American Petroleum Institute

STEP Strategies for Today's Environmental Partnership

Health and Environmental Sciences Department

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

TSCA Guideline §797.1930

FEBRUARY 1995

TOXICOLOGY REPORT NUMBER 407
CAIS ABSTRACT NO. 42-1520

One of the most significant long-term trends affecting the future vitality of the petroleum industry is the public's concerns about the environment. Recognizing this trend, API member companies have developed a positive, forward-looking strategy called STEP: Strategies for Today's Environmental Partnership. This program aims to address public concerns by improving our industry's environmental, health and safety performance; documenting performance improvements; and communicating them to the public. The foundation of STEP is the API Environmental Mission and Guiding Environmental Principles.

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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
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. 94-5-5269

Study Sponsor: American Petroleum Institute

Study Director: Mark W. Machado

Final Report Modifications and / or Additions:

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

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

Approval Signatures:

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

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

TSCA Guideline § 797.1930

Submitted to:

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SLI Report #94-5-5269

SLI Study #12827.0394.6110.510

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29 December 1994

AMENDED FINAL REPORT

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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

SPRINGBORN LABORATORIES, INC.

Mark W. Machado 12/29/94

Mark W. Machado Date
Study Director

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SUMMARY

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

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

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 $\pm$ 11% for filtered seawater. Conditions and procedures used throughout the analysis of exposure solution samples and QC samples during this study were similar to those described in Appendix V.

4.0 STATISTICS

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

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

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 12/29/94
Mark W. Machado Date
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

REFERENCES

APHA, AWWA, WPCF. 1989. *Standard Methods for the Examination of Water and WasteWater.* 17th Edition, Washington, D.C.

ASTM, 1985. Standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. Standard E1022-84. American Society for Testing and Substances, 1916 Race Street, Philadelphia, PA 19103.

ASTM, 1989. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-88a. American Society for Testing and Substances, 1916 Race Street, Philadelphia, PA 19103.

Reitsema. L.A. and J.M. Neff. 1980. A recirculating seawater system for the laboratory culture of *Mysidopsis bahia* (Crustacea: Pericaridea). *Estuaries* 3(4):321-323.

U.S. EPA. 1975. Methods for Acute Toxicity Tests with Fish, Microinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009). 61 pp.

U.S. EPA. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. October 1982. EPA-540/9-85-024.

U.S. EPA. 1985. Standard evaluation procedures for acute toxicity test for estuarine and marine organisms. Hazard Evaluation Division, Office of Pesticide Programs. Draft June 7, 1985.

U.S. EPA. 1989. *Toxic Substances Control; Good Laboratory Practice Standards;* Final Rule (40 CFR, Part 792) Federal Register, Part III, 48(230): 53922-53944, August 17, 1989.

TABLES

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
1.6	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)
1.6	7.7 (105)	7.8 (109)	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 (°/oo)[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			96-Hour		
	A	B	Mean	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	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]	10 (1)	0 (0)	5[e]
5.0	0 (0)	0 (0)	0	30 (3)	0 (0)	15[fg]	30 (3)	0 (0)	15[fg]	30 (3)	10 (1)	20[ef]
9.5	0 (0)	0 (0)	0	10 (1)	0 (0)	5[h]	10 (1)	10 (1)	10[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]	70 (7)	50 (5)	60[b]
35	0 (0)	0 (0)	0[b]	30 (3)	40 (4)	35[b]	80 (8)	90 (9)	85[b]	90 (9)	100 (10)	95[b]
65	0 (0)	20 (2)	10[c]	40 (4)	30 (3)	35[i]	90 (9)	90 (9)	90[j]	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)[ab]				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.**

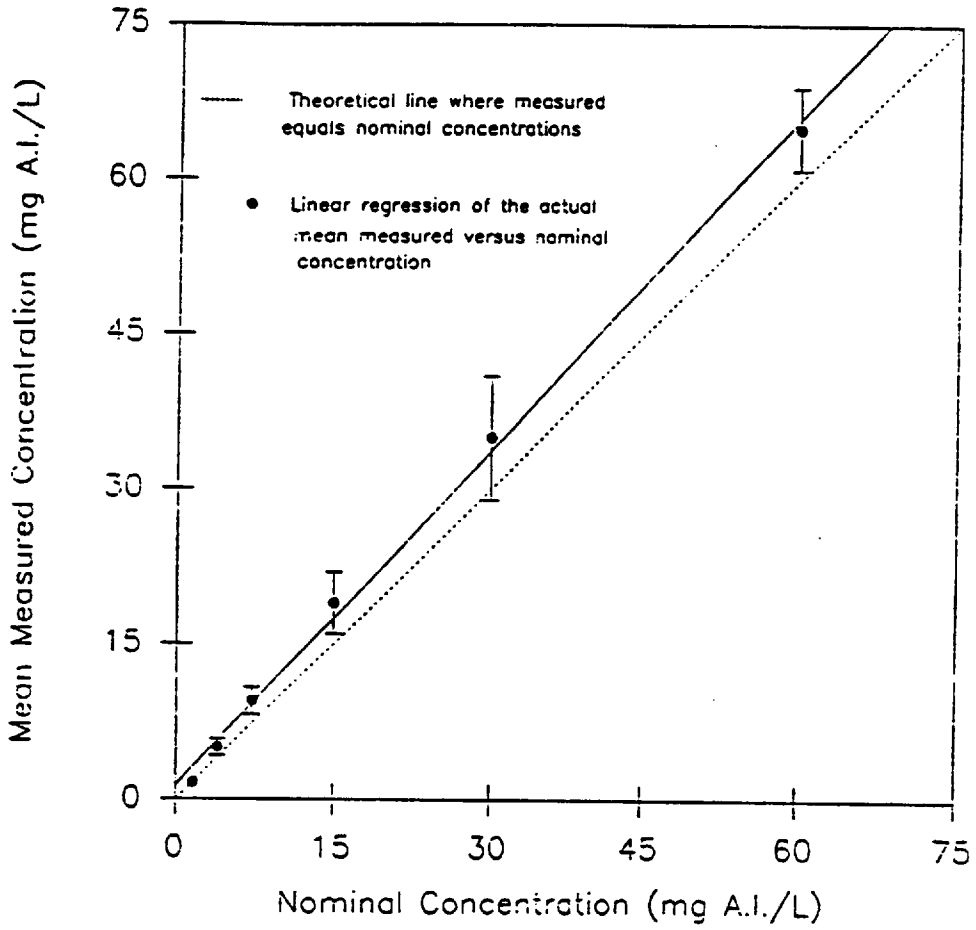
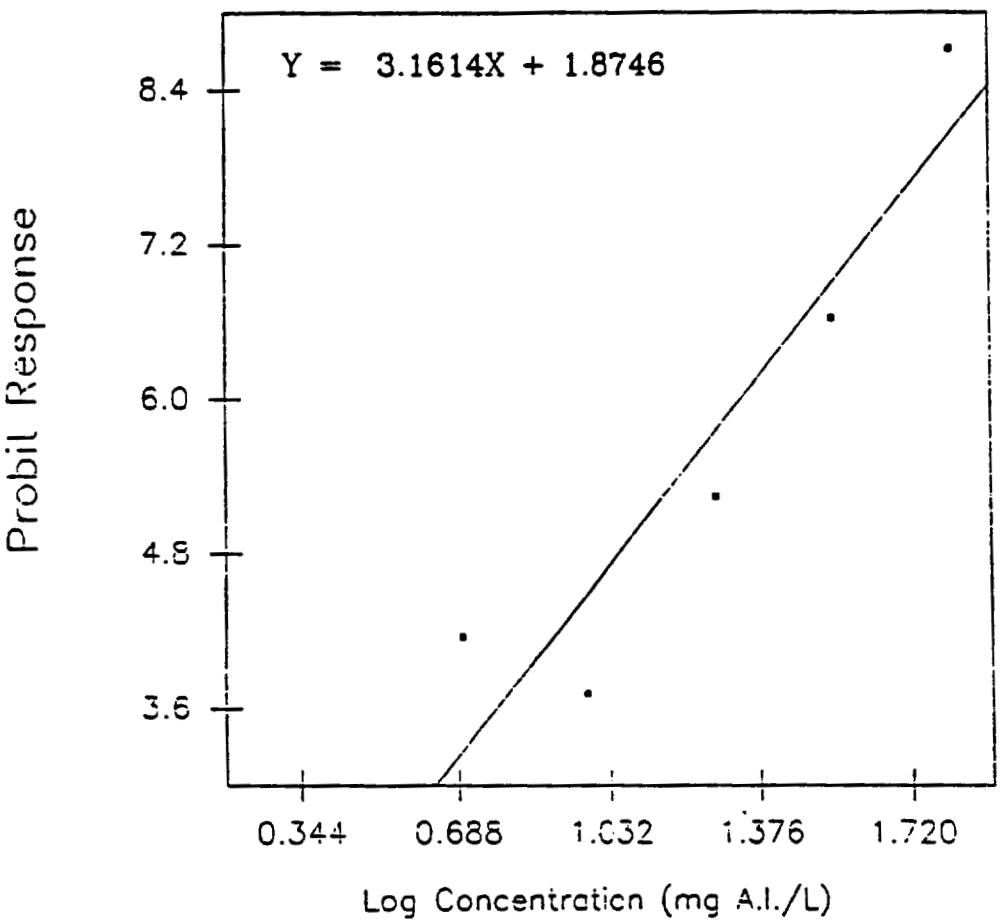


Figure 2. **The 96-hour concentration-response (mortality) curve for the static renewal toxicity test exposing mysid shrimp (*Mysidopsis bahia*) to TAME.**



SIGNATURES AND APPROVAL

SUBMITTED BY:

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

PREPARED BY:

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

Robin H. Dyer — Date 12/29/94 for RHD
Principal Investigator

Mark DaSilva — Date 12-29-94
Analytical Chemist

Lisa M. Thibault — Date 29 Dec 94
Coordinator, Data Management
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APPROVED BY:

Donald C. Surprenant — Date 12/29/94
Program Manager
Environmental Toxicology

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

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

6.0 APPENDIX I - STUDY PROTOCOL

FROM:API-HESD WASH DC TO:67005150829581O7 APR 25, 1994 9:48AM #625 P.01

RE

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street • Wareham, Massachusetts 02571-1075 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: TAME - Acute Toxicity to Mysids (*Mysidopsis bahia*) Under Static-Renewal Conditions, Following TSCA Guideline 797.1930.

TO BE COMPLETED BY THE STUDY SPONSOR:

Study Sponsor: American Petroleum Institute

Address: 1220 L Street Northwest, Washington D.C. 20005

Phone: (202) 682-8300

Sponsor Protocol/Project No.: ____

Test Substance: Tert-Amyl Methyl Ether (TAME) Purity: 94% CAS# or LOT#: [illegible]

Analytical Standard: ____ Purity: ____ CAS# or LOT#: ____

Additional Comments and/or Modifications: ____

[signature] 4/25/94
Sponsor Approval Date

TO BE COMPLETED BY SPRINGBORN LABORATORIES, INC., PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc., 790 Main Street, Wareham, MA 02571

Study Director: MARK W. MICHAEL Study Number: 12727-0394-6110-~~[illegible]~~ ① 510

Test Concentrations: *

Carrier Used: * CAS# or LOT#: *

Proposed Experimental Schedule: (Start) * (Completion) * (Draft Report) *

[signature] 4/25/94
Study Director Date

*To be provided by protocol amendment.

① INCORRECT ENTRY. MWM 5/16/94.

Springborn

TAME - ACUTE TOXICITY TEST TO MYSIDS (*Mysidopsis bahia*) UNDER STATIC-RENEWAL CONDITIONS, FOLLOWING TSCA GUIDELINE 797.1930.

1.0 OBJECTIVE

The purpose of this test will be to determine the 96-hour acute toxicity of a test substance to a representative marine invertebrate, the mysid (*Mysidopsis bahia*), under static-renewal conditions. During the conduct of the study, all test solution renewals will be performed at 24-, 48- and 72-hours of exposure. LC50 values with 95% confidence limits and a No-Observed-Effect Concentration (NOEC) of TAME to mysids will be determined. 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) will be followed during the conduct of the study.

2.0 STUDY DESIGN

Groups of ten mysids (twenty mysids per treatment level and control) will be exposed in a static system for 96 hours to various concentrations of TAME. During the conduct of the study, each exposure vessel will be sealed, maintaining little or no headspace, in an effort to minimize volatilization of the test substance. Dilution water controls will be included. Test concentrations will be selected based upon results of range-finding experiments. During the course of the study, water quality will be monitored and daily observations for visible abnormalities and mortality will be made and recorded. All test solutions will be renewed at 24-, 48 and 72-hours of exposure. The concentration of the test substance in each vessel will be verified by a GC - purge and trap analytical method. At the end of the 96-hour exposure period, an LC50 and a No-Observed-Effect Concentration (NOEC) will be determined.

3.0 MATERIALS AND METHODS

3.1 CHEMICAL SYSTEM

3.1.1. Test Substance. Upon arrival at Springborn Laboratories, Inc., the external packaging of the test substance will be inspected for damage. The packaging will be removed and the primary storage container will also be inspected for leakage or damage. The sample identity and percent activity will be recorded and, unless different arrangements are made with the Study Sponsor, the test substance will be stored in the dark at approximately 20°C until used. The Study Sponsor will be responsible for the characterization of the test substance.

3.1.2. Test Substance Concentration Selection. Test substance concentrations will be based on the results of a preliminary range-finding test. The preliminary range- finding test will be conducted with both newly hatched (≤ 24 hours old) and young adult (5 - 6 days old) mysids. A minimum of ten mysids will be exposed to a series of widely spaced concentrations of test substance. The age class which is most sensitive to the test substance will be used to perform the definitive test. If no apparent differences are found, ≤ 24 hours old mysids will be used. The range of concentrations selected for the definitive test is intended to include both 100% effect and no-effect levels, but due to the nature of some test substances, one or both levels may not be observed. No attempt will be made to determine the degree of adsorption of the test substance by the test system, as this falls outside the scope and intent of this study. Six test concentrations and a negative control will be used. Each test substance concentration will be 1.5 to 2 times the concentration of the next lower concentration of test substance. A negative control consists of dilution water without the test substance.

3.1.3. Stock Solution Preparation. The test substance will be weighed on an analytical balance for which a calibration log is maintained. A Chemical Usage Log will also be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used.

3.1.4. Exposure Solution Preparation. Each replicate solution of TAME will be prepared by adding the test substance directly into each respective test vessel. Each test vessel will be filled with dilution water to approximately 90% capacity and an appropriate aliquot of the test substance will be added. The solutions will be gently stirred for approximately 30 seconds, the test vessel will be filled to capacity with dilution water (no headspace) and then sealed with a screw-top lid. The procedure will be followed at test initiation and at the 24-, 48- and 72-hour renewal.

3.2 TEST ORGANISMS

3.2.1. Species. Mysids, *Mysidopsis bahia*, will be used to conduct this acute toxicity test. Test organisms will be ≤ 24 hours old or 5 to 6 days old at the initiation of the test. Mysids will be obtained from in-house cultures by isolating sexually mature adults prior to initiating the test. Young produced by these isolated adults will be collected and subsequently pipetted into the test vessel.

3.2.2. Justification of Species. Characteristics which make this test organism suitable for acute toxicity testing are their ease of culturing and handling, their sensitivity to a variety of chemical substances, and the extensive data base for this common marine invertebrate.

3.2.3. Origin and Acclimation. Mysid cultures will be maintained at Springborn Laboratories, Inc. Water used to culture mysids will be similar to the characteristics described for dilution water. Culture water will be maintained at 25 ± 2°C.

3.2.4. Feeding. Mysids will be fed live brine shrimp nauplii, *Artemia salina*, at least twice daily prior to and once daily during the 96-hour test. Periodic analyses of representative samples of the food will be conducted to ensure the absence of potential toxicants,

including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to mysids.

3.2.5. Handling. Fire-polished, wide-bore pipets will be used to transfer mysids, taking care to minimize possible stress due to handling. Mysids that are damaged or dropped during transfer will not be used.

3.2.6. Loading. Biomass loading will not exceed 30 mysids per liter of test solution.

3.3 PHYSICAL SYSTEM

3.3.1. Test Vessels. Test vessels will be 940 mL glass jars. The test vessels will be chemically cleaned before the test is started following standard laboratory procedures. The vessels will be washed with hot water and a detergent, rinsed with acetone, and then rinsed extensively with water. The test vessels will be completely filled with test solution (no headspace) and covered with a metal screw-top lid with a teflon liner to minimize evaporation and loss of test substance due to volatilization. The test solution volume in each replicate vessel will be 940 mL. Test vessels will be labeled to identify the treatment/control and the replicate designation.

3.3.2. Replication and Control of Bias. Two replicates will be included with each exposure concentration and control. Test vessels will be positioned impartially inside a waterbath. Each replicate vessel will contain ten mysids (20 mysids per concentration and control). Mysids will be added impartially to the test vessels, two at a time, until each vessel contains ten mysids.

3.3.3. Dilution Water. Natural filtered seawater from Cape Cod Canal will be used as dilution water for the test. The water will be filtered through a series of polypropylene core filters as fine as 5-µm and heated to the required test temperature. The water is characterized as having a salinity range of 30 to 35 °/oo and a pH range of 7.7 to 8.3. Salinity and pH of each new batch of seawater will be measured to ensure that these parameters are within the normal acceptable ranges. The salinity of the seawater used in the test will be adjusted to 20 °/oo ± 3 percent by diluting with laboratory dilution water. This water is routinely used in freshwater toxicity tests. Periodic analyses of representative samples of dilution water source and the freshwater used to adjust salinity will be conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the test organisms.

3.4 TEST CONDITIONS

3.4.1. Temperature. Water temperature of the test solutions will be maintained at 25 ± 2°C by placing the test vessels in a waterbath at the appropriate test temperature.

3.4.2. Dissolved Oxygen. Total dissolved oxygen concentration will not be allowed to drop below 60% or go above 105% of saturation during the test. Test solutions will not be aerated.

3.4.3. Salinity. Salinity of the seawater will be maintained at 20°/oo ± 3 percent

3.4.4. Lighting. Light intensity at the water surface will be 20 - 100 footcandles. Fluorescent bulbs will be used to provide lighting. The photoperiod will be maintained at 14 hours light:10 hours dark using an automatic timer.

3.4.5. Test Initiation. The test begins when all mysids have been impartially placed in the test vessels and terminates after 96 hours of exposure. Mysids will be fed once daily during the 96-hour exposure period.

3.4.6. Renewal Scheme. Fresh test solutions will be prepared every 24 hours at each treatment level and control. Test organisms will be carefully transferred into the freshly prepared test solutions at each renewal interval using a fire-polished wide-bore pipet.

3.5 SAMPLING AND OBSERVATIONS

3.5.1. Sampling. Samples from both replicate test vessels of each concentration and control will be taken at the initiation (new solutions) and termination (old solutions) of the test (0 and 96 hours) for determination of test substance concentrations. Water samples will be taken from a point approximately midway between the surface, bottom and sides of each test vessel and either analyzed immediately after sampling or appropriately preserved and stored until analysis can be performed.

Three quality control (QC) samples will be prepared at each sampling interval and stored and analyzed with the set of study samples. The QC samples will be prepared in diluent water at test substance concentrations similar to the treatment level range. Results of these analyses indicate the accuracy of the analytical method for measuring test substance concentration at each sampling period. The analytical method used to measure test substance concentration in the exposure solutions will be validated a Springborn Laboratories at the expected nominal concentration range prior to test initiation.

3.5.2. Water Quality Measurements. At test initiation and daily thereafter, water quality variables (temperature, pH, salinity and dissolved oxygen concentrations) will be measured in each test vessel. Measurement techniques will follow methods described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1989). The temperature range will be monitored continuously in one test solution by using a minimum-maximum thermometer. Readings of temperature extremes will be recorded daily.

3.5.3. Biological Observations. At the start of the test and at 24 hour intervals thereafter, observations of stress, abnormal behavioral activity and mortality will be made. Dead mysids will be removed from test solutions at these intervals. In addition, characteristics of the test solutions (such as precipitated materials, cloudiness, etc.) will be also observed and recorded.

3.5.4. Acceptability Criterion. During the definitive test, mortality or test organisms exhibiting abnormal benavior in the control must not exceed 10% at termination, or the test will be considered unacceptable.

4.0 STATISTICAL ANALYSIS

Mortality data derived from the acute test will be used to statistically estimate a median lethal concentration (LC50) and its 95% confidence interval after each 24-hour interval of exposure. The LC50 is the measured concentration of the test substance in dilution water which produces 50% mortality in the test population at the stated times of exposure. LC50 values will be computed using mean measured concentrations.

A computer program will be used to estimate LC50 values using one of three statistical methods: probit analysis, moving average method, or binomial probability. The method selected is determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.). An LC50 value cannot be calculated if the mortality data derived is insufficient according to any of the three statistical methods. The probit method provides values of the slope, including 95% confidence intervals, as well as appropriate statistical tests to evaluate goodness-of-fit. In addition, the highest test concentration that shows no difference from the control (No-Observed-Effect Concentration, NOEC) will be determined and reported.

5.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

6.0 REPORTING

The raw data and final draft of the report will be reviewed by the Quality Assurance Unit and the Study Director. Chemical and water quality measurements will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will be initially submitted to the Study Sponsor for review. Upon acceptance by the Sponsor, three copies of the final report will be submitted. All reports will include, but are not be limited to, the following information:

- Springborn Laboratories, Inc., report and project numbers and if applicable, Sponsor protocol and project numbers and the dates of when the definitive test was conducted.
- Laboratory and site, the dates of testing and personnel involved in the study, i.e., Quality Assurance Unit, Program Coordinator, Study Director, Principal Investigator.

- All information pertaining to the test substance which appears on the sample bottle, e.g., its empirical formula, molecular structure, source, percent active ingredient, physical properties, Sponsor's test substance I.D., and sample number if available.
- Characterization and origin of the dilution water.
- Scientific name of the test organisms, source and culturing information (including salinity and temperature).
- Exposure system description, dilution water volume, number of organisms per treatment, construction materials used, depth and volume of test containers, and test conditions.
- Description of the test substance delivery system and stock solution preparation.
- Information regarding test temperatures, dissolved oxygen concentration, pH, salinity, photoperiod and light intensity used.
- Observations of insolubility of the test substance, including the test levels and when observed.
- Definition of criteria used to determine sublethal effects and general observations on non-quantifiable effects.
- Number and percentage of organisms that showed lethality in the controls and in each treatment at each observation period, in tabular form.
- Description or reference (or inclusion as an appendix) to chemical and statistical procedures applied.
- Analytical results of test concentration measurements and QC samples.
- If applicable, means and standard deviations of measured concentrations of the test substance, as well as nominal test concentrations.
- The 24- 48- 72- and 96-hour LC50 with 95 percent confidence limits, and the No-Observed-Effect Concentration (NOEC).
- Concentration-response curves for mortality data collected at 24, 48, 72 and 96 hours.
- Deviations from the protocol not addressed in protocol amendments, together with a discussion of the impact on the study.
- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- Dates of Quality Assurance reviews, signed by the QA Unit.
- Location of the raw data and report.

7.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor. Protocol amendments and deviations must include the reasons for the change and the impact of the change on results of the study, if any. If necessary, amendments initially may be in the form of verbal authorization, followed by Springborn's written documentation of the amendment. In such a case, the effective date of the amendment will be the date of verbal authorization.

8.0 SPECIAL PROVISIONS

GOOD LABORATORY PRACTICES (GLP): All test procedures, documentation, records, and reports will comply with the U. S. Environmental Protection Agency's Good Laboratory Practices as promulgated under the Toxic Substance Control Act (FEDERAL REGISTER, Part III, 17 August, 1989)

TEST SUBSTANCE DISPOSAL: After 60 days from the issuance of the final test report for this or related studies, the test substance will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

ARCHIVAL: All raw data and the final report will be archived by the Study Sponsor unless different arrangements are made.

9.0 REFERENCES

APHA, AWWA, WPCF. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, Washington, DC.

U.S. Environmental Protection Agency. 1989. *Toxic Substances Control; Good Laboratory Practice Standards; Final Rule*. (40 CFR, Part 792) Federal Register, Part III, 48(230): 53922-53944, August 17, 1989.

U.S. Environmental Protection Agency. 1985. *Toxic Substance Control Act Test Guidelines*. Federal Register 50(188): 39252-39516, September 27, 1985. Amended May 20, 1987, July 1, 1991 and July 1, 1992.

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

PROTOCOL AMENDMENT

AMENDMENT #: 1

DATE: ~~25 April 1994~~ 17 MAY 1994 INCORRECT DATE CORRECTED BY HLM 12/28/94

PROTOCOL TITLE: "TAME - Acute Toxicity to Mysids (*Mysidopsis bahia*) Under Static-Renewal Conditions, Following TSCA Guideline 797-1930."

STUDY SPONSOR: American Petroleum Institute

TEST MATERIAL: Tert Amyl Methyl Ether (TAME)

SLI STUDY NO: 12827.0394.6110.~~596~~ 510 INCORRECT STUDY NUMBER CORRECTED BY HLM 12/28/94

AMENDMENT(S):

1. The following information has been provided as specified on page one of the protocol.

   Test Concentrations: 60, 30, 15, 7.3, 4.0, 1.6 and control.

   Carrier Used: NA CAS# or Lot#: NA

   Proposed Experimental Schedule:

   (Start) 5-3-94 (Completion) 5-7-94 (Draft Report) 5-27-94

Amendment:

The Study Protocol states that test organisms will be carefully transferred into the freshly prepared test solutions at each renewal interval using a fire-polished wide-bore pipet. In this study, the mysids will not be transferred. The solutions will be renewed by carefully siphoning the old solution out of the test vessels using an inverted glass powder funnel and a length of silastic tubing. The open end of the funnel will be covered with 363 micron Nitex screen attached with silicone adhesive to prevent removal of the mysids. Approximately 90% of the old solution will be removed leaving an adequate amount of solution remaining for the mysids. Freshly prepared solution will be added to the test vessel in the same manner by siphoning into the test vessel using the funnel.

Reason for Change:

Transferral of mysids is time-consuming and can be injurious due to the mysids' highly mobil activity. This change is instituted to reduce the risk of mortality to the mysids due to handling in transferral procedures.

Springborn Laboratories Protocol #: ~~942694~~ 942594/TSCA/510/TAME INCORRECT PROT. NUMBER CORRECTED BY HLM 12/28/94

Impact:

This change will not have an impact on the study.

Amendment:

The protocol states that each replicate solution of TAME will be prepared by adding the appropriate aliquot of test substance directly into each test vessel pre-filled to approximately 90% capacity with dilution water. The solutions will be gently stirred for approximately 30 seconds and then the vessel will be filled to capacity with dilution water and sealed with a screw-top lid to maintain zero headspace.

In this study, the procedure for preparation of the solutions was modified. The appropriate aliquot of test substance was added directly to the test vessel which was pre-filled with dilution water to approximately 90% of capacity. The test vessels were filled to capacity in order to maintain zero headspace, sealed with a screw-top lid and inverted several times.

Reason for Change:

An initial attempt at conducting a definitive study yielded analytical results which were inconsistent with expectation based on prior preliminary exposures with the test substance. Analytical results for this initial attempt were low (i.e., approximately 25% of nominal) and quite variable. This initial attempt was eventually aborted. Through additional analytical investigations it was determined that mixing of the solutions by simply inverting the test vessels several times yielded better dissolution of the test substance and resulted in a higher measured to nominal concentration ratio.

Impact:

This change is not expected to have had an impact on the study.

Approval Signatures: Mark W. Machado 5/17/94

Mark W. Machado
SLI Study Director

Richard A. Rhoden 6/2/94

Richard A. Rhoden, Ph.D.
Sponsor Study Monitor

042594/TSCA/510/TAME
~~042694/TSCA/510/TAME~~

INCORRECT PROTOCOL NUMBER CORRECTED BY MWM 12/28/94

Springborn Laboratories Protocol #: ~~070793/FDA 4.02~~

MWM 8/31/94

Springborn

7.0 APPENDIX II - CERTIFICATE OF ANALYSIS

aldrich chemical co.
chemists helping chemists in research & industry

SPRINGBORN LABORTORIES
508 295 8107
PAULA LECONTE

PO NBR:

PRODUCT INFORMATION

PRODUCT NUMBER: 28309-6 LOT NUMBER: 02814BZ

PRODUCT NAME: TERT-AMYL METHYL ETHER, 94%

FORMULA: C6H14O FORMULA WEIGHT: 102.18

APPEARANCE	COLORLESS LIQUID
REFRACTIVE INDEX AT 20 DEG C	1.3685
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS ILLUSTRATED ON PAGE 268A OF EDITION I, VOLUME 3 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".
GAS LIQUID CHROMATOGRAPHY	98.8 %

Aldrich Chemical Company
DAVID SWESSEL
NOVEMBER 11, 1992

aldrich chemical co.
P.O. Box 355, Milwaukee, Wisconsin 53201 USA • (414) 273-3850

aldrich chemical co.
chemists helping chemists in research & industry

928

AMER PETRO INST
202 682 8270
DR DICK RHODEN

PO NBR: 820003008A13

12527.0692.6052-45

PRODUCT INFORMATION

PRODUCT NUMBER: 28309-6 LOT NUMBER: 07905KZ

PRODUCT NAME: TERT-AMYL METHYL ETHER, 94%

FORMULA: C6H14O FORMULA WEIGHT: 102.18

APPEARANCE	COLORLESS LIQUID
REFRACTIVE INDEX AT 20 DEG C	1.3876
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS ILLUSTRATED ON PAGE 268A OF EDITION 1, VOLUME 3 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".
GAS LIQUID CHROMATOGRAPHY	98.7 %

Aldrich Chemical Company
DAVID SWEBSEL
NOVEMBER 6, 1992

aldrich chemical co.
P.O. Box 355, Milwaukee, Wisconsin 53201 USA • (414) 273-3850

8.0 APPENDIX III - CULTURE FOOD ANALYSIS

Hatched Brine Shrimp Grab Sample*		
Date Collected:7/28/93 Date Reported: 9/6/93		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 mg/kg	0.01
Beta BHC	< 0.01 mg/kg	0.01
Gamma BHC - Lindane	< 0.01 mg/kg	0.01
Delta BHC	< 0.01 mg/kg	0.01
Heptachlor	< 0.01 mg/kg	0.01
Aldrin	< 0.01 mg/kg	0.01
Heptachlor Epoxide	< 0.01 mg/kg	0.01
DDE	< 0.01 mg/kg	0.01
DDD	< 0.01 mg/kg	0.01
DDT	< 0.01 mg/kg	0.01
HCB	< 0.01 mg.kg	0.01
Mirex	< 0.01 mg/kg	0.01
Methoxychlor	< 0.05 mg/kg	0.05
Dieldrin	< 0.01 mg/kg	0.01
Endrin	< 0.01 mg/kg	0.01
Telodrin	< 0.01 mg/kg	0.01
Chlordane	< 0.05 mg/kg	0.05
Toxaphene	< 0.1 mg/kg	0.1
PCBs	< 0.2 mg/kg	0.2
Ronnel	< 0.01 mg/kg	0.01
Ethion	< 0.02 mg/kg	0.02
Trithion	< 0.05 mg/kg	0.05
Diazinon	< 0.1 mg/kg	0.1
Methyl Parathion	< 0.02 mg/kg	0.02
Ethyl Parathion	< 0.02 mg/kg	0.02
Malathion	< 0.05 mg/kg	0.05
Endosulfan I	< 0.01 mg/kg	0.01
Endosulfan II	< 0.01 mg/kg	0.01
Endosulfan Sulfate	< 0.03 mg/kg	0.03
Chlorpyrifos	< 0.01 mg/kg	0.01
* Analyzed by Lancaster Laboratories, Inc.		

Hatched Brine Shrimp Grab Sample*		
Date Collected: 7/28/93 Date Reported: 9/6/93		
Analysis	Result As Received	Limit of Quantitation
Mercury	< 0.30 mg/kg	0.30
Pesticide Screen [illegible]	attached	
Boron	< 20. mg/kg	20.
Thallium	< 100. mg/kg	100.
Aluminum	< 60. mg/kg	60.
Antimony	< 40. mg/kg	40.
Barium	< 20. mg/kg	20.
Beryllium	< 1.0 mg/kg	1.0
Cadmium	< 4.0 mg/kg	4.0
Calcium	243. mg/kg	60.
Chromium	< 8.0 mg/kg	8.0
Cobalt	< 10. mg/kg	10.
Copper	< 8.0 mg/kg	8.0
Iron	< 20. mg/kg	20.
Lead	< 20. mg/kg	20.
Magnesium	718. mg/kg	30.
Manganese	< 4.0 mg/kg	4.0
Molybdenum	< 10. mg/kg	10.
Nickel	< 10. mg/kg	10.
Potassium	1,100. mg/kg	100.
Silver	< 4.0 mg/kg	4.0
Sodium	8,010. mg/kg	200.
Titanium	< 2.0 mg/kg	2.0
Vanadium	< 4.0. mg/kg	4.0
Zinc	< 40. mg/kg	40.
* Analyzed by Lancaster Laboratories, Inc.		

9.0 APPENDIX IV - DILUTION WATER ANALYSIS

Filtered Seawater Grab Water Sample*		
Date Collected: 7/29/93 Date Reported: 9/17/93		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.3 µg/l	0.3
Toxaphene	< 4. µg/l	4.
PCBs	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
* Analyzed by Lancaster Laboratories, Inc.		

Filtered Sea Water Grab Water Sample*		
Date Collected: 8/9/93 Date Reported: 8/26/93		
Analysis	Result As Received	Limit of Quantitation
Mercury	< 0.00020 mg/l	0.00020
Arsenic	< 0.20 mg/l	0.20
Selenium	< 0.20 mg/l	0.20
Boron	3.20 mg/l	0.040
Thallium	< 0.30 mg/l	0.30
Aluminum	< 0.20 mg/l	0.20
Antimony	< 0.20 mg/l	0.20
Barium	< 0.10 mg/l	0.10
Beryllium	< 0.010 mg/l	0.010
Cadmium	< 0.010 mg/l	0.010
Calcium	294. mg/l	0.20
Chromium	< 0.050 mg/l	0.050
Cobalt	< 0.050 mg/l	0.050
Copper	< 0.020 mg/l	0.020
Iron	< 0.10 mg/l	0.10
Lead	< 0.10 mg/l	0.10
Magnesium	1,080. mg/l	1.0
Manganese	0.068 mg/l	0.010
Molybdenum	< 0.10 mg/l	0.10
Nickel	< 0.050 mg/l	0.050
Potassium	317. mg/l	0.50
Silver	< 0.020 mg/l	0.020
Sodium	9,620. mg/l	20.
Titanium	< 0.010 mg/l	0.010
Vanadium	< 0.010 mg/l	0.010
Zinc	< 0.040 mg/l	0.040
Total Organic Carbon ***	< 1. mg/l	1.
* Analyzed by Lancaster Laboratories, Inc.		
*** Represents "non-purgeable TOC"		

Well[1] Water Sample*		
Date Collected: 7/29/93 Date Reported: 9/17/93		
Pesticide Screen I;II;III	Result As Received	Limit of Quantitation
Alpha BHC	< 0.01 µg/l	0.01
Beta BHC	< 0.01 µg/l	0.01
Gamma BHC - Lindane	< 0.01 µg/l	0.01
Delta BHC	< 0.01 µg/l	0.01
Heptachlor	< 0.01 µg/l	0.01
Aldrin	< 0.01 µg/l	0.01
Heptachlor Epoxide	< 0.01 µg/l	0.01
DDE	< 0.01 µg/l	0.01
DDD	< 0.01 µg/l	0.01
DDT	< 0.01 µg/l	0.01
HCB	< 0.01 µg/l	0.01
Mirex	< 0.01 µg/l	0.01
Methoxychlor	< 0.05 µg/l	0.05
Dieldrin	< 0.01 µg/l	0.01
Endrin	< 0.01 µg/l	0.01
Telodrin	< 0.01 µg/l	0.01
Chlordane	< 0.3 µg/l	0.3
Toxaphene	< 4. µg/l	4.
PCBs	< 1. µg/l	1.
Ronnel	< 0.01 µg/l	0.01
Ethion	< 0.02 µg/l	0.02
Trithion	< 0.05 µg/l	0.05
Diazinon	< 0.1 µg/l	0.1
Methyl Parathion	< 0.02 µg/l	0.02
Ethyl Parathion	< 0.02 µg/l	0.02
Malathion	< 0.05 µg/l	0.05
Endosulfan I	< 0.01 µg/l	0.01
Endosulfan II	< 0.01 µg/l	0.01
Endosulfan Sulfate	< 0.03 µg/l	0.03
[1] Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		

Well[1] Water Sample*		
Date Collected: 8/9/93 Date Reported: 8/26/93		
Analysis	Result As Received	Limit of Quantitation
Mercury	< 0.00020 mg/l	0.00020
Arsenic	< 0.20 mg/l	0.20
Selenium	< 0.20 mg/l	0.2
Boron	< 0.040 mg/l	0.04
Thallium	< 0.30 mg/l	0.3
Aluminum	< 0.20 mg/l	0.2
Antimony	< 0.20 mg/l	0.2
Barium	< 0.10 mg/l	0.1
Beryllium	< 0.010 mg/l	0.01
Cadmium	< 0.010 mg/l	0.01
Calcium	7.71 mg/l	0.2
Chromium	< 0.050 mg/l	0.05
Cobalt	< 0.050 mg/l	0.05
Copper	< 0.020 mg/l	0.02
Iron	< 0.10 mg/l	0.1
Lead	< 0.10 mg/l	0.1
Magnesium	2.31 mg/l	0.1
Manganese	< 0.010 mg/l	0.01
Molybdenum	< 0.10 mg/l	0.1
Nickel	< 0.050 mg/l	0.05
Potassium	1.07 mg/l	0.5
Silver	< 0.020 mg/l	0.02
Sodium	14.0 mg/l	0.4
Titanium	< 0.010 mg/l	0.01
Vanadium	< 0.010 mg/l	0.01
Zinc	<0.040 mg/l	0.04
Total Organic Carbon ***	< 1. mg/L	1.
[1] Well water supplemented by Town of Wareham water		
* Analyzed by Lancaster Laboratories, Inc.		
*** Represents "non-purgeable TOC"		

10.0 APPENDIX V - ANALYTICAL METHODOLOGY

SUMMARY

An analytical methodology is presented for the measurement of TAME (Tert-Amyl Methyl Ether) in AAP media, filtered seawater and freshwater (reconstituted to increase hardness). All water samples were analyzed either by direct sampling into a purge and trap liquid sample concentrator or vial sampling system. The water phase was stripped of TAME with a high flow of helium gas and trapped on an active support substance. The TAME was then thermally desorbed from the support and transferred through a heated line onto a gas chromatograph for separation and quantitation. TAME was detected utilizing a flame ionization detector. Quantitation was performed using various fitting techniques both on and off the instrument.

Mean recovery from AAP media was 89.7 ± 2.3%, 104 ± 11% for filtered seawater and 102 ± 5% for freshwater, however, the analyte purging efficiency from a hard reconstituted water matrix presents a greater degree of instrumental variability. Therefore the standard deviation acceptance criteria has been increased to 10% to more acutely represent the recovery data. Repeatability of TAME analysis showed a 5.4% relative standard deviation (%RSD) at 0.026 mg/L from water.

EQUIPMENT AND REAGENTS

Equipment

1. Balance: Mettler AE 200 182, four-place analytical
2. Volumetric flask: grade A, assorted sizes
3. Wheaton vials with teflon-lined crimp top lids, assorted sizes
4. Syringes: Hamilton, assorted sizes, gas tight and valved
5. Absorbent Trap: 25 cm x 0.125 O.D. stainless steel column packed with 1 cm 3% OV -1, 15 cm tenax and 8 cm silica gel.
6. Purge and Trap Liquid Sample Concentrator: Tekmar model LSC-2000
7. Vial Sampling System: Tekmar Model ALS2050
8. Gas chromatograph: Hewlett-Packard 5890A equipped with a capillary injection port and 105 m x 0.53 mm I.D. 3 μm film RT_x 502.2 column and Flame Ionization detector.

Reagents

1. Methanol: reagent grade solvent
2. TAME: Lot # 02814BZ, was received from Experimental Pathology Labs, Inc., on 17 August 1992 and was identified by the Aldrich Chemical Company to be 98.8% pure.
3. Water: All solutions were prepared using water from a Sybron/Barnstead NANOpure II® (meets ASTM Type IIA specifications) filtered and sterilized water purification system. The filtered sterilized water typically shows greater than 16.7 Mohm-cm resistivity and less than 1 mg/L total organic carbon.
4. AAP Media
5. Filtered seawater
6. Hard Reconstituted water

PROCEDURE

Preparation of Stock Solution

Primary standards were prepared by placing approximately nine and a half milliliters (mL) of methanol into a 10 mL volumetric flask. The flask was allowed to stand unstoppered to allow any methanol along the neck to evaporate and was weighed to the nearest 0.1 milligram (mg). TAME was immediately added to the flask using a microliter syringe, making sure the primary substance fell directly into the alcohol. The vessel was reweighed, diluted to the mark, stoppered, and finally mixed by inverting the flask several times.

The solution was transferred to a 10 mL crimp top bottle with a Teflon lined lid and stored in a refrigerator until used. This stock was used with further dilution for sample fortification and standard(s) preparation. All stock solutions and dilutions were stored in Wheaton vials with Teflon lined crimp tops in a refrigerator.

Preparation of Standards for Purge & Trap

Secondary standards (104, 26.0 and 5.20 mg/L in methanol) were drawn into a microliter syringe and spiked directly into water in a 5 mL gas tight Luer lock syringe. These aqueous standards were added directly to the purge vessel and analyzed immediately. Calibration and

check standards were prepared just prior to analysis. Standards were prepared in a 5 mL gas-tight syringe using TAME working standards. Examples of formulation working standard formulation are outlined below:

Stock Concentration (mg/L)	Volume Taken (μL)	Nominal Concentration (mg/L)
5.20	25.0	0.026
26.0	25.0	0.130
26.0	50.0	0.260
26.0	100	0.520
26.0	250	1.30
104	250	5.20
104	500	10.4

Sample Fortification

Method validation/recovery samples were prepared using AAP media, filtered seawater and freshwater (reconstituted to increase hardness). Samples were fortified with dilutions of the TAME stock in volumetric flasks and loaded onto a automatic liquid sample autosampler (LSC 2050). The fortified levels produced were 0.052, 4.16 and 10.4 mg/L TAME in AAP media, 0.026, 4.16 and 10.4 mg/L in filtered seawater and 49.7, 248 and 695 mg/L in freshwater (reconstituted to increase hardness). Three replicates at each level were prepared for each experiment along with three unfortified matrix blanks.

Liquid Sampler

Samples were loaded into 40 mL vials. Vials were placed in vial sampler. Five milliliters sample was transferred from the vial samples into the purge vessel attached in-line with the activated sorbent support matrix (EPA method 624 trap) and the stripping program initiated with a high flow of helium (60 mL/min) bubbled through the vessel. The sorbent trapped gaseous TAME from the helium carrier gas. This approach was effective because the compound is highly volatile. After the water phase had been stripped for four or six minutes, the sorbent trap was heated and TAME stripped into the carrier and brought through a heated capillary transfer line

(0.53 mm I.D fused silica) onto the top of the gas chromatographic column located in a capillary injection port of the gas chromatograph.

TAME was separated chromatographically using a temperature program after splitless injection from the purge and trap liquid sample concentrator.

Liquid Sample Concentrator: Tekmar LSC-2000.

Programmed Purge & Trap Conditions

Standby Temperature: 40 ° C

	Time (minutes)	Temperature (° C)
Purge:	4 or 6	< 40
Desorption Preheat:	NA	175
Desorption:	4.0	180
Bake:	8.0	225

Heating Zones

	Temperature °C
Valve:	200
Mount:	40
Transfer Line:	200

Gas Chromatography

Gas chromatographic analysis was conducted utilizing a directly coupled liquid sample concentrator (purge and trap) into the capillary injection port. The samples were introduced by programmed injection from the purge and trap. The refocusing of sample entered the column occurred at the head of the column as a function of the film thickness of the RT_x 502.2 column.

Gas Chromatograph: Hewlett Packard 5890A gas chromatograph equipped with a split/splitless capillary injection port operated in the splitless mode.

Column:	105 m x 0.53 mm ID x 3 μm film
	Temperature (°C): Injector: 200
	column temperature programmed: 40 - 250
	Rate: 10 °C per minute from 40 to 70 °C
	25 °C per minute from 70 - 250 °C
Gas (mL/minute):	Helium
	Carrier Gas: ca. 9
Makeup gas(mL/minute):	Helium (28)
	Run Time: 16 minutes
	Retention Time: ca. 12.4 minutes

Integrator: Hewlett Packard 3396A II programmable integrator

Analysis

TAME was analyzed utilizing purge and trap thick film capillary (0.53 mm I.D.) gas chromatography flame ionization detection (GC/FID). Water samples were loaded onto the purge vessel (5 mL) of the LSC-2000 using a 5 mL gas tight syringe or vial transfer line from the vial sampler. The purge program was initiated and the systems allowed to sequence through the preprogrammed methods (purge and trap, gas chromatograph and integrator).

RESULTS AND DISCUSSION

Analytical results for the recovery of TAME from AAP media, filtered seawater and freshwater (reconstituted to increase hardness) are presented in Table 1A, 2A and 3A, respectively. System performance was tested for system repeatability in water. Results of repeatability studies are presented in Table 4A. Run time for samples was approximately 27 minutes. Samples were introduced through the capillary injection port operated in the splitless mode onto the gas chromatographic column. The split vent was closed for the 4 minutes of

desorb on the purge and trap. Figure 1A is a representative chromatogram of TAME analysis by purge and trap GC-FID.

TAME analysis was generally linear (correlation coefficient, r^2, greater than 0.98) from 0.25 mg/L TAME in water through 5.0 mg/L (Figure 2A). Detector response was not linear, rather there is a notable curve apparent in detector response from 0.026 though 10.4 mg/L TAME (Figure 3A). The integrator had software to fit calibration data to polynomial fit. Recovery samples for AAP media and filtered seawater were calculated using a least squares polynomial analysis performed on the height response. Recovery from freshwater (reconstituted to increase hardness) samples were calculated using a least squares linear regression analysis performed on the height response.

The reports generated by the integrator were categorized in a report with concentration (mg/L) calibrated from a 5-mL sample. Check standards were evaluated periodically and providing up-to-date evaluation of system calibration. Calibration was monitored utilized a series of stock standards in methanol. Evaluation was based on the trend of results and the reported value for that standard. Working standards were prepared around the concentration range of interest and stored along with other operating information on the integrator. Calibration could be conducted using linear, polynomial or point to point fitting techniques.

Table 1A. Analytical results for the recovery of TAME from AAP media.

Nominal Concentration (mg/L)	Volume Purged (mL)	Concentration Recovered (mg/L)	Percent Recovered (%)
10.4	5.00	8.92	85.8
10.4	5.00	9.17	88.1
10.4	5.00	9.39	90.3
4.16	5.00	3.79	91.1
4.16	5.00	3.88	93.2
4.16	5.00	3.84	92.3
0.052	5.00	0.0462	88.9
0.052	5.00	0.0462	88.9
0.052	5.00	0.0462	88.9
Control	5.00	< 0.026	NA
Control	5.00	< 0.026	NA
Control	5.00	< 0.026	NA

Mean Recovery: 89.7 ± 2.3%

The minimum detectable concentration was 0.026 mg/L for a 5.00 mL sample which is the lowest standard used in the polynomial fit.

Table 2A. Analytical results for the recovery of TAME from filtered seawater.

Nominal Concentration (mg/L)	Volume Purged (mL)	Concentration Recovered (mg/L)	Percent Recovered (%)
10.4	5.00	10.0	96.3
10.4	5.00	12.1	116
10.4	5.00	12.1	117
10.4	5.00	11.9	114
4.16	5.00	3.79	91.1
4.16	5.00	3.78	90.9
4.16	5.00	3.79	91.2
0.026	5.00	0.027	105
0.026	5.00	0.027	105
0.026	5.00	0.028	109
Control	5.00	< 0.026	NA
Control	5.00	< 0.026	NA
Control	5.00	< 0.026	NA

Mean Recovery: 104 ± 11%

The minimum detectable concentration was 0.026 mg/L for a 5.00 mL sample which is the lowest calibration standard used in the polynomial fit.

Table 3A. Analytical results for the recovery of TAME from freshwater (reconstituted to increase hardness).

Nominal Concentration (mg/L)	Dilution Factor	Volume Purged (mL)	Concentration Recovered (mg/L)	Percent Recovered (%)
695	200	5.00	694	99.8
695	200	5.00	693	99.6
695	200	5.00	705	101
248	100	5.00	268	108
248	100	5.00	258	104
248	100	5.00	265	107
49.7	20.0	5.00	50.9	102
49.7	20.0	5.00	44.9	90.3
49.7	20.0	5.00	51.7	104
Control	1.00	5.00	< 0.248	NA
Control	1.00	5.00	< 0.248	NA
Control	1.00	5.00	< 0.248	NA

Mean Recovery: 102 ± 5% (10)[1]

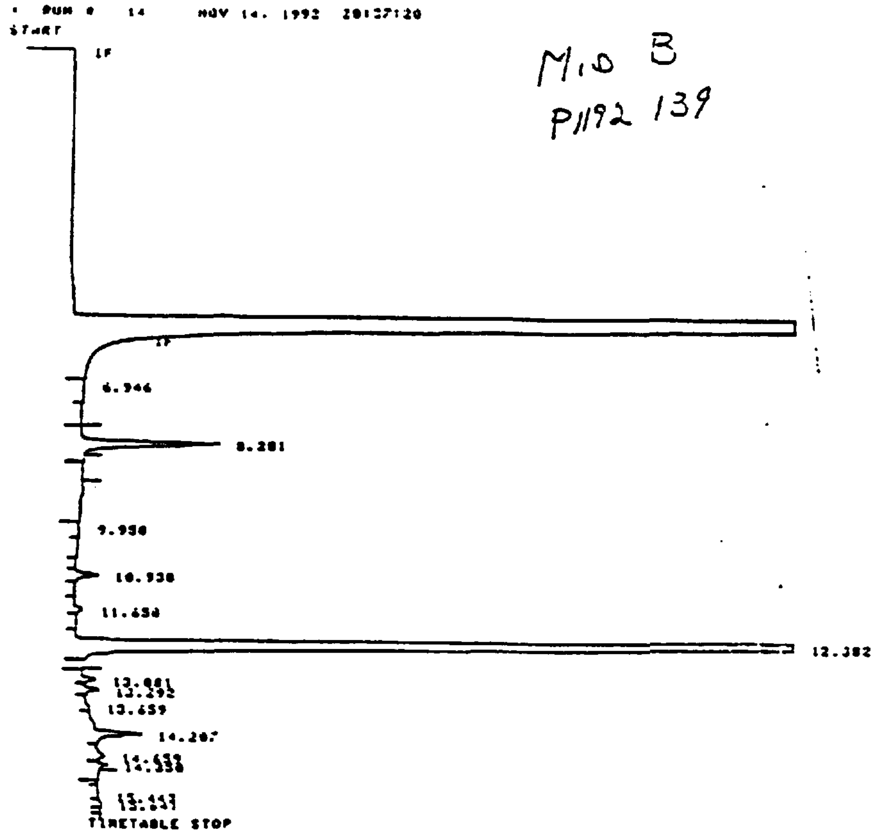
The minimum detectable concentration was 0.248 mg/L for a 5.00 mL sample which is the lowest standard used in the linear regression analysis.

[1] The analyte purging efficiency from a hard reconstituted water matrix presents a greater degree of instrumental variability. Therefore the standard deviation acceptance criteria has been increased to 10% to more accurately represent the recovery data.

Table 4A. Repeatability of TAME analysis from ASTM Type II water at 0.026 mg/L.

Replicate	Area	Height
1	47510	5725
2	54711	6099
3	46909	5631
4	36628	5646
5	36305	5699
6	55640	6292
7	54256	6365
Mean:	47423	5922
Std Dev.:	8243	320
% RSD:	17.4	5.4

Figure 1A. A representative chromatogram of TAME purge and trap GC/FID analysis.



RUN# 14 NOV 14, 1992 20:37:20

METHOD NAME: M-TAMEPGTP.MET

IDENTIFIER : 13140A39449

TAME 12.Dec.92

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMT/L	NAME
8.281	PB	156375	.107	24263		.000	
10.930	PP	18711	.082	3817		.000	
12.382	PB	2787170	.103	612842	1R	3.596	TAME
13.292	VP	11101	.071	2618		.000	
14.207	VV	69006	.129	9723		.000	
14.659	VV	17942	.164	1825		.000	

TOTAL HEIGHT= 654200
MUL FACTOR=1.0000E+00

Figure 2A. A representative linear regression analysis from standard TAME analysis.

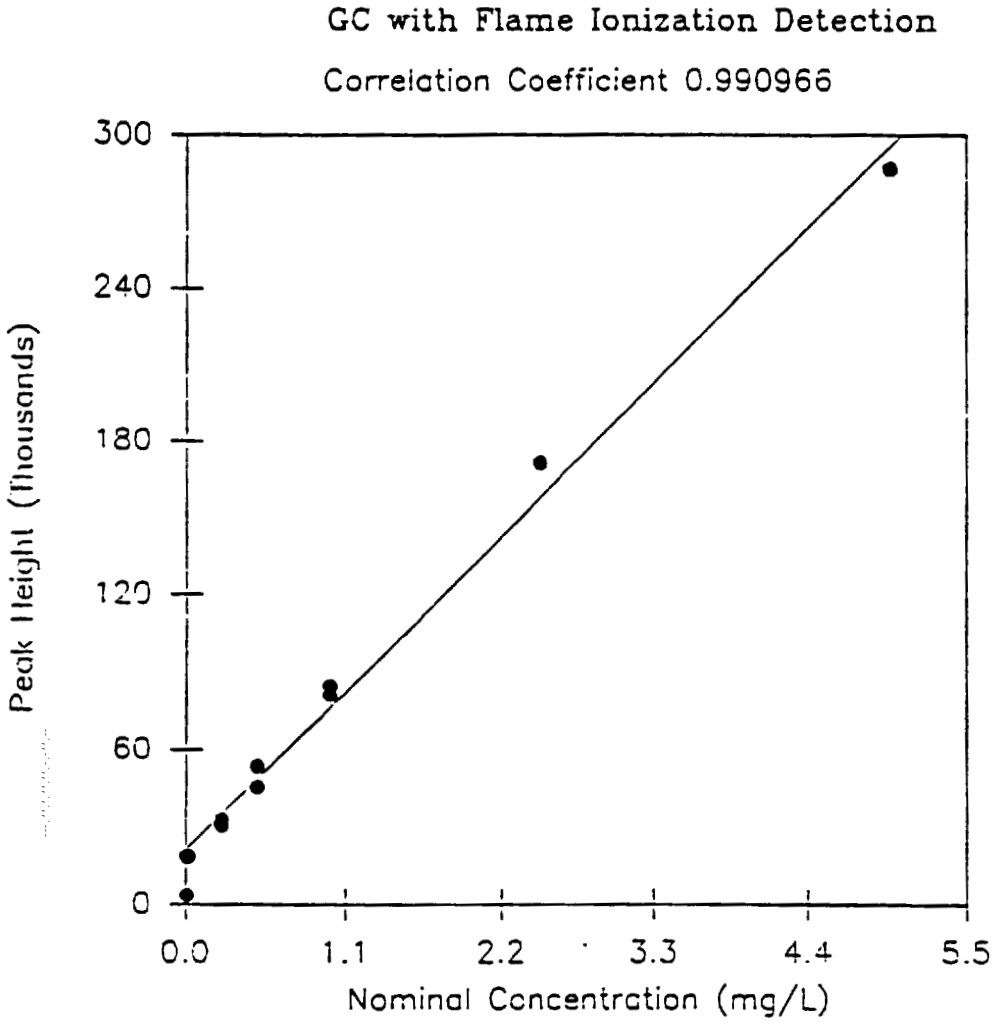


Figure 3A. **A representative polynomial regression analysis from standard TAME analysis.**

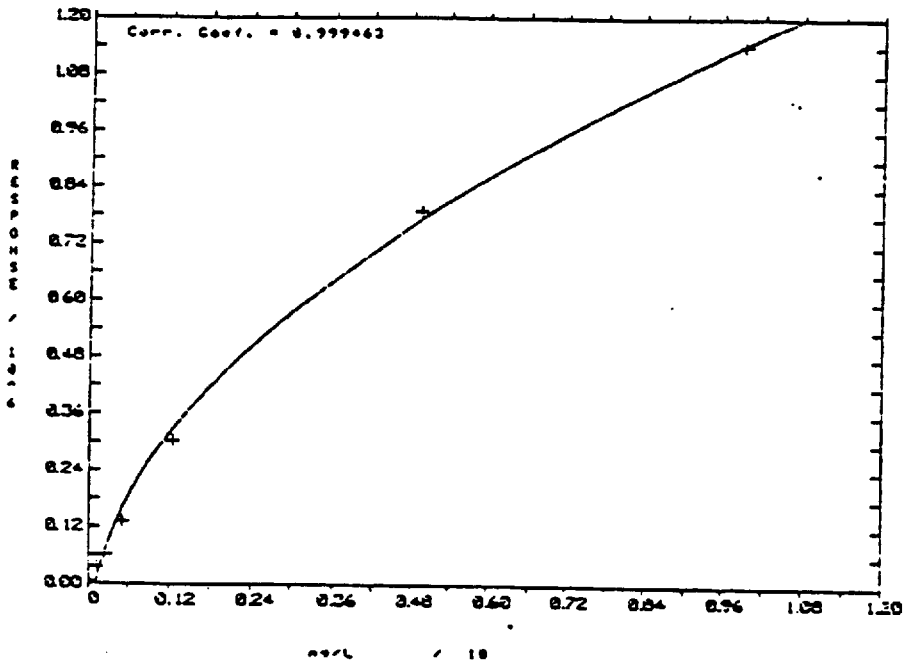
```
WELCOME TO THE HP3396 CALIBRATION CURVE PLOTTING PROGRAM Rev. 8.02.00

At any prompt:     '0' [ENTER] Quits
                   '9' [ENTER] Starts Over

Load which method or calib. file [Current active]:

Plot the calibration curve for which CAL # [All]:

                        ng/L        vs. Response for Cal # 1
ng/L        = +1.16E-01 +1.37E-06 (RESPONSE) +6.26E-12 (RESPONSE^2)
```




```
Plot additional points [Y=/N]: N

* EDIT CALIB #

1 = CALIB PROCEDURE
2 = RETENTION TIME WINDOWS
3 = TABLE ENTRIES
4 = PEAK GROUPS
5 = CALIB OPTIONS
```

11.0 APPENDIX VI - RAW DATA

Springborn Laboratories, Inc.

STATIC ACUTE TOXICITY DATA SHEET: 96 HOUR DEFINITIVE

SPONSOR: American Petroleum Institute

STUDY NO.: 12427.0294.6110.510

SPECIES: Mysidopsis bahia

SAMPLE IDENTIFICATION: Tert-Amyl Methyl Ether (TAME)

PRINCIPAL INVESTIGATOR: Robin H. Dyer

BIOLOGICAL OBSERVATION AND MORTALITY

TEST CONC. % (mg/L) µg/L	REP	OBSERVATION 0 H	NO. DEAD	OBSERVATION 24 H	NO. DEAD	CUM. DEAD	OBSERVATION 48 H	NO. DEAD	CUM. DEAD	OBSERVATION 72 H	NO. DEAD	CUM. DEAD	OBSERVATION 96 H	NO. DEAD	CUM. DEAD
Control	A	None	0	None	0	0	None	0	0	None	0	0	None	0	0
Control	B	None	0	None	0	0	None	0	0	None	0	0	None	0	0
1.6	A	None	0	None	0	0	1-PLE	0	0	1-PLE	0	0	None	1	1
1.6	B	None	0	None	0	0	2-LETH	0	0	2-LETH	0	0	2-LETH	0	0
4.0	A	None	0	None	0	0	2 DEK	3	3	2 DEK	0	3	2-DEK	0	3
4.0	B	None	0	None	0	0	3 LETH	0	0	3 LETH	0	0	2 LETH	1	1
7.3	A	None	0	None	0	0	1 DEK	1	1	1 DEK	0	1	1 DEK	0	1
7.3	B	None	0	None	0	0	2 DEK	0	0	2 DEK	[illegible]	1	2 DEK	0	1
15	A	None	0	None	0	0	2 DEK	2	2	LETH	4	6	LETH	1	7
15	B	None	0	None	0	0	3 DEK	3	3	LETH	2	5	LETH	0	5
30	A	None	0	LETH	0	0	LETH	3	3	LETH	5	8	LETH	1	9
30	B	None	0	LETH	0	0	LETH	4	4	LETH	5	9	None	1	10
60	A	None	0	4 DECILETH	0	0	DECILETH	4	4	PLE	5	9	None	[illegible]	10
60	B	None	0	2 DECILETH	2	2	DECILETH	1	3	PLE	6	9	None	1	10

N/A RD 5/10/94

DATA RECORDING INFO

OBSERVATION INTERVAL	DATE/TIME AND INITIALS
0 HOUR	5/3/94 / RD
24 HOUR	5/4/94
48 HOUR	5/5/94 RD
72 HOUR	5/6/94
96 HOUR	5/7/94

NOTES

N/A RD 5/10/94

TA914

Springborn Laboratories, Inc.

Springborn Laboratories, Inc.
12827 0394 6110 510

OBSERVATION KEY

NONE - Observation was made and nothing out of the ordinary was observed.

AS - AT THE SURFACE

MSP - MUSCLE SPASM

CLE - COMPLETE LOSS OF EQUILIBRIUM

PLE - PARTIAL LOSS OF EQUILIBRIUM

LETH - LETHARGIC

PFAE - PECTORAL FINS ANTERIORLY EXTENDED

EXO - EXOPHTHALMUS

EA - EXTENDED ABDOMEN

HYP - HYPERACTIVE

ERR - ERRATIC

CS - ON BOTTOM

LT - LIGHT

DRK - DARK

EMP - EXCESSIVE MUCUS

HEM - HEMORRHAGIC

RA - RAPID

RE - REDUCED

CLDY - CLOUDY

PRE - PRECIPITATE

FOS - FILM ON SURFACE

UN - UNDISSOLVED CHEMICAL

PM - PARTICULATE MATTER

SPRINGBORN LABORATORIES, INC.

RESULTS OF CHROMATOGRAPHIC ANALYSIS
MEAN MEASURED TABLE

Sponsor: API
Test Material: TAME
Project No.: 12827-0394-6110-510
Test Type: 96 HR STATIC RENEWAL M/MYSIDOPSIS BAHIA
Data Entered By: JMH/JLG
Data Program Run: 12-May-95

Sample ID	Nominal Conc. (MG/L)	Interval	Analytical Result (MG/L)		Percent of Nominal	MEAN Percent of Nominal	MEAN Analytical Result (MG/L)	Std.Dev. Analytical Result	N	C.V.
S-94-37CNT	0	0 HR (5-3-94)	< -0.1598	1	NA	NA	NA	NA	4	NA
S-94-38CNT	0	0 HR (5-3-94)	< -0.1598	1	NA					
S-94-324CNT	0	96 HR (5-7-94)	< -0.0002	2	NA					
S-94-325CNT	0	96 HR (5-7-94)	< -0.0002	2	NA					
S-94-39	1.6	0 HR (5-3-94)	< -0.1598	1	/// 3	NA	NA	NA	NA	NA
S-94-40	1.6	0 HR (5-3-94)	1.312E+00		/// 3					
S-94-164	1.6	24 HR (5-4-94)	5.375E-01		/// 3					
S-94-165	1.6	24 HR (5-4-94)	1.535E+00		/// 3					
S-94-326	1.6	96 HR (5-7-94)	4.259E+00		/// 3					
S-94-327	1.6	96 HR (5-7-94)	3.727E+00		/// 3					
S-94-41	4	0 HR (5-3-94)	3.586E+00		97.2	124.9	4.99	0.504	4	16.1
S-94-42	4	0 HR (5-3-94)	4.974E+00		124					
S-94-328	4	96 HR (5-7-94)	5.752E+00		144					
S-94-329	4	96 HR (5-7-94)	5.367E+00		134					
S-94-43	7.3	0 HR (5-3-94)	9.527E+00		131	130	9.51	1.27	4	13.3
S-94-44	7.3	0 HR (5-3-94)	7.713E+00		106					
S-94-330	7.3	96 HR (5-7-94)	1.032E+01		141					
S-94-331	7.3	96 HR (5-7-94)	1.047E+01		143					
S-94-45	15	0 HR (5-3-94)	1.654E+01		110	127	19.0	2.72	4	14.3
S-94-46	15	0 HR (5-3-94)	1.740E+01		116					
S-94-332	15	96 HR (5-7-94)	1.952E+01		130					
S-94-333	15	96 HR (5-7-94)	2.266E+01		151					
S-94-47	30	0 HR (5-3-94)	2.894E+01		96.5	116	34.7	6.31	4	18.2
S-94-48	30	0 HR (5-3-94)	2.955E+01		98.5					
S-94-334	30	96 HR (5-7-94)	3.942E+01		131					
S-94-335	30	96 HR (5-7-94)	4.081E+01		136					
S-94-49	60	0 HR (5-3-94)	6.485E+01		108	109	65.2	4.23	4	6.49
S-94-50	60	0 HR (5-3-94)	5.966E+01		99.3					
S-94-336	60	96 HR (5-7-94)	7.315E+01		117					

SPRINGBORN LABORATORIES, INC.

RESULTS OF CHROMATOGRAPHIC ANALYSIS
MEAN MEASURED TABLE

Sponsor: API
Test Material: TAME
Project No.: 12827-0394-6110-510
Test Type: 96 HR STATIC RENEWAL W/MYSIDOPSIS BAHIA
Data Entered By: JMM/JLG JMM 7/20
Date Program Run: 12-May-96

Sample ID	Nominal Conc. (MG/L)	Interval	Analytical Result (MG/L)	Percent of Nominal	MEAN Percent of Nominal	MEAN Analytical Result (MG/L)	Std.Dev. Analytical Result	N	C.V.
5-94-337	60	96 HR (5-7-94)	6.600E+01	110					

Samples from the 24 hour sampling interval (1.6 mg/L) were calculated by point-to-point calculation.

1,2: The linear regression analysis has calculated a negative X-intercept and a positive Y-intercept, consequently, a response (height) less than the value of the Y-intercept results in a negative 'less than' value for the controls. A more accurate representation is determined using the following equation:

1. less than 1/2 of the lowest standard (0.250 mg/L) x dilution factor of sample (4) = < 0.500 mg/L
2. less than 1/2 of the lowest standard (0.250 mg/L) x dilution factor of sample (1) = < 0.125 mg/L

3. Due to poor precision per interval and per sample replicate for the 1.6 mg/L samples, the data obtained for this concentration level will not be reported.

TOTAL MEAN % RECOVERY 121
TOTAL MEAN C.V. 13.7

SPRINGBORN LABORATORIES, INC. Page 12

RESULTS OF CHROMATOGRAPHIC ANALYSIS
QUALITY CONTROL SUMMARY TABLE

Sponsor: API
Test Material: TAME
Project No.: 12827-0394-6110-510
Test Type: 96 HR STATIC RENEWAL W/MYSIDOPSIS BAHIA
Data Entered By: JOH
Date Program Run: 02-Jun-94

Sample ID	Nominal Concentration (MG/L)	Interval	Analytical Result (MG/L)	Percent of Nominal	
5-94-510C1	1.5	0 HR (5-3-94)	1.206E+00	80.4	
5-94-520C2	30	0 HR (5-3-94)	3.236E+01	108	
5-94-530C3	60	0 HR (5-3-94)	6.220E+01	104	
5-94-1660C1	1.5	24 HR (5-4-94)	1.214E+00	80.9	1
5-94-1670C2	1.5	24 HR (5-4-94)	1.225E+00	31.7	1
5-94-1680C3	1.5	24 HR (5-4-94)	1.389E+00	92.6	1
5-94-3380C1	1.5	96 HR (5-7-94)	1.578E+00	105	
5-94-3390C2	30	96 HR (5-7-94)	2.858E+01	95.3	
5-94-3400C3	60	96 HR (5-7-94)	5.893E+01	98.2	

1. Because the 1.6 mg/L samples will not be reported, the additional data for QC samples obtained on the 24 hour interval (used only for the 1.6 mg/L test samples) will not be included in the statistical analysis.

MEAN	98.4
N =	6
STD.DEV.	10.0
C.V.	10.13

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

Order No. 848-00407