT STATEMENT

The raw data and report "**Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions**" were inspected by the Springborn Laboratories, Inc., Environmental Sciences Division, Quality Assurance Unit (QAU) to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. Dates of study inspections, dates reported to Study Director and to Management are listed below.

It is the opinion of the QAU that this report accurately reflects the raw data generated during this study.

Inspection Date	Reported to Study Director	Reported to Management
3/1/93	3/1/93	3/12/93
3/11/93	3/12/93	3/12/93
3/12/93	3/12/93	3/12/93
3/15/93	3/15/93	3/26/93
9/19/94	9/19/94	9/23/94
9/26-28/94	9/28/94	10/7/94
12/19/94	12/19/94	12/19/94

SPRINGBORN LABORATORIES, INC.

12/19/94

Patricia D. Royal Date
Manager, Regulatory Affairs
and Quality Assurance Unit

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TABLES

Table 1. The water quality parameters measured during the 96-hour flow-through toxicity test exposing rainbow trout (*Oncorhynchus mykiss*) to TAME.

Nominal Concentration (mg A.I./L)	0-Hour A	0-Hour B	24-Hour A	24-Hour B	48-Hour A	48-Hour B	72-Hour A	72-Hour B	96-Hour A	96-Hour B
					pH					
950	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
570	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
340	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
210	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
120	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Control	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
					Dissolved Oxygen Concentration, mg/L (% Saturation)					
950	9.8 (91)	9.6 (89)	9.6 (87)	9.4 (85)	9.0 (83)	9.3 (86)	9.8 (89)	9.6 (87)	9.0 (83)	9.1 (84)
570	9.6 (89)	9.7 (90)	9.6 (87)	9.6 (87)	9.2 (85)	9.0 (83)	9.7 (88)	9.4 (85)	9.1 (82)	8.9 (82)
340	9.8 (91)	9.8 (91)	9.6 (87)	9.4 (85)	9.3 (86)	9.4 (87)	9.6 (87)	9.6 (87)	9.1 (82)	9.1 (82)
210	9.8 (91)	9.7 (90)	9.5 (86)	9.4 (85)	9.4 (87)	9.4 (87)	9.8 (89)	9.7 (88)	9.6 (87)	9.0 (81)
120	9.8 (91)	9.8 (91)	9.4 (85)	9.6 (87)	9.0 (83)	9.2 (83)	9.6 (87)	9.7 (88)	8.9 (81)	9.0 (81)
Control	9.6 (89)	9.7 (90)	9.5 (86)	9.6 (87)	9.1 (84)	9.2 (83)	9.5 (86)	9.6 (87)	8.9 (82)	8.9 (81)
					Temperature (°C)[a]					
	12		11		11 - 12		11		11 - 12	

[a] Value presented represents the daily range of temperature measured (Brooklyn alcohol thermometer) in all test concentrations and the controls at the stated observation interval. Continuous monitoring of replicate B of the dilution water control established a test solution temperature range of 11 - 13 °C throughout the exposure period.

Table 2. **Concentrations of TAME measured in replicate (A,B) test solutions during the 96-hour flow-through exposure of rainbow trout (*Oncorhynchus mykiss*).**

Nominal Concentration (mg A.I./L)	0-Hour Measured Concentration (mg A.I./L)		96-Hour Measured Concentration (mg A.I./L)		Mean Measured Concentration[a] (mg A.I./L)
	A	B	A	B	
950	740	700	560	580	640 (87)
570	510	110[b]	560	610	560[c] (51)
340	280	340	320	280	310 (29)
210	150	150	160	140	150 (6.7)
120	56	89	76	93	78 (17)
Control	< 5.3	< 5.3	< 5.2	< 5.2	
QC #1[d]	1215 (950)[e]		898 (950)		
QC #2	357 (350)		454 (350)		
QC #3	128 (120)		118 (120)		

[a] Mean measured concentrations are presented with the standard deviations in parentheses and were calculated using the unrounded analytical results and not the rounded (two significant figures) values presented in this table.

[b] The lower than expected concentration for this sample is due to an error during the analytical process and is not considered representative of exposure conditions. This value was not included in the calculation of the mean measured concentration.

[c] N = 3

[d] QC = Quality Control sample.

[e] Value in parentheses represents the nominal fortified concentration for the corresponding QC sample.

Table 3. **Mean measured concentrations tested, corresponding mortalities and observations made during the 96-hour flow-through exposure of rainbow trout (*Oncorhynchus mykiss*) to TAME.**

Mean Measured Concentration (mg A.I./L)	Cumulative Mortality (%)											
	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
640	100	70	85[a]	100	70	85[d]	100	100	100	100	100	100
560	30	0	15[bc]	40	0	20[ce]	40	0	20[d]	40	20	30[d]
310	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0

[a] All of the surviving fish exhibited complete loss of equilibrium,
[b] Several of the surviving fish exhibited complete loss of equilibrium.
[c] Several of the surviving fish were observed to be lethargic.
[d] All of the surviving fish exhibited darkened pigmentation and complete loss of equilibrium.
[e] Several of the surviving fish exhibited darkened pigmentation and complete loss of equilibrium.

Table 4. **The LC50 values (95% confidence interval) and No-Observed-Effect Concentration for rainbow trout (*Oncorhynchus mykiss*) exposed to TAME under flow-through conditions.**

LC50 (mg A.I./L)[ab]				No-Observed-Effect Concentration Through 96 Hours (mg A.I./L)[a]
24-Hour[c]	48-Hour[c]	72-Hour[d]	96-Hour[de]	
600 (580 - 620)	600 (570 - 620)	580 (560 - 640)	580 (310 - 640)	310

[a] Based on mean measured concentrations of TAME (as active ingredient).
[b] Corresponding 95% confidence interval is presented in parentheses.
[c] LC50 value (95% confidence interval) calculated by probit analysis.
[d] LC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.
[e] Since the 96-hour LC50 value was not less than 50% of the 48-hour value, the study was not extended beyond 96-hours to determine the incipient LC50.

FIGURES

Figure 1. **Graphical illustration of the relationship between mean measured concentrations (analyses at 0- and 96-hours) and the nominal treatment levels established during the 96-hour flow-through exposure of rainbow trout (*Oncorhynchus mykiss*) to TAME.**

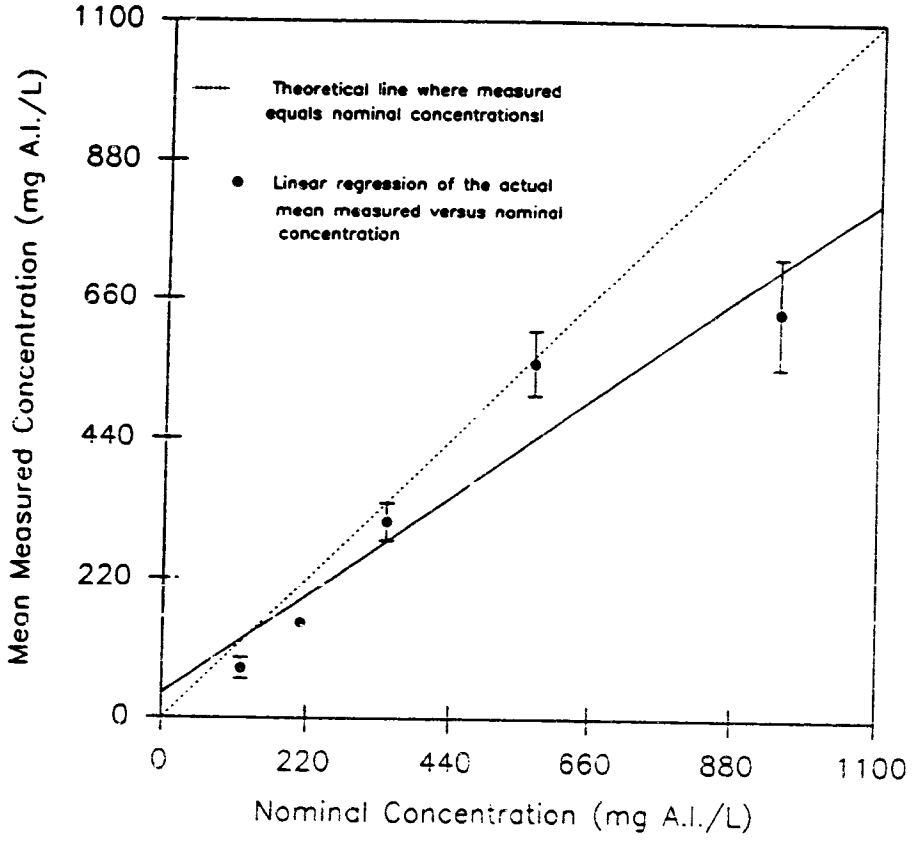
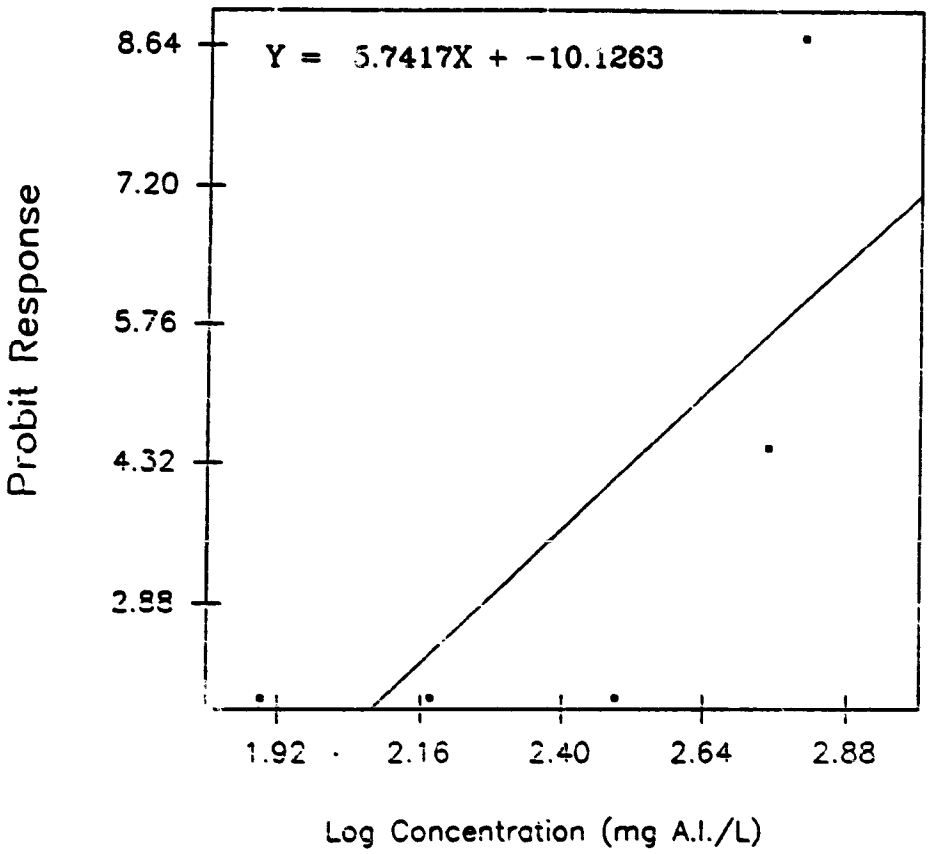


Figure 2. The 96-hour concentration-response (mortality) curve for rainbow trout (*Oncorhynchus mykiss*) exposed to TAME.



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This final report has been signed in accordance with SLI SOP No. 4.3.07(1).