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FYI-1094-1052

Robert T. Drew, Ph.D.  
Director, Health and  
Environmental Sciences  
(202) 882-4308  
(202) 882-8270 (FAX)



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FYI Coordinator  
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October 21, 1994

Dear FYI Coordinator:

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

**(Identification no: FYI not assigned) Tert-Amyl Methyl Ether (TAME): Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) 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.

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Springborn Laboratories, Inc.

Environmental Sciences Division

788 Main Street • Waltham, Massachusetts 02571-1075 • (508) 295-2500 • Telex 982041 • Facsimile (508) 295-8107

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5 October 1984

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:

803-3-4682 entitled "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) 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 8, 18 and 26. The LC50 value for the 96-hour interval was corrected to be 580 mg/L.

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

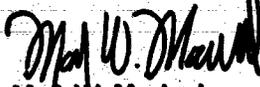
Page 19. Protocol deviation # 2 was added to this page.

Page 54 and 62. Explanation was made for the recovery of  $102 \pm 5\%$  in the freshwater section of this method validation/recovery study.

Page 55. 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.

Very truly yours,  
SPRINGBORN LABORATORIES, INC.



Mark W. Machado  
Study Director

MWM/eeb  
enclosure(s)

cc: Ms. Christine Saxsmith

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

**TSCA GUIDELINE § 797.1400**

**Submitted to:**

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

**SLI Report # 93-3-4682**

**Study # 12827.0692.6104.108**

**Study Director: Mark W. Machado**

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

**29 September 1994**

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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:

893-3-4882 entitled "Tert-Amyl Methyl Ether (TAME) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) 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."

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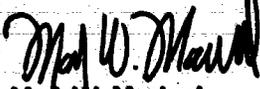
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Very truly yours,  
SPRINGBORN LABORATORIES, INC.



Mark W. Machado  
Study Director

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Environmental Sciences Division  
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Wareham, Massachusetts 02571-1075**

**29 September 1994**

**REVISED DRAFT REPORT**

10/6/94

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**GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT**

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

SPRINGBORN LABORATORIES, INC.

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Mark W. Machado  
Study Director

Date

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**SUMMARY**

The purpose of this study was to estimate the acute toxicity (LC50) of Tert-Amyl Methyl Ether (TAME) to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. The LC50 is defined as the concentration of the test material in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. Twenty organisms (ten per replicate) were exposed in duplicate test aquaria to each of five concentrations of TAME and a dilution water control for 96-hours. During the test, nominal concentrations of 950, 570, 340, 210 and 120 mg A.I./L were maintained by introducing approximately 6.5 aquarium volumes per day of newly prepared test solution via a modified constant-flow serial diluter apparatus (Benoit, 1962). Each replicate solution was sampled and analyzed for TAME concentration at 0-hour (test initiation) and 96-hours (test termination) of exposure. Based on the results of these analyses, the mean measured exposure concentrations were defined as 640, 560, 310, 150 and 78 mg A.I./L. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and every 24 hours thereafter until the test was terminated. Throughout the exposure period, treatment level solutions were observed to be clear and colorless and contained no visible sign of undissolved test material (e.g., precipitate).

Following 72-hours of exposure, 100% mortality was observed among fish exposed to the highest mean measured concentration tested (640 mg A.I./L). At test termination (96-hours), mortality of 30% was observed among fish exposed to the 560 mg A.I./L treatment level. In addition, sublethal effects (e.g., loss of equilibrium, darkened pigmentation) were observed among all of the surviving fish exposed to this treatment level. No mortality or sublethal effects were observed among fish exposed to the remaining concentrations tested (310, 150 and 78 mg A.I./L). The LC50 values and the 95% confidence intervals determined throughout the exposure period are summarized in the following table. The No-Observed-Effect Concentration (NOEC) established during this study was 310 mg A.I./L.

**TEST RESULTS**

LC50 (mg A.I./L) <sup>a,b</sup>				No-Observed- Effect Concentration Through 96 Hours (mg A.I./L) <sup>c</sup>
24-Hour <sup>a</sup>	48-Hour <sup>a</sup>	72-Hour <sup>d</sup>	96-Hour <sup>a,b</sup>	
600 (580 - 620)	600 (570 - 620)	580 (560 - 640)	580 (310 - 640)	310

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

## 1.0 INTRODUCTION

The purpose of this study was to determine the acute toxicity of Tert-Amyl Methyl Ether (TAME) to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. The LC50 is defined as the concentration of the test material in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of the potential acute hazards resulting from release of the test material into aquatic environments. The study was initiated on 14 October 1992, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the 96-hour definitive flow-through toxicity test was conducted from 27 February - 3 March 1993 at Springborn Laboratories, Inc. (SLI), Environmental Sciences Division, Wareham, Massachusetts. All original raw data and the final report produced for this study are stored at SLI.

## 2.0 MATERIALS AND METHODS

### 2.1 Protocol

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

### 2.2 Test Material

Two samples of Tert-Amyl Methyl Ether (TAME) (CAS # 994-05-8), a clear liquid, were received from Experimental Pathology Labs, Inc., Herndon, Virginia. The first sample, Lot # 02814BZ, was received at SLI on 17 August 1992 and was used to prepare analytical standards during the method validation/recovery study and to prepare Quality Control samples during the definitive exposure. The sample was identified by Aldrich Chemical to contain 98.8% active ingredient A.I. (Certificate of Analysis, Appendix II). The second sample, Lot # 07905KZ, was

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

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

### 2.3 Test Organisms

Rainbow trout (*Oncorhynchus mykiss*) were selected as the test species since it is a recommended species (U.S. EPA, 1985) and a commonly used cold water fish in flow-through freshwater fish toxicity tests. The rainbow trout (SLI lot #93B3) used during the study were obtained from Spring Creek Trout Hatchery, a commercial supplier located in Lewistown, Montana. Prior to testing, these fish were held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. The well water which flowed into this holding tank was characterized as having total hardness and total alkalinity ranges as calcium carbonate ( $\text{CaCO}_3$ ) of 25 - 27 mg/L and 20 - 22 mg/L, respectively, and a specific conductance of 110  $\mu\text{mhos/cm}$  (Gravity Feed Tank Water Quality Log). Other parameters monitored in the holding tank were pH with a range of 6.8 - 6.9, dissolved oxygen concentration with a range of 80 - 87% of saturation

and the flow rate with 6.2 - 11 tank volume replacements/day (Weekly Record of Fish Holding Characteristics). Fish used during the definitive exposure were maintained under similar conditions for a minimum of 14 days prior to testing. The temperature in the holding tank ranged from 11 - 12 °C during this 14-day period. All fish were fed a dry commercial pelleted food, *ad libitum*, daily except during the 48 hours prior to, and during the definitive test. Representative samples of the food source were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix III). Food sources were considered to be of acceptable quality since the total concentration of pesticides was less than 0.3 mg/kg. Mortality of 0.20% was observed in the test fish population during the two days prior to testing (Daily Record of Fish Holding Conditions). The rainbow trout used during this study were all of the same year class and a representative sample (N = 30) had a mean (range) wet weight and total length of 0.47 (0.28 - 0.75) grams and 37 (30 - 48) millimeters, respectively (Fish Lengths and Weights Log).

#### 2.4 Test Dilution Water

The dilution water used during this study was from the same source as the water which flowed into the fish holding tank and was characterized as having total hardness and total alkalinity (as CaCO<sub>3</sub>) ranges of 20 - 22 and 25 - 27 mg/L, respectively, a pH range of 6.9 - 7.0 and a specific conductance of 110  $\mu$ mhos/cm (Gravity Feed Tank Water Quality Log). Representative samples of the dilution water source were analyzed for the presence of pesticides, PCBs and toxic metals (Appendix IV). None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with ASTM standard practice. In addition, representative samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentration. The results of these analyses demonstrated that the TOC concentration of the dilution water ranged from 0.82 - 1.3 mg/L for the months of September 1992 - February 1993 (TOC and TSS Master Log, Vol 1). Several species of daphnids (a representative freshwater organism generally recognized to be sensitive to chemical challenges) are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over several generations. This, in combination with the previously mentioned analyses, confirms the acceptability of this dilution water for bioassays.

### 2.5 Test Conditions

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

### 2.6 Test Concentrations

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

### 2.7 Test Solution Preparation and Delivery

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

individual delivery tube also received 0.050 L/min of dilution water. Delivery of the test material and dilution water at this point in the system aided in the mixing and solubilization of the test material. Proportional dilution (80%) across the range of nominal concentrations established (i.e., 950, 570, 340, 210 and 120 mg A.I./L) was accomplished by adjusting the size of syringes used (i.e., 20 - 50 mL) and the flow of each pump (0.0625, 0.0375, 0.0225, 0.0135 and 0.0081 mL/min).

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

### 3.0 TEST PROCEDURES

#### 3.1 Test Initiation

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

#### 3.2 Test Monitoring

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

### 3.3 Water Quality Measurements

Dissolved oxygen concentration, temperature and pH were measured once daily in each replicate of each treatment level and the controls throughout the exposure period. The pH was measured using a Jenco Model 801A 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 Brookdyn alcohol thermometer. Light intensity was measured with a General Electric Model 214 light meter. Test solution temperature was continuously monitored in one replicate (B) of the dilution water control solution using a Brookdyn Min/Max thermometer.

### 3.4 Analytical Measurements

Both replicate solutions of the high, middle and low treatment levels and the control were sampled and analyzed for TAME concentration prior to the start of the definitive exposure. Results of these pretest analyses were used to judge whether sufficient quantities of test material were being delivered and maintained in the exposure aquaria to initiate the definitive test. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls at 0- and 96-hours of exposure for analysis of TAME concentration. Each exposure solution sample was collected from the approximate midpoint of the aquarium with a volumetric pipet. In addition, three Quality Control (QC) samples were prepared at each sampling interval and remained with the exposure solution samples throughout the analytical process. These QC samples were prepared in dilution water at TAME concentrations similar to the exposure concentration range. Results of the analyses of the QC samples were used to judge the precision and quality control maintained during the analysis of exposure solution samples. All samples were analyzed for TAME using a gas chromatographic (GC) procedure according to the methodology described in Appendix V. A method validation study, conducted at SLI prior to the initiation of the definitive test, established an average recovery of TAME of  $102 \pm 10\%$  from hard reconstituted water.

#### 4.0 STATISTICS

The mean measured concentrations (0- and 96-hour analysis) and the corresponding mortality data derived from the definitive test were used to estimate the median lethal concentration (LC50) and 95% confidence interval at each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test material in dilution water lethal to 50% of the test animal population at the stated exposure interval. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Stephan, 1982, personal communication) was used to calculate the LC50 values and 95% confidence interval. Additionally, the data was evaluated to estimate an incipient LC50. The incipient LC50 is defined as the concentration that is lethal to 50% of a test population when exposure to the test substance is continued until the mean increase in mortality does not exceed 10% in any concentration over a 24-hour period. Since the 96-hour LC50 was not less than 50% of the estimated 48-hour LC50, the duration of the exposure was not continued to determine the incipient LC50 value (U.S. EPA, 1987).

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

## 5.0 RESULTS

### 5.1 Preliminary Testing

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

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

## 5.2 Definitive Test

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

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

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

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

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

**PROTOCOL DEVIATION**

1. The study protocol states that dissolved oxygen concentration exceeds 90% of saturation at the initiation of the test. For this study, dissolved oxygen concentration ranged from 89 - 91% at test initiation.
  
2. The study protocol states that the dilution water used during this study had a total hardness range of 25 to 40 mg/L (as CaCO<sub>3</sub>). During this study, the total hardness of the dilution water ranged from 20 to 22 mg/L (CaCO<sub>3</sub>).

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

SPRINGBORN LABORATORIES, INC.

\_\_\_\_\_  
Mark W. Machado  
Study Director

\_\_\_\_\_  
Date

\_\_\_\_\_  
Springborn Laboratories, Inc.

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**QUALITY ASSURANCE UNIT STATEMENT**

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

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

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

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

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Springborn Laboratories, Inc.

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**TABLES**

0 0 2 5

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

Nominal Concentration (mg A.I./L)	0-Hour		24-Hour		48-Hour		72-Hour		96-Hour	
	A	B	A	B	A	B	A	B	A	B
<b>pH</b>										
950	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
570	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
340	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
210	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
120	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Control	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
<b>Dissolved Oxygen Concentration, mg/L (% Saturation)</b>										
950	9.8 (91)	9.6 (89)	9.6 (87)	9.4 (85)	9.0 (83)	9.3 (86)	9.8 (89)	9.6 (87)	9.0 (83)	9.1 (84)
570	9.6 (89)	9.7 (90)	9.6 (87)	9.6 (87)	9.2 (85)	9.0 (83)	9.7 (88)	9.4 (85)	9.1 (82)	8.9 (82)
340	9.8 (91)	9.8 (91)	9.6 (87)	9.4 (85)	9.3 (86)	9.4 (87)	9.6 (87)	9.6 (87)	9.1 (82)	9.1 (82)
210	9.8 (91)	9.7 (90)	9.5 (86)	9.4 (85)	9.4 (87)	9.4 (87)	9.8 (89)	9.7 (88)	9.6 (87)	9.0 (81)
120	9.8 (91)	9.8 (91)	9.4 (85)	9.6 (87)	9.0 (83)	9.2 (83)	9.6 (87)	9.7 (88)	8.9 (81)	9.0 (81)
Control	9.6 (89)	9.7 (90)	9.5 (86)	9.6 (87)	9.1 (84)	9.2 (83)	9.5 (86)	9.6 (87)	9.9 (82)	9.9 (81)
<b>Temperature (°C)<sup>a</sup></b>										
	12		11		11 - 12		11		11 - 12	

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

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

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

- <sup>a</sup> Mean measured concentrations are presented with the standard deviations in parentheses and were calculated using the unrounded analytical results and not the rounded (two significant figures) values presented in this table.
- <sup>b</sup> The lower than expected concentration for this sample is due to an error during the analytical process and is not considered representative of exposure conditions. This value was not included in the calculation of the mean measured concentration.
- <sup>c</sup> N = 3
- <sup>d</sup> QC = Quality Control sample.
- <sup>e</sup> Value in parentheses represents the nominal fortified concentration for the corresponding QC sample.

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

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

- <sup>a</sup> All of the surviving fish exhibited complete loss of equilibrium.
- <sup>b</sup> Several of the surviving fish exhibited complete loss of equilibrium.
- <sup>c</sup> Several of the surviving fish were observed to be lethargic.
- <sup>d</sup> All of the surviving fish exhibited darkened pigmentation and complete loss of equilibrium.
- <sup>e</sup> Several of the surviving fish exhibited darkened pigmentation and complete loss of equilibrium.

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

LC50 (mg A.I./L) <sup>a,b</sup>				No-Observed- Effect Concentration Through 96 Hours (mg A.I./L) <sup>a</sup>
24-Hour <sup>c</sup>	48-Hour <sup>c</sup>	72-Hour <sup>c</sup>	96-Hour <sup>c,d</sup>	
600 (580 - 620)	600 (570 - 620)	580 (560 - 640)	580 (310 - 640)	310

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

**FIGURES**

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

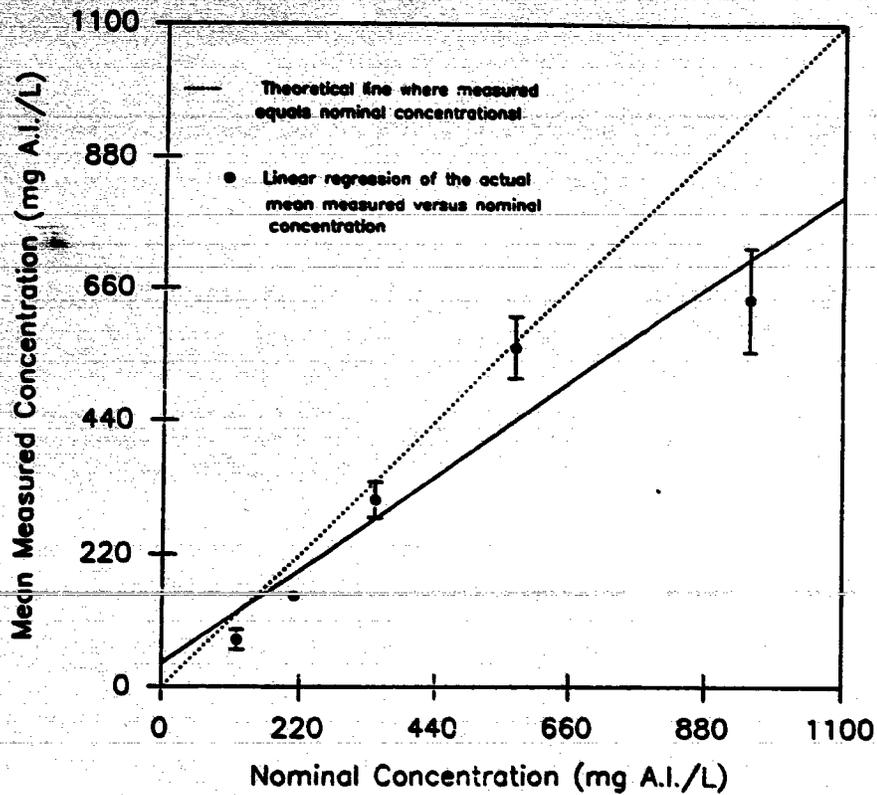
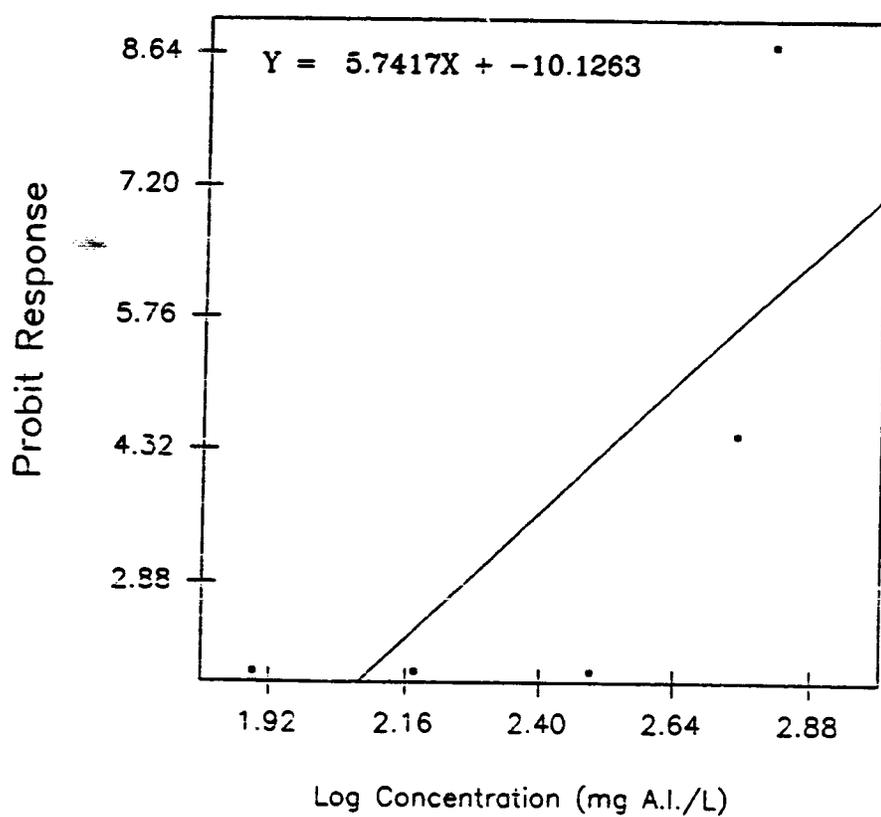


Figure 2. The 96-hour concentration-response (mortality) curve for rainbow trout (*Oncorhynchus mykiss*) exposed to TAME.



**SIGNATURES AND APPROVAL**

**SUBMITTED BY:**

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

**PREPARED BY:**

Mark W. Machado

Lisa M. Thibault

\_\_\_\_\_  
Study Director

\_\_\_\_\_  
Date

\_\_\_\_\_  
Coordinator, Data Management  
and Reporting Unit

\_\_\_\_\_  
Date

Mark da Silva

\_\_\_\_\_  
Analytical Chemist

\_\_\_\_\_  
Date

**APPROVED BY:**

Patricia D. Royal

\_\_\_\_\_  
Manager, Regulatory Affairs  
and Quality Assurance Unit

\_\_\_\_\_  
Date

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

\_\_\_\_\_  
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**6.0 APPENDIX I - STUDY PROTOCOL**

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LOT NUMBER: 028148Z

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FORMULA: C<sub>6</sub>H<sub>14</sub>O

FORMULA WEIGHT: 102.18

APPEARANCE

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REFRACTIVE INDEX AT  
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LOT NUMBER: 07908KZ

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FORMULA: C<sub>6</sub>H<sub>14</sub>O

FORMULA WEIGHT: 102.18

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