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Environmental Sciences
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FYI Coordinator
OTS Document Processing Center (TS-790)
U.S. EPA, Room 421-B East Tower
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Washington DC 20460

October 21, 1994



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Dear FYI Coordinator:

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

(Identification no: FYI not assigned) Tert-Amyl Methyl Ether (TAME): Acute Toxicity to Daphnia (*Daphnia magna*) Under Flow-Through Conditions. Draft Report.

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

Sincerely,

Robert T. Drew, Ph.D.

Springborn Laboratories, Inc.

Environmental Sciences Division

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5 October 1994

Richard A. Rhoden, Ph.D.
American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

Dear Dr. Rhoden:

Please find below our responses to your review of the following draft report:

#92-12-4545 entitled "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Daphnids (Daphnia magna) Under Flow-Through Conditions".

Page 2. Third sentence changed to read: "Storage stability, characterization and verification of the test material identity and maintenance of these records on the test material are the responsibility of the Study Sponsor."

Page 9. 2.2 Test Material - 1992 was added after 17 August in this section, 3rd line.

Page 10. Study Sponsor changed to read Aldrich Chemical Company in lines 2 and 5.

Page 10. Mass Spectral analysis of TAME samples are reported in the 2.2 Test Material section.

Page 14. Average recovery of TAME changed to 10%.

Page 15. 5.1 Preliminary Test - 3rd line - "All test vessels during" added. 7th line - "several" changed to "all" and "...this treatment level." changed to "... the 410 mg A.I./L nominal treatment" 9th line "250" deleted.

Page 18. Protocol Deviation - 4th line "buy" corrected to "by".

Page 24. Table 3 - subtitle changed from "24-Hour" to "0-Hour".

Page 41. Study number corrected on Protocol Amendment.

Page 55 and 63. Explanation was made for the recovery of $102 \pm 5\%$ in the freshwater section of this method validation/ recovery study.

Page 56. Reagents # 2 "...identified by the Sponsor..." has been changed to "... identified by Aldrich Chemical Company..."

Please feel free to contact me with any questions or comments you may have.

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10/6/94

Very truly yours,
SPRINGBORN LABORATORIES, INC.



Arthur E. Pugh
Study Director
ALP/eah
enclosure(s)

cc: Ms. Christine Saxmith

Springborn

0005

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

TSCA GUIDELINE § 797.1300

Submitted to:

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

SLI Report # 92-12-4545

SLI Study # 12827.0692.6102.115

Study Director: Arthur E. Putt

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

29 September 1994

REVISED DRAFT REPORT

19/6/94

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

The data and report for "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity To Daphnia (Daphnia magna) Under Flow-Through Conditions" were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 792) with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs and metals are conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, PA. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.). Storage stability, characterization and verification of the test material identity and maintenance of these records on the test material are the responsibility of the Study Sponsor. At the termination of the testing program, all remaining test material will be sent to the Study Sponsor. Maintenance of a sample of the test material is the responsibility of the Study Sponsor.

SPRINGBORN LABORATORIES, INC.

Arthur E. Putt
Study Director

Date

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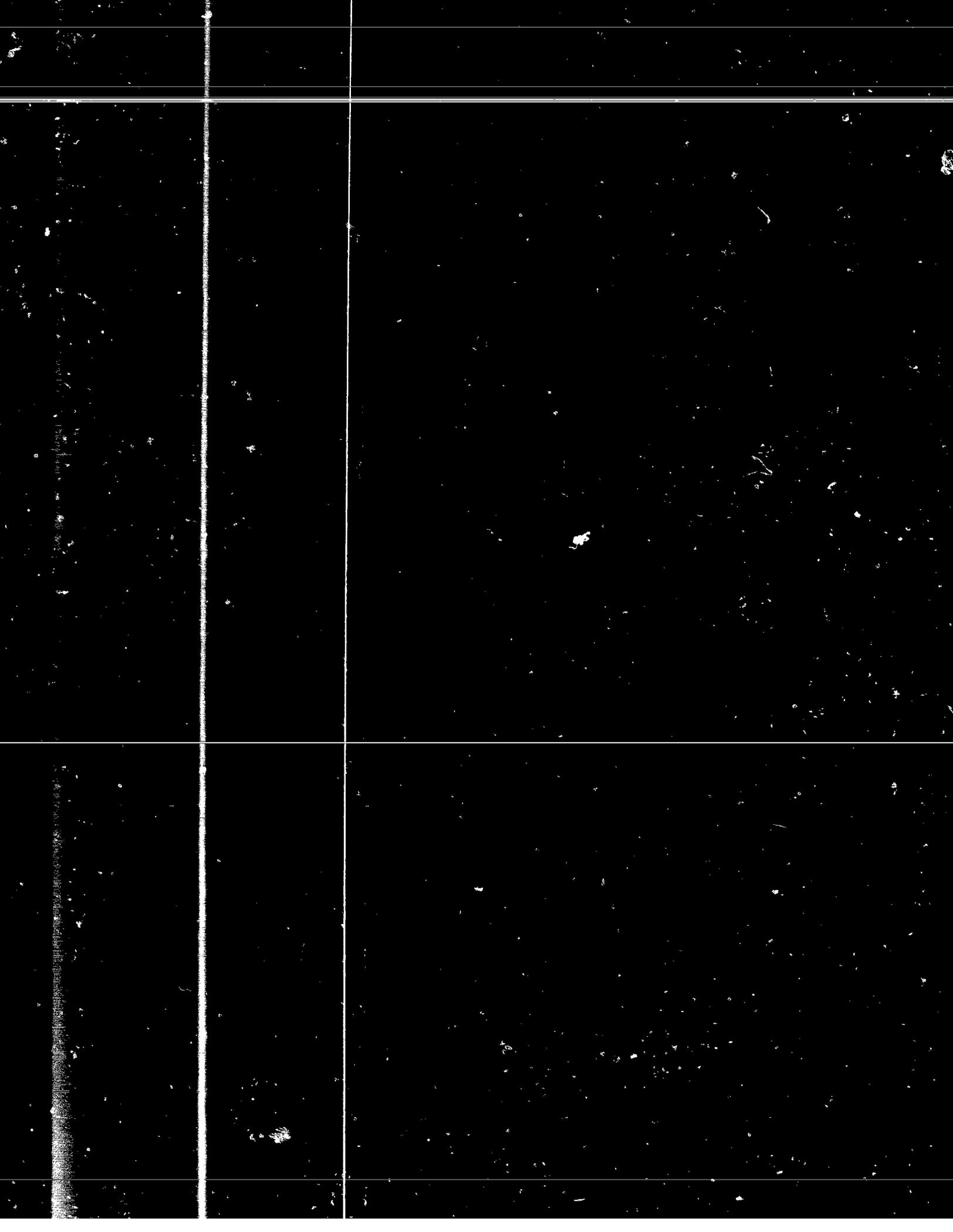
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SUMMARY

The purpose of this study was to estimate the acute toxicity (EC50) of Tert-Amyl Methyl Ether (TAME) to *Daphnia magna* under flow-through conditions. The EC50 is defined as the concentration of test material estimated to cause immobilization among 50% of a test population at the stated exposure interval. Twenty organisms (ten per replicate) were exposed in duplicate test vessels to five concentrations of TAME and a dilution water control for 48 hours. During the test, nominal concentrations of 690, 410, 250, 150 and 89 mg A.I./L were maintained in the exposure vessels by introducing approximately 6.0 test chamber volumes per day of newly prepared test solution via intermittent-flow proportional diluter apparatus. Each replicate solution was sampled and analyzed for TAME concentration at 0-hour (initiation) and 48-hours (termination) of the exposure period. Based on the results of these analyses, the mean measured exposure concentrations were defined as 120, 83, 55, 28 and 15 mg A.I./L. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation, 3, 6, 24 and 48 hours. Throughout the exposure period, no visible signs of undissolved test material (e.g., precipitate) were observed in either the diluter system or in the exposure solutions.

At test termination (48-hours), immobilization of 90% was observed among daphnids exposed to the highest mean measured concentration tested (120 mg A.I./L). In addition, sublethal effects (e.g., lethargy) were observed among all of the mobile daphnids exposed to this treatment level. No immobilization or sublethal effects were observed among daphnids exposed to the remaining concentrations tested (83, 55, 28 and 15 mg A.I./L). The EC50 values and the corresponding 95% confidence intervals determined throughout the exposure period are summarized in the following table. The No-Observed-Effect Concentration (NOEC) through 48-hours of exposure was established to be 83 mg A.I./L.

TEST RESULTS

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

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

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1.0 INTRODUCTION

The purpose of this study was to estimate the acute toxicity (EC50) of Tert-Amyl Methyl Ether (TAME) to daphnids (*Daphnia magna*) under flow-through conditions. The EC50 is defined as the concentration of test material in dilution water which causes immobilization of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from the release of the test substance into aquatic environments. The study was initiated on 26 October 1992, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the 48-hour definitive toxicity test was conducted from 10 - 12 December 1992 at Springborn Laboratories, Inc. (SLI), Environmental Sciences Division, Wareham, Massachusetts. All original raw data and the final report produced for this study are stored at SLI.

2.0 MATERIALS AND METHODS

2.1 Protocol

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

2.2 Test Material

Two samples of Tert-Amyl Methyl Ether (TAME) (CAS # 994-05-8), a clear liquid, were received from Experimental Pathology Labs, Inc., Herndon, Virginia. The first sample, Lot # 02814BZ, was received at SLI on 17 August 1992 and was used to prepare exposure solutions during the preliminary exposure, analytical standards during the method validation/recovery study and to prepare Quality Control samples during the definitive exposure. The sample was identified

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by Aldrich Chemical Company to contain 98.8% active ingredient A. (Certificate of Analysis, Appendix II). The second sample, Lot # 07905KZ, was received at SLI on 2 November 1992 and was used to prepare exposure solutions during the definitive exposures. The sample was identified by Aldrich Chemical Company to contain 98.7% active ingredient A.I. (Certificate of Analysis, Appendix II). Upon receipt at SLI, the samples of test material were stored in a dark, ventilated cabinet at room temperature (approximately 20 °C). Test concentrations are expressed as milligrams of test material (as active ingredient) per liter of test solution and are reported as mg A.I./L.

At the request of the Study Sponsor, mass spectral analysis was conducted on the initial batch of TAME received at program initiation, and the additional batches received throughout the course of the program. The purpose of the mass spectral analysis evaluation was to determine test material integrity throughout the duration of the program. Initial evaluation of test material (i.e., lot # 02814BZ) was conducted on 3 December 1992. Following completion of the flow-through acute toxicity test with mysids (SLI Report # 94-5-5269), spectral analysis was conducted on 20 July 1994 on each of the remaining two lots (lot # 02814BZ and lot # 07905KZ). The spectral analysis conducted on 20 July 1994 on the two remaining lots in comparison to the initial spectral analyses of lot # 02814BZ established that negligible change in test material composition had occurred during storage at Springborn Laboratories, Inc. (i.e., approximately 24 months).

2.3 Test Organisms

The *Daphnia magna* used in this toxicity test were obtained from laboratory cultures maintained at Springborn Laboratories, Inc., Wareham, Massachusetts. The culture water was prepared by fortifying well water based on the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column and a carbon filter. Two weeks prior to test initiation, this water had total hardness and total alkalinity as calcium carbonate (CaCO₃) of 160 mg/L and 110 mg/L, respectively, a pH of 8.1, a specific conductivity range of 400 to 500 μ mhos/cm and a dissolved oxygen concentration of greater than 60% of saturation. The daphnid culture area received a regulated photoperiod of 16 hours of light and 8 hours of

clarity. Light intensity in the culture area ranged from 32 - 48 footcandles (Invertebrate Culture Log, Vol. VII). A waterbath in the culture area was used to maintain the culture solution temperature at 20 ± 2 °C. Daphnids were fed a combination of a trout food suspension and a unicellular green algae (*Aristrodesmus falcatus*) once daily. The food solution contained 5.0 mg/mL trout food and approximately 4×10^7 cells/mL of algae. Representative samples of the food source were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix III). Food sources were considered to be of acceptable quality since the total concentration of pesticides measured was less than 0.3 mg/kg (ASTM, 1985).

2.4 Test Dilution Water

The dilution water used during this study was from the same source as the culture water described above and had a total hardness and total alkalinity (CaCO_3) of 160 and 110 mg/L, respectively, a pH range of 8.1 - 8.2 and a specific conductivity of 500 $\mu\text{mhos/cm}$ (IWQ Log Book, Vol. 13). During holding and prior to use, the dilution water was continuously aerated. Representative samples of the dilution water source were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix IV). None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with ASTM standard practice (ASTM, 1985). In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentrations. The results of these analyses demonstrated that the TOC concentration of the dilution water source ranged from 0.97 - 2.2 mg/l for the months of June - November 1992 (TOC and TSS master log, Volume I). Daphnid cultures are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over several generations. This, in combination with the previously mentioned analyses, confirms the acceptability of this dilution water for bioassays.

2.5 Test Conditions

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

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

2.6 Test Concentrations

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

2.7 Exposure Solution Preparation

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

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

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

3.0 TEST PROCEDURES

3.1 Test Initiation

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

3.2 Test Monitoring

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

3.3 Water Quality Measurements

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

presented in this report were measured by the EDTA titrimetric method and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA *et al.*, 1985) using a Jenco Model 601A pH meter and combination electrode. Specific conductivity was measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe; the pH was measured with a Jenco Model 601A pH meter and combination electrode; the dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe; and the daily solution temperature was measured with a Fisher alcohol thermometer. Continuous temperature monitoring was performed in one replicate (B) of the 690 mg A.I./L test solution (nominal) using the Omega Data Acquisition System (ODAS). Light intensity was measured with a General Electric type 214 light meter.

3.4 Analytical Measurements

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

4.0 STATISTICS

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

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

5.0 RESULTS

5.1 Preliminary Test

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

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

5.2 Definitive Test

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

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

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

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

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

PROTOCOL DEVIATION

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

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

SPRINGBORN LABORATORIES, INC.

Arthur E. Putt Date
Study Director

Springborn Laboratories, Inc.

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QUALITY ASSURANCE UNIT STATEMENT

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

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

<u>Inspection Date</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
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SPRINGBORN LABORATORIES, INC.

Patricia D. Royal Regulatory Affairs and Quality Assurance Unit	Date
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- U.S. EPA. 1985, 1987. Toxic Substance Control Act Test Guidelines. Federal Register 50 (198): 39252-39516, September 27, 1985, with modifications promulgated on May 20, 1987 (Fed. Reg. 52).**

TABLES

0 0 2 . 6

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

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

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

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

Nominal Concentration (mg A.I./L)	Total Hardness^a (mg/L CaCO₃)	Total Alkalinity^a (mg/L CaCO₃)	Specific Conductance^a (µmhos/cm)
660	190	110	500
410	180	110	500
250	180	110	500
150	180	110	500
89	170	120	500
Control	170	120	500

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

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

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

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

^b QC = Quality Control sample.

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

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

Mean Measured Concentration (mg A.I./L)	Cumulative Percent Immobilized Organisms											
	3-Hour			6-Hour			24-Hour			48-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
120	0	0	0	0	0	0	10	20	15 ^a	100	80	90 ^b
63	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	10	0	5

^a All of the mobile daphnids were observed to be lethargic.

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

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

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

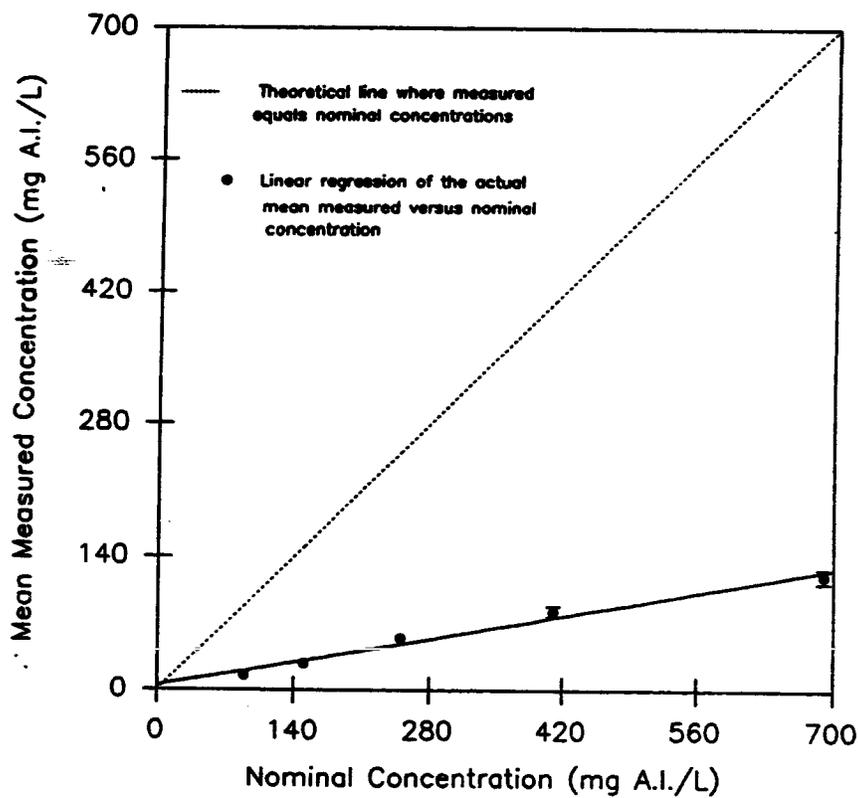
- ^a Based on mean measured concentrations of TAME (as active ingredient).
^b Corresponding 95% confidence interval is presented in parentheses.
^c EC50 value empirically estimated as greater than the highest concentration tested.
^d EC50 value estimated by nonlinear interpolation; 95% confidence interval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

FIGURES

Springborn Laboratories, Inc.

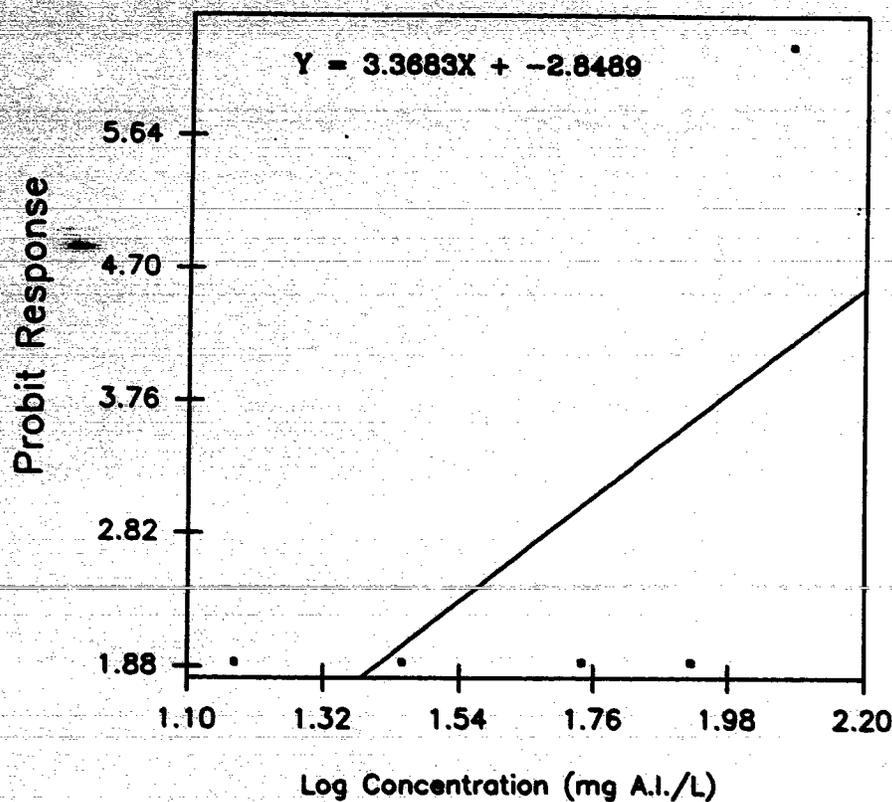
0032

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



0 0 3 3

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



0 0 3 4

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	86.8
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: $89.7 \pm 2.3\%$

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: $104 \pm 11\%$

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 2A. 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: $102 \pm 5\%$ (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 efficiently 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.

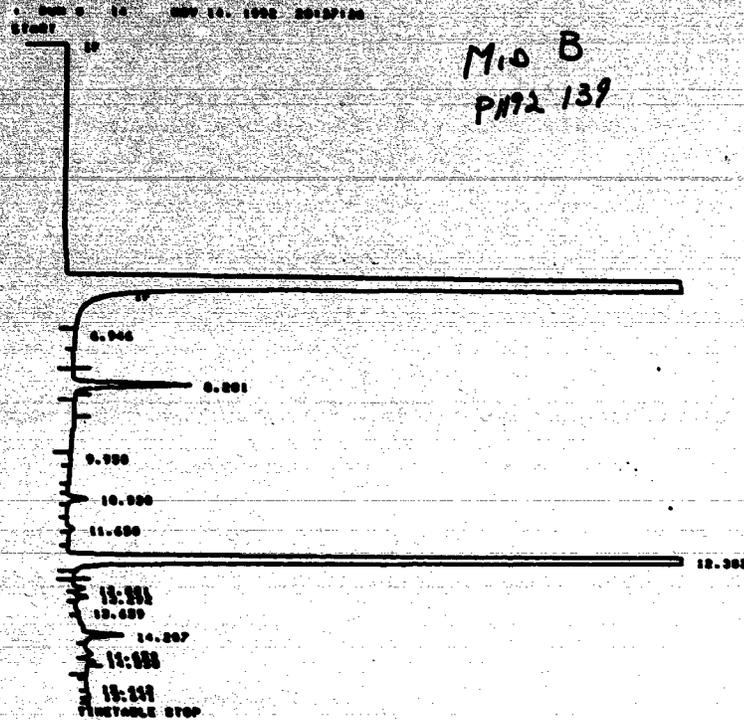
0068

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

0 0 6 9

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



RUN# 14 NOV 10, 1992 20:37:20

METHOD NAME: H-TAMEPSTP.MET

IDENTIFIER: 13100039449

ACQUISITION TIME: 11/10/92 20:37:20

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CALL	OFFL	NAME
0.001	PP	104373	.107	24243			.000
10.930	PP	10711	.002	3017			.000
12.302	PP	3707170	.103	612042	12		3.536 TAME
12.507	VP	11101	.071	2610			.000
13.658	VV	60004	.129	0922			.000
15.148	VV	17942	.164	1023			.000

TOTAL HEIGHT= 634206

NULL FACTOR=1.0000E-00

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

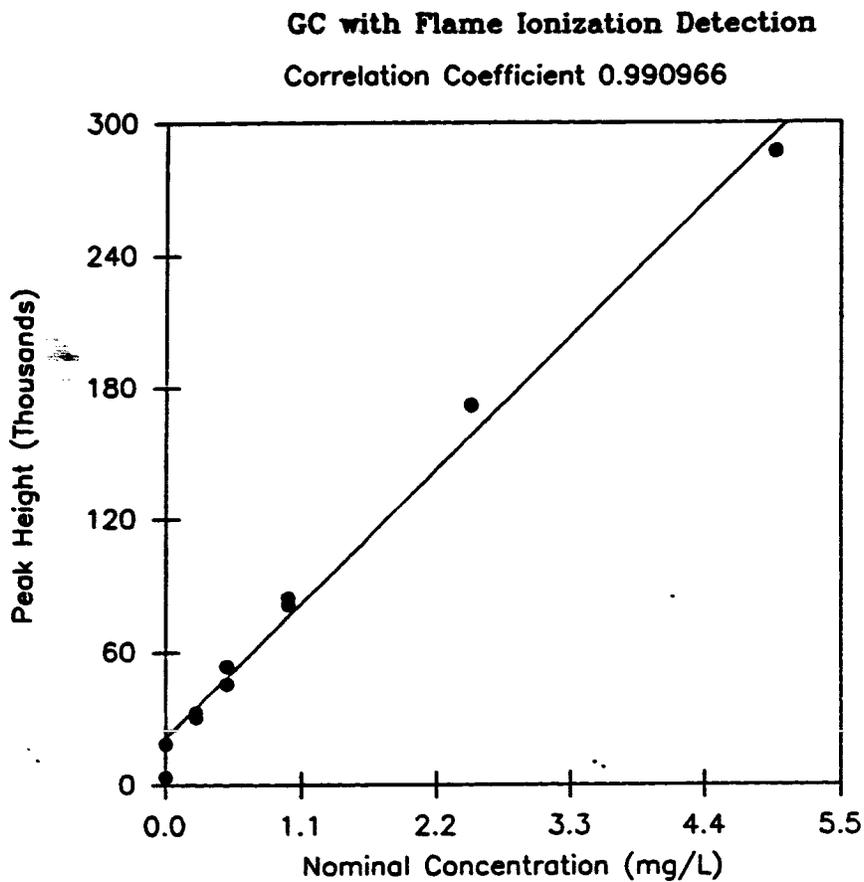


Figure 3A. A representative polynomial regression analysis from standard TAME analysis.

API
TAME

13E27-0492-6100-250
Metro-Viljoen

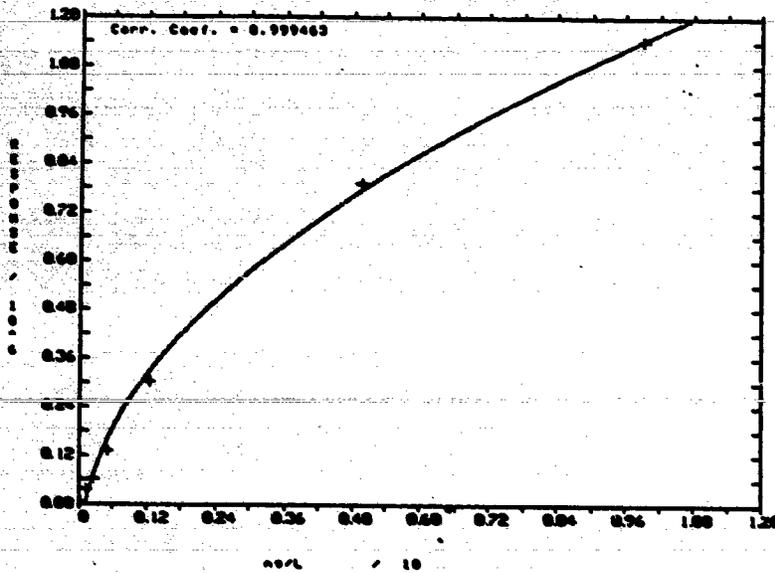
WELCOME TO THE HP3396 CALIBRATION CURVE PLOTTING PROGRAM Rev. 8.02.00

At any prompt: *0* (ENTER) Quit
0 (ENTER) Starts Over

Load which method or calib. file (Current active):

Plot the calibration curve for which CAL # (all):

no/L vs. Response for Cal # 1
no/L * 1.16E-01 + 1.37E-06 (RESPONSE) + 6.26E-12 (RESPONSE^2)



Plot additional peaks (Y=H): N

- * EDIT CALIB #
- 1 = CALIB PROCEDURE
- 2 = RETENTION TIME WINDOWS
- 3 = TABLE ENTRIES
- 4 = PEAK GROUPS
- 5 = CALIB OPTIONS

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REVISED FINAL DRAFT REPORT ACCEPTANCE

The revised final draft report from the study titled, "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Daphnids Under Flow-Through Conditions" was reviewed today by API QA. All items requiring attention as noted in the QA audit report have been addressed. The report may now be considered final.

C. Sexsmith

CS
10/15/94

10/10/94

0073