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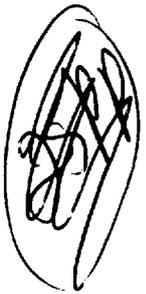
American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005



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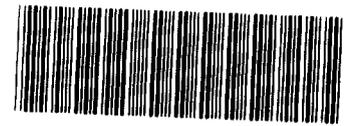
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Robert T. Drew, Ph.D.
Director, Health and
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(202) 682-8308
(202) 682-8270 (FAX)



FYI-93-000881
INIT 04/09/93

FYI Coordinator
OTS Document Processing Center (TS-790)
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Washington DC 20460



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April 2, 1993

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 copies of the following reports:

(Identification No. FYI not assigned). Closed-Patch Repeated Insult Dermal Sensitization Study of TAME in Guinea Pigs. Draft Report from Bio/Dynamics.

(Identification No. FYI not assigned). Tert-Amyl Methyl Ether (TAME) Acute Toxicity to Daphnids Under Flow-Through Conditions. Draft Report from Springborn Laboratories.

(Identification No. FYI not assigned). Tert-Amyl Methyl Ether (TAME) Acute Toxicity to Rainbow Trout Under Flow-Through Conditions. Draft Report from Springborn Laboratories.

These documents do not contain confidential information. If you have any questions, please communicate with me.

Sincerely

Robert T. Drew, Ph.D.

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PHARMACO :: LSR

Contains No CBT

January 26, 1993

Robert T. Drew, Ph.D.
American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

Ref: Project No. 92-6222 - Closed-Patch Repeated Insult Dermal
Sensitization Study of TAME in
Guinea Pigs

Dear Dr. Drew:

Enclosed please find one copy of the audited draft final report
for the above referenced study.

We will issue a final report after receipt of your comments. The
final report will reflect our name change from Bio/dynamics, Inc. to
Pharmaco LSR International Inc.

Please feel free to contact me with any questions and/or comments.

Sincerely,



Donna L. Blaszcak, B.S.
Study Director
Toxicology Services USA

DLB/ljk

Enclosures

29 Jan 93

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BIO/DYNAMICS PROJECT NO.: 92-6222

CLOSED-PATCH REPEATED INSULT
DERMAL SENSITIZATION STUDY OF TAME IN GUINEA PIGS
(Buehler Method)

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

Attn: Robert T. Drew, Ph.D.

Date:

Bio/dynamics Study No.: 92-6222

Closed-Patch Repeated Insult
Dermal Sensitization Study of TAME in Guinea Pigs
(Buehler Method)

ABSTRACT

This study was conducted for American Petroleum Institute in order to evaluate the allergic contact sensitization potential of Tertiary Amyl Methyl Ether (TAME) in guinea pigs. This study was performed at Bio/dynamics, Inc., P.O. Box 2360, Mettlers Road, East Millstone, New Jersey 08875-2360.

TAME was administered as received to twenty Dunkin Hartley guinea pigs (10/sex). Animals were clipped free of hair, the test material was applied to saturation (approximately 0.3 mls) beneath a Hilltop Chamber®. The chamber was occluded and left in place for six hours. This was performed once a week, for three weeks, for a total of three induction exposures. Twenty control animals (10/sex/control material) were similarly treated with Light Mineral Oil (vehicle control) or Dinitrochlorobenzene (DNCB; positive control). Challenge treatments followed the same administration procedure as the Induction Phase but at naive sites. In order to differentiate dermal reactions produced by irritation from those produced by sensitization, ten (5/sex) previously untreated animals were subjected to the same challenge procedures, with Light Mineral Oil, DNCB and TAME applied at three separate sites.

Observations for mortality were made twice daily. Body weights were obtained pretest and two days after challenge. Animals were also observed prior to treatment and weekly during the study for general health. Dermal evaluations were made approximately 24 and 48 hours after the first induction exposure and 24 and 48 hours after the challenge exposure.

All animals survived throughout the study. Most animals gained weight throughout the study; Animal No. 8082 (found dead one week after study termination) lost 18 grams of weight during the study.

All ten vehicle control animals challenged with 100% light mineral oil were free of significant dermal responses, as were the irritation control animals. The Incidence Index of sensitization to the vehicle was 0%. The Severity Indices at 24 and 48 hours were 0, for both vehicle-treated animals and irritation control animals.

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All ten positive control animals treated with 0.3% DNCB exhibited clear dermal responses which were of greater incidence and severity than the responses seen in the irritation control animals to the same concentration. The Incidence Index of sensitization to DNCB was 100%. The Severity Indices at 24 and 48 hours were 1.8 and 2.1, respectively, for the positive control animals, compared the indices of 0.2 and 1.4 for the irritation control animals. This positive response to a known sensitizer demonstrated the susceptibility of this shipment of animals to sensitization.

All twenty animals challenged with 100% TAME were free of dermal responses as were the irritation control animals. The Incidence Index of sensitization to TAME was 0%. The Severity Indices at 24 and 48 hours were 0, for test material-treated animals and irritation control animals.

Under conditions of this study, TAME did not exhibit any potential to produce dermal sensitization in guinea pigs.

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I. INTRODUCTION

This study was conducted for American Petroleum Institute in order to evaluate the allergic contact sensitization potential of Tertiary Amyl Methyl Ether (TAME) in guinea pigs. This study was performed at Bio/dynamics, Inc., P.O. Box 2360, Mettlers Road, East Millstone, New Jersey 08875-2360, and used procedures based on the methods described by E.V. Buehler in "Delayed Contact Hypersensitivity in the Guinea Pig", Arch. Dermatol. 91: 171-175, (1965) and H.L. Ritz and E.V. Buehler in "Planning, Conduct and Interpretation of Guinea Pig Sensitization Patch Tests", in Current Concepts in Cutaneous Toxicity (Victor A. Drill and Paul Lazar, eds.), pp. 25-40; Academic Press, 1980.

This study was designed to follow the Buehler Test method which is the method specified in the following guideline:

TSCA (Toxic Substances Control Act): Health Effects Test Guidelines; Office of Toxic Substances; Office of Pesticides and Toxic Substances, United States Environmental Protection Agency, September 1985, Section 798.4100: Dermal Sensitization.

This report has been reviewed by the Quality Assurance Unit of Bio/dynamics, Inc. to assure its conformance with the protocol and the raw data. All raw data and the original study protocol and final report will be retained on file in the Bio/dynamics, Inc. Archives.

II. EXPERIMENTAL DESIGN

<u>Group</u>	<u>Test/Control Material</u>	<u>Number of</u>	<u>Concentration (%)</u>	
			<u>Animals</u>	<u>Induction Challenge</u>
IA	Light Mineral Oil	10	100%	100%
IB	Light Mineral Oil (Irritation Control) ^c	10	-	100%
IIA	DNCB	10	0.5% ^a	0.3% ^b
IIB	DNCB (Irritation Control) ^c	10	-	0.3% ^b
IIIA	TAME	20	100%	100%
IIIB	TAME (Irritation Control) ^c	10	-	100%

^aVehicle: 80% ethanol.

^bVehicle: acetone.

^cIrritation control groups were treated at challenge only. The same ten animals served as irritation controls for all three materials.

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III. DATES OF STUDY

Study Initiation: 21 October 1992
Animal Receipt: 5 October 1992
Range-Finding: 21 through 24 October 1992
Induction:
First: 27 October 1992
Second: 3 November 1992
Third: 10 November 1992
Challenge: 24 November 1992
Study Termination: 26 November 1992

IV. STUDY PERSONNEL

Study Director: Donna L. Blaszcak, B.S., AALAS LATG
Supervisor: Thomas D. Jones, B.A., AALAS LATG
Technician-in-Charge: Daniel Walters
Study Monitor
(Report Preparation): Laura J. Kurowski, A.S.

V. MATERIALS

A. Test and Control Materials:

1. Test Material: TAME (TAME-2)
Lot/Batch Number: MZ07905K2
Description: Colorless liquid
Date of Receipt: 20 October 1992
Expiration Date: Not provided
Received From: Experimental Pathology Laboratory, Inc.
Storage: Room temperature. Refrigerated after 2 November 1992.
Sampling: An archival sample of approximately 10 mls of the test material is stored in the archives of Bio/dynamics, Inc.
2. Positive Control Material: 1-chloro, 2,4-dinitrobenzene (DNCB)
Lot Number: A11T
Date of Receipt: 7 December 1989
Description: Yellow granules
Supplier: Eastman Kodak Company, Rochester, New York
Storage: Room temperature
Sampling: An archival sample of approximately 5 g of positive control material is stored in the archives of Bio/dynamics, Inc.

V. MATERIALS (cont.)

A. Test and Control Materials (cont.):

3. Control Material: Light Mineral Oil
Lot Number: 6358 KHVY
Date of Receipt: 15 April 1992
Description: Clear colorless viscous liquid
Supplier: Mallinckrodt, Paris, Kentucky
Storage: Room temperature
Sampling: An archival sample of approximately 10 g of control material is stored in the archives of Bio/dynamics, Inc.

4. Vehicle: Reagent Alcohol (Induction)
Lot Number: 7006 KHNE
Date of Receipt: 13 December 1991
Description: Clear, colorless liquid
Supplier: Mallinckrodt, Paris, Kentucky
Storage: Room temperature
Preparation: 160 mls of reagent ethanol was added to 40 mls of distilled water to produce an 80% v/v ethanol mixture.

5. Vehicle: Acetone (Challenge)
Lot Number: KDSC
Date of Receipt: 4 December 1989
Description: Clear liquid
Supplier: Baxter Healthcare Corporation
McGaw Park, Illinois
Storage: Room temperature; away from heat, sparks and open flame.

B. Test Animals: Albino Guinea Pigs
Stock: Dunkin Hartley Haz: (DH)fBR
Reason for Selection: Standard laboratory animal for dermal sensitization studies. The Hartley Albino stock was used because of its availability and because of the existing historical data base available for comparative evaluation.
Supplier: HRP, Inc.
Denver, Pennsylvania

V. MATERIALS (cont.)

B. Test Animals (cont.):

Number/Sex of Animals: 1. Range-Finding: 6 females
2. Sensitization Study:
40 (20 males, 20 females)
3. Irritation Controls:
10 (5 males, 5 females)

Age: 3-4 weeks at receipt.
5-6 weeks old at study initiation.

Weight Range at
Initiation of Treatment
(sensitization
animals): Males: 399 - 555 grams
Females: 357 - 460 grams

Equilibration Period: Range-Finding Study: 16 days
Sensitization Study: 22 days

Observations: All animals were checked for viability
twice daily. Prior to assignment to study,
all animals received a physical examination
to ascertain suitability for study.

Husbandry: Currently acceptable practices of good
animal husbandry were followed, e.g., Guide
for the Care and Use of Laboratory Animals;
NIH Publication No 86-23, Revised 1985.

Housing: Individually housed in suspended, stainless
steel cages with wire mesh bottoms.

Environmental
Conditions: 1. Temperature: monitored and recorded
twice daily.
2. Humidity: monitored and recorded
daily.
3. Light Cycle: 12 hours light, 12 hours
dark (controlled by an automatic
timer).

Food: Agway ProLab Guinea Pig Diet, ad libitum

Water: Automatic watering system, ad libitum,
Municipal water supply (Elizabeth Water
Company)

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V. MATERIALS (cont.)

B. Test Animals (cont.):

- Contaminants: There were no known contaminants reasonably expected to be found in the food or water which would be expected to interfere with the results of this study.
- Identification: Each animal was identified with a monel ear tag, bearing a unique number, prior to testing.
- Selection: More animals than required for the study were purchased and equilibrated. Animals were randomly placed into groups using a computer generated random sort. Any animals considered unsuitable because of poor health, outlying body weights, or unacceptable skin were excluded.

VI. METHODS

A. Route of Administration:

Dermal, to the clipped skin of the back and sides.

B. Justification for Route of Administration:

This study was intended to provide information on the health hazards likely to arise from exposure to the test material by the dermal route; skin content is a possible worker and consumer exposure route. The Buehler method is an acceptable method for evaluation of the potential of test materials to produce dermal sensitization.

C. Range-Finding Study:

Prior to initiation of the study, a range-finding study was performed in order to select a slightly irritating concentration for topical induction and a non-irritating concentration for the challenge application. Six animals were treated topically with undiluted test material (100%) and with concentrations of 50%, 25% and 10% v/v of the test material in light mineral oil (4 chambers per animal). The test material mixtures were applied beneath a 25 mm Hilltop Chamber® in a volume of 0.3 ml. The chamber was then occluded with impermeable plastic and secured by an elastic adhesive bandage (Elastoplast®) which was wound around the torso of the animal. The chambers were left in place for six hours, after which they were removed and the skin wiped free of any excess material with distilled water and gauze. Observations for irritation were made at 24 and 48 hours.

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VI. METHODS (cont.):

D. Doses:

Based on results of the range-finding study (presented in Appendix A), the undiluted material was found to be non-irritating and was, therefore, administered at 100% concentration for both induction and challenge.

E. Preparation of Animals:

The hair on the application site (back and sides) was clipped short with an electric clipper on the day prior to each application.

F. Preparation of Test and Control Materials:

1. Positive Control:

- a. Induction: 0.05 g of DNCB was added to 80% ethanol and brought to a total volume of 10 ml to produce a 0.005 g/ml (0.5% w/v) mixture.
- b. Challenge: 0.03 g of DNCB was added to acetone and brought to a total volume of 10 ml to produce a 0.003 g/ml (0.3% w/v) mixture.

2. Test Material:

The test material was administered as received; no preparation was required.

G. Induction Phase:

The hair on the application sites (back and sides) was clipped short with an electric clipper on the day prior to each application. The test materials were applied to saturation (approximately 0.3 mls) beneath a 25 mm Hilltop Chamber[®] placed directly on the test site. The test site was to one side of the midline, as close to the midline as possible. The chamber was covered by overlapping, impermeable plastic. This was firmly secured by an elastic adhesive bandage which was wound around the torso of the animal. The chamber was left in place for six hours after which it was removed and the skin was wiped free of any excess material. This was performed once a week, for three weeks, for a total of three exposures. Note: Due to technician oversight, Female No. 8291's (Group IIIA) second induction exposure was approximately 48 hours. Since no irritation was evident when the wrappings were removed, and there was no subsequent sensitization, this error did not affect the integrity of the study.

VI. METHODS (cont.)

H. Challenge:

a. Test Animals:

Fourteen days after the last induction exposure, the challenge treatment was administered. The test materials were administered in the same manner as in the induction phase, but at a site on the opposite side of the midline from the site used for induction. After six hours of exposure, the chambers were removed and the skin wiped free of any excess material.

b. Irritation Control Animals:

In order to differentiate dermal reactions produced by irritation from those produced by sensitization, 10 animals (previously untreated) were subjected to the same challenge procedure as the animals which received the induction exposures.

VII. EXPERIMENTAL EVALUATION

A. Viability Check:

Twice daily.

B. Body Weights:

Pretest (day prior to first induction)
Terminal (two days after challenge)

C. Observations:

Pretest and weekly during the study for general health; unusual observations were recorded.

D. Evaluation of Dermal Response:

1. Intervals:

Induction:

Dermal evaluations were made approximately 24 and 48 hours after the first induction exposure to confirm that a slightly-irritating concentration of DNCB and an appropriate concentration of the test material had been selected.

Challenge: 24 and 48 hours after dosing

2. Methods:

Dermal responses were scored according to the scoring system presented in Appendix B.

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VIII. POSTMORTEM

A macroscopic examination was performed on the animal which was found dead. Abnormal observations were recorded but no tissues were saved. All animals surviving at termination of the study were killed by carbon dioxide inhalation; no postmortem examinations were performed.

IX. EVALUATION OF RESULTS

Redness at the challenge site which is clearly greater than that seen in the irritation control animals is considered an allergic response. In general, dermal scores of 1 or greater (in the absence of dermal response in irritation control animals) are considered clearly indicative of sensitization. Scores of 0.5 (barely perceptible erythema) are considered equivocal, although a high percentage of scores of 0.5 in treated animals with no dermal response in irritation control animals is considered suggestive of sensitization.

In order to evaluate the responses seen for both test and control animals, two indices were used; one for incidence and one for severity of scores seen. The Incidence Index is a percentage of positive responses [(number of animals per group with a score of 1 or greater at 24 and/or 48 hours) per (total number of animals in the group) x 100]. The Severity Index is the mean value of the male and female dermal scores and is calculated for both the 24- and 48-hour evaluations.

X. RESULTS AND DISCUSSION

A. Mortality

All animals survived throughout the study. Note: Animal No. 8082 (Test Material Group IIIA) was found dead after study termination (Test Day 35). Postmortem macroscopic examination revealed changes only in the heart (1.0 cm diameter white area). Since this death occurred one week after the study terminated, it does not appear to be due to the test material.

B. Body Weights (Table I)

Most animals gained weight throughout the study; Animal No. 8082 (found dead after study termination) lost 18 grams of weight during the study.

C. Dermal Responses

1. Induction

Animals treated with light mineral oil or 100% TAME (Groups IA and IIIA), were free of dermal irritation after the first induction. Most animals treated with 0.5% DNCB (Group IIA) exhibited mild dermal irritation after the first induction.

2. Challenge (Incidence of Dermal Response at Challenge - Table II;
Individual Dermal Response at Challenge - Table III)

All ten vehicle control animals (Group IA) challenged with 100% light mineral oil were free of significant dermal responses, as were the irritation control animals (Group IB). The Incidence Index of sensitization to the vehicle was 0%. The Severity Indices at 24 and 48 hours were 0, for both vehicle-treated animals and irritation control animals.

All ten positive control animals treated with 0.3% DNCB (Group IIA) exhibited clear dermal responses which were of greater incidence and severity than the responses seen in the irritation control animals (Group IIB)

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X. RESULTS AND DISCUSSION (cont.)

C. Dermal Responses (cont.)

2. Challenge (cont.)

to the same concentration. The Incidence Index of sensitization to DNCB was 100%. The Severity Indices at 24 and 48 hours were 1.8 and 2.1, respectively, for the positive control animals, compared the indices of 0.2 and 1.4 for the irritation control animals. This positive response to a known sensitizer demonstrated the susceptibility of this shipment of animals to sensitization.

All twenty animals challenged with 100% TAME (Group IIIA) were free of dermal responses as were the irritation control animals (Group IIIB). The Incidence Index of sensitization to TAME was 0%. The Severity Indices at 24 and 48 hours were 0, for test material-treated animals and irritation control animals.

XI. CONCLUSION

Under conditions of this study, TAME did not exhibit any potential to produce dermal sensitization in guinea pigs.

Donna L. Blaszcak, B.S., AALAS LATG
Study Director/Toxicology

Date

Carol S. Auletta, B.A., D.A.B.T.
Associate Director of Toxicology

Date

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TABLE I
CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY
OF TAME IN GUINEA PIGS
BODY WEIGHTS (GRAMS)

	Animal No. and Sex	Pretest	Terminal	Weight Gain
Group IA Mineral Oil	8106 M	490	642	152
	8113 M	510	721	211
	8083 M	493	699	206
	8123 M	480	781	301
	8089 M	470	698	228
	8282 F	359	498	139
	8257 F	380	543	163
	8259 F	360	476	116
	8308 F	460	614	154
	8267 F	412	618	206
Group IIA DNCEB	8079 M	457	677	220
	8116 M	420	647	227
	8100 M	505	707	202
	8110 M	459	620	161
	8137 M	470	733	263
	8303 F	398	552	154
	8314 F	420	557	137
	8299 F	400	573	173
	8265 F	420	539	119
	8297 F	410	537	127
Group IIIA TAME	8081 M	417	633	216
	8097 M	555	869	314
	8119 M	450	677	227
	8094 M	412	677	265
	8138 M	490	767	277
	8128 M	480	777	297
	8076 M	460	621	161
	8082 M	480	462	-18
	8088 M	399	606	207
	8078 M	530	850	320
	8263 F	410	595	185
	8271 F	359	486	127
	8293 F	368	509	141
	8268 F	357	479	122
	8256 F	378	513	135
	8286 F	390	610	220
	8295 F	405	562	157
	8291 F	371	529	158
	8279 F	456	658	202
	8311 F	409	618	209
Group IB/IIB/ IIIB Challenge Irritation Controls	8080 M	439	672	233
	8093 M	460	704	244
	8132 M	426	618	192
	8085 M	399	626	227
	8099 M	478	693	215
	8255 F	372	544	172
	8290 F	372	526	154
	8254 F	445	674	229
	8313 F	410	560	150
	8294 F	331	436	105

M=Male; F=Female.

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TABLE II
CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY
OF TAME IN GUINEA PIGS

INCIDENCE OF DERMAL RESPONSES AT CHALLENGE

Group	Material	Interval		Dermal Scores ^a							pb	Total No. of Animals		
		Conc. ^c	Hrs	0	0.5	1	2	3	Ed	N			E	
IA	Light Mineral Oil	100%	24	9	1	0	0	0	0	0	0	0	0	10
			48	10	0	0	0	0	0	0	0	0		10
IB	Light Mineral Oil (Irritation Control) ^d	100%	24	10	0	0	0	0	0	0	0	0	0	10
			48	10	0	0	0	0	0	0	0	0		10
IIA	DNCB	0.3%	24	0	0	4	4	2	10	2	0	10	10	
			48	0	0	3	3	4	10	3	0		10	
IIB	DNCB (Irritation Control) ^d	0.3%	24	7	3	0	0	0	0	0	0	9	10	
			48	0	1	6	2	1	2	1	0		10	
IIIA	TAME	100%	24	20	0	0	0	0	0	0	0	0	20	
			48	20	0	0	0	0	0	0	0		20	
IIIB	TAME (Irritation Control) ^d	100%	24	10	0	0	0	0	0	0	0	0	10	
			48	10	0	0	0	0	0	0	0		10	

^aScored using the scoring system presented in Appendix B.

^bP=Positive response; number of animals with a score of 1 or greater at 24 and/or 48 hours, out of the 10 (or 20) animals per group.

^cConc.=Concentration administered at challenge.

^dIrritation control groups were treated at challenge only.

Ed=Edema; N=Necrosis; E=Eschar.

TABLE III
CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY
OF TAME IN GUINEA PIGS

INDIVIDUAL DERMAL SCORES^a AT CHALLENGE

GROUP: I MATERIAL: LIGHT MINERAL OIL
INDUCTION CONCENTRATION: 100%
CHALLENGE CONCENTRATION: 100%

Group IA			Group IB		
Animal No. and Sex	Interval		Animal No. and Sex	Interval	
	24 Hrs	48 Hrs		24 Hrs	48 Hrs
8106 M	0	0	8080 M	0	0
8113 M	0	0	8093 M	0	0
8083 M	0	0	8132 M	0	0
8123 M	0.5	0	8085 M	0	0
8089 M	0	0	8099 M	0	0
8282 F	0	0	8255 F	0	0
8257 F	0	0	8290 F	0	0
8259 F	0	0	8254 F	0	0
8308 F	0	0	8313 F	0	0
8267 F	0	0	8294 F	0	0
Sum of Scores:	0.5	0		0	0
Mean ^c :	0	0		0	0

^aScored using the scoring system presented in Appendix B.

^bIrritation control animals were treated at challenge only.

^cMean=Severity Index.

M=Male; F=Female.

TABLE III (cont.)

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY
OF TAME IN GUINEA PIGS

INDIVIDUAL DERMAL SCORES^a AT CHALLENGE (cont.)

GROUP: II

MATERIAL: DNCB

INDUCTION CONCENTRATION: 0.5%

CHALLENGE CONCENTRATION: 0.3%

Group IIA			Group IIB		
Animals Treated During Induction			Irritation Control Animals ^b		
Animal No. and Sex	Interval		Animal No. and Sex	Interval	
	24 Hrs	48 Hrs		24 Hrs	48 Hrs
8079 M	2 Ed	2 Ed	8080 M	0	3 N,Ed
8116 M	3 Ed,N	3 Ed,N	8093 M	0	1
8100 M	3 Ed,N	3 Ed,N	8132 M	0	2
8110 M	2 Ed	2 Ed	8085 M	0.5	1
8137 M	1 Ed	1 Ed	8099 M	0	1
8303 F	1 Ed	1 Ed	8255 F	0	1
8314 F	1 Ed	3 Ed,N	8290 F	0	0.5
8299 F	1 Ed	1 Ed	8254 F	0	2 Ed
8265 F	2 Ed	2 Ed	8313 F	0.5	1
8297 F	2 Ed	3 Ed	8294 F	0.5	1
Sum of Scores:	18.0	21.0		1.5	13.5
Mean ^c :	1.8	2.1		0.2	1.4

^aScored using the scoring system presented in Appendix B.

^bIrritation control animals were treated at challenge only.

^cMean=Severity Index.

M=Male; F=Female; N=Necrosis; Ed=Edema.

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TABLE III (cont.)

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY
OF TAME IN GUINEA PIGS

INDIVIDUAL DERMAL SCORES^a AT CHALLENGE (cont.)

GROUP: III

MATERIAL: TAME

INDUCTION CONCENTRATION: 100%

CHALLENGE CONCENTRATION: 100%

Group IIIA					
Animals Treated During Induction					
Animal No. and Sex	Challenge		Animal No. and Sex	Challenge	
	24 Hrs	48 Hrs		24 Hrs	48 Hrs
8081 M	0	0	8263 F	0	0
8097 M	0	0	8271 F	0	0
8119 M	0	0	8293 F	0	0
8094 M	0	0	8268 F	0	0
8138 M	0	0	8256 F	0	0
8128 M	0	0	8286 F	0	0
8076 M	0	0	8295 F	0	0
8082 M	0	0	8291 F	0	0
8088 M	0	0	8279 F	0	0
8078 M	0	0	8311 F	0	0
Sum of Scores:	0	0		0	0
Mean ^c :	0	0		0	0
Irritation Control ^b					
Group IIIB					
Challenge					
Animal No. and Sex	Interval		Animal No. and Sex	Interval	
	24 Hrs	48 Hrs		24 Hrs	48 Hrs
8080 M	0	0	8255 F	0	0
8093 M	0	0	8290 F	0	0
8132 M	0	0	8254 F	0	0
8085 M	0	0	8313 F	0	0
8099 M	0	0	8294 F	0	0
Sum of Scores:	0	0		0	0
Mean ^c :	0	0		0	0

^aScored using the scoring system presented in Appendix B.

^bIrritation control animals were treated at challenge only.

^cMean=Severity Index.

M=Male; F=Female.

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Appendix A

Closed-Patch Repeated Insult Dermal Sensitization Study
of TAME in Guinea Pigs

Range-Finding Study - Individual Dermal Scores^a

Animal No. and Sex	Concentration: Interval:	100%		50% ^b		25% ^b		10% ^b	
		24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours
8171 F		0	0	0	0	0	0	0	0
8172 F		0	0	0	0	0	0	0	0
8173 F		0	0	0	0	0	0	0	0
8174 F		0	0	0	0	0	0	0	0
8175 F		0	0	0	0	0	0	0	0
8176 F		0	0	0	0	0	0	0	0

^aScored using scoring system presented in Appendix B.

^bVehicle: Light mineral oil.

F=Female

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Appendix B

Closed-Patch Repeated Insult Dermal Sensitization Study
of TAME in Guinea Pigs

Evaluation of Dermal Irritation

No reaction.....	0
Very slight (barely perceptible) erythema, usually non-confluent.....	0.5
Slight (well-defined) erythema, usually confluent.....	1
Moderate erythema.....	2
Severe erythema, with or without edema, necrosis or eschar formation...	3

If edema, necrosis or eschar formation occurred, they were also indicated using the following code:

Edema..... Ed
Necrosis.. N
Eschar.... E

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Appendix C

Closed-Patch Repeated Insult Dermal Sensitization Study
of TAME in Guinea Pigs

Quality Assurance Statement

Listed below are dates that this study was inspected by the Quality Assurance Unit of Bio/dynamics, Inc. and the dates findings were reported to the Study Director and Management.

<u>Dates of Inspection</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
10/28/92 01/20/93 to 01/21/93	10/28/92 01/25/93	11/09/92 01/26/93

Jane Pasquito
Jane Pasquito, B.S.
Group Leader, Quality Assurance

1/26/93
Date

**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

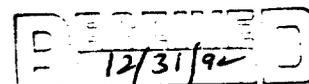
SLI Study # 12827.0692.6102.115

Study Director: Arthur E. Putt

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

30 December 1992

DRAFT REPORT



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GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

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

Springborn Laboratories, Inc.

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

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

1.0 INTRODUCTION

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

2.0 MATERIALS AND METHODS

2.1 Protocol

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

2.2 Test Material

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

to prepare Quality Control samples during the definitive exposure. The sample was identified by the Study Sponsor to contain 98.8% active ingredient A.I. (Certificate of Analysis, Appendix II). The second sample, Lot # 07905KZ, was received at SLI on 2 November 1992 and was used to prepare exposure solutions during the definitive exposures. The sample was identified by the Study Sponsor 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.

2.3 Test Organisms

The *Daphnia magna* used in this toxicity test were obtained from laboratory cultures maintained at Springborn Laboratories, Inc., Wareham, Massachusetts. The culture water was prepared by fortifying well water based on the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column and a carbon filter. Two weeks prior to test initiation, this water had total hardness and total alkalinity as calcium carbonate (CaCO_3) of 160 mg/L and 110 mg/L, respectively, a pH of 8.1, a specific conductivity range of 400 - 500 $\mu\text{mhos/cm}$ 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 darkness. Light intensity in the culture area ranged from 32 - 48 footcandles (Invertebrate Culture Log, Vol. VIII). A waterbath in the culture area was used to maintain the culture solution temperature at 20 ± 2 °C. Daphnids were fed a combination of a trout food suspension and a unicellular green algae (*Ankistrodesmus falcatus*) once daily. The food solution contained 5.0 mg/mL trout food and approximately 4×10^7 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 pipet. In addition, three Quality Control (QC) samples were prepared at each sampling interval and remained with the samples of the exposure solutions throughout the analytical process. Results of the analyses of the QC samples were used to judge the precision and the quality control maintained during the analysis of exposure solution samples. All samples were analyzed for TAME using a gas chromatography (GC) procedure according to the methodology described in Appendix V. A method validation recovery study, conducted at SLI prior to the initiation of the definitive test, established an average recovery of TAME of $102 \pm 5\%$ (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. 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 several of the mobile daphnids exposed to this treatment level. No immobilization or sublethal effects were observed among daphnids exposed to the remaining nominal concentrations tested (250, 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 Daphnids (*Daphnia magna*) Under Flow-Through Conditions" were inspected by the Springborn Laboratories, Inc., Environmental Sciences Division, Quality Assurance Unit (QAU) to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations. Dates of study inspections, Dates reported to Study Director and to Management are listed below.

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

<u>Inspection Date</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
------------------------	-----------------------------------	-------------------------------

SPRINGBORN LABORATORIES, INC.

Patricia D. Royal
Regulatory Affairs
and Quality Assurance Unit

Date

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TABLES

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

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

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

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

Nominal Concentration (mg A.I./L)	Total Hardness ^a (mg/L CaCO ₃)	Total Alkalinity ^a (mg/L CaCO ₃)	Specific Conductance ^a (μmhos/cm)
690	190	110	500
410	180	110	500
250	180	110	500
150	180	110	500
89	170	120	500
Control	170	120	500

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

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

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

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

^b QC = Quality Control sample.

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

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

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

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

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

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

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

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

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

^c EC50 value empirically estimated as greater than the highest concentration tested.

^d EC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.

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FIGURES

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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.

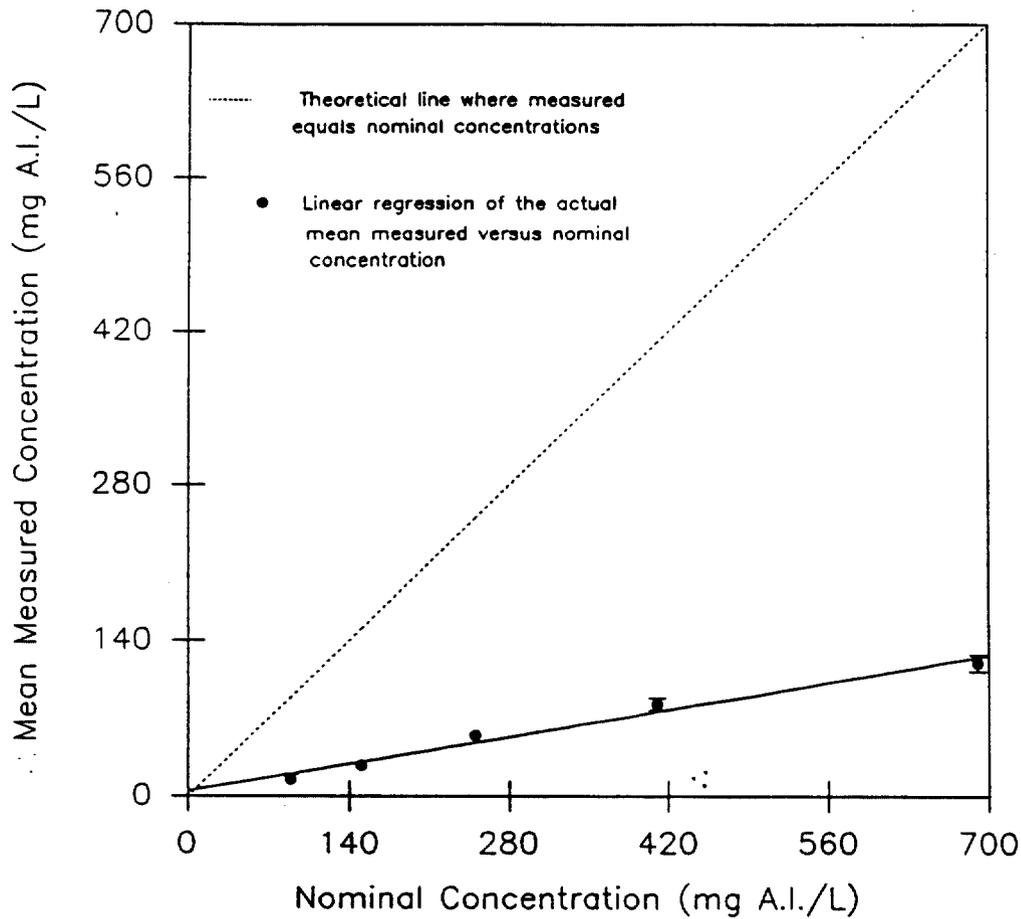
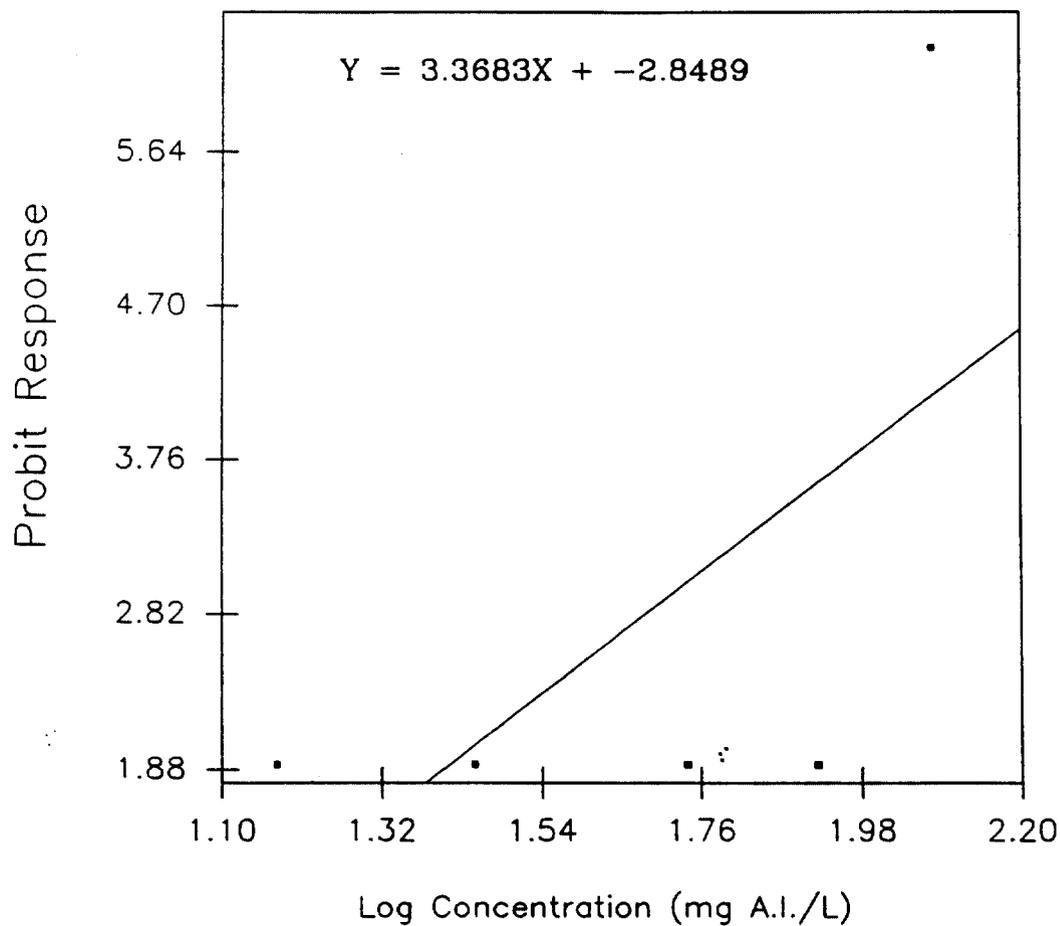


Figure 2. The 48-hour concentration-response (immobilization) curve for daphnids (*Daphnia magna*) exposed to TAME.



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SIGNATURES AND APPROVAL

SUBMITTED BY: Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

PREPARED BY:

Arthur E. Putt

Mark J. Brown

Study Director Date

Principle Investigator Date

Joseph P. St Laurent

Susan P. Shepherd

Senior, Analytical Chemist Date

Coordinator, Data Date
Management and Reporting Unit

APPROVED BY:

Patricia D. Royal

Regulatory Affairs and Date
Quality Assurance Unit

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6.0 APPENDIX I - STUDY PROTOCOL

RE

Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Protocol for Conducting a Flow-Through Acute Toxicity Test with *Daphnia magna* Following TSCA §797.1300.

TO BE COMPLETED BY THE STUDY SPONSOR

Study Sponsor: American Petroleum Institute

Address: 2201 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#: 028148Z

Additional Comments and/or Modifications: _____

[Signature] _____
Sponsor Approval Date

TO BE COMPLETED BY SLI PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc.

Study Director: Arthur E. Pott

Test Concentrations: 690, 410, 250, 150, 89 mg A.I./L

Solvent Used: NA CAS# or LOT#: NA

Proposed Schedule: (Start) 12/10/92 (Completion) 12/12/92

Additional Comments and/or Modifications: _____

[Signature] 10/26/92
Study Director Date

Springborn Laboratories Protocol #: 091192/TSCA 797.1300 DM-FA

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Springborn
LABORATORIES

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PROTOCOL FOR CONDUCTING A FLOW-THROUGH ACUTE TOXICITY TEST WITH
DAPHNIA MAGNA FOLLOWING TSCA §797.1300

OBJECTIVE

The purpose of this test is to determine the acute effects of a test material on the water flea, *Daphnia magna*, under flow-through conditions. Test results are reported as 3-, 6-, 24- and 48-h EC50 values, i.e., the median concentration that will immobilize 50% of the number of daphnids exposed, with 95% confidence limits and no observed effect concentrations. These test procedures 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.

MATERIALS AND METHODS

TEST ORGANISMS:

1. **Species.** The water flea, *Daphnia magna*, is the species used in this test. Test organisms are \leq 24 hours old at the initiation of the test. Daphnids are obtained by removing all immature daphnids from the culture vessel, thus isolating sexually mature daphnids 24 hours prior to initiating the test. Young produced by these isolated organisms are subsequently used for test initiation. Daphnids are not used if the culture contains any ephippia, if adults in the cultures do not produce young before day 12, if adults in the cultures do not produce an average of at least 3 young per adult per day over the 7 day period prior to the test, if more than 20% of the culture stock die in the two days preceding the start of the test, or if organisms have been used in any portion of a previous test either in a treatment or control vessel.
2. **Source.** *D. magna* cultures are maintained at the Aquatic Toxicology Laboratory of Springborn Laboratories, Inc. Daphnids are cultured in 2-L glass vessels containing 1 L of water. Water used to culture the daphnids is prepared in the same manner and has the same characteristics as described for dilution water. Culture water is maintained at $20 \pm 2^\circ\text{C}$. Each culture aquarium is cleaned once weekly.
3. **Feeding.** While being maintained in culture prior to the test, organisms are fed daily a combination of a yeast suspension and a unicellular green algae, *Ankistrodesmus falcatus*. The food solution is prepared to contain 5 mg/mL yeast and approximately 4×10^7 cells/mL of algae. An aliquot of 0.5 mL of yeast and 2 mL of algae is manually introduced to each aquarium once daily. Additionally, daphnids are fed a Selco[®] suspension (0.6 mg/mL) twice weekly at a rate of 0.5 mL. Routine analysis are conducted on the food source to ensure the absence of contamination which would be expected to alter the results of the study. Daphnids are not fed during the 48-h exposure period.

4. Handling. Wide-bore pipets are used to transfer the daphnids, taking care to minimize possible stress due to handling. Daphnids that are damaged or dropped during transfer are not used.

PHYSICAL SYSTEM:

1. Test Containers. The test chambers used in the flow-through acute toxicity tests are 2-L clear glass battery jars which are chemically clean. Each jar has a 3 x 8 cm notch cut out on the upper edge, covered with Nitex 40-mesh screen for drainage, and the test solution volume is thus maintained at approximately 1.8 liters. Test chambers are loosely covered to minimize volatilization of the test material and prevent dust from falling in the test solution.
2. Cleaning. The diluter is disassembled and cleaned before use. The water cell is brushed and siphoned in place. The chemical cells, mixing chamber, splitters, delivery tubes and test vessels are removed from the unit and washed with hot water and soap, then cleaned by an appropriate method to remove residue of the test material previously used (i.e., acid to remove metal and bases; detergent and organic solvents to remove organic compounds) and rinsed several times with diluent water. The diluter is then reconstructed and allowed to cycle for at least 24 hours for further rinsing.
3. Dilution Water. Dilution water consists of hard fortified unchlorinated well water with a total hardness of 160 to 180 mg/L CaCO₃. The well water (total hardness of approximately 30 mg/L as CaCO₃) is fortified according to the formulation for hard water presented in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975). Hard water is used in the chronic daphnid test, because the survival and reproduction of *D. magna* is enhanced under these conditions. Dilution water is filtered through an amberlite XAD-7 resin column and an activated carbon bed prior to delivery to the diluter. The column is about 15 cm long and 1.6 cm wide. This filtration effectively removes any potential organic contaminants from the water. The resin is replaced in the column prior to initiation of each study.

Quality of the dilution water used to conduct daphnid chronic tests is judged by the ability of the daphnid cultures to survive and reproduce in the water free of stress. The dilution water is prepared in 1,900-L batches. New batches of diluent water are prepared when the previous batch is exhausted, when a water quality parameter (total hardness, alkalinity, etc.) has varied from the normal ranges, or after two weeks of holding. The diluent water is aerated with an air pump and air stones to bring the pH and dissolved gases into equilibrium with the atmosphere. Fiberglass containers are used to hold the diluent water, and water is pumped from this holding tank to the diluter. At least twice each year analyses of representative samples of dilution water source are conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the daphnids. In addition, TOC, COD, particulate matter unionized ammonia, and organic chlorine analyses are conducted at least twice each year in the dilution water.

Total hardness, total alkalinity, pH and specific conductance of the diluent water are monitored on each batch prior to use to assure that these parameters are within the normal acceptable ranges. Total hardness and alkalinity are determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1985). Ranges for these parameters generally are: total hardness, 160 - 180 mg/L CaCO₃; alkalinity, 110 - 130 mg/L CaCO₃; specific conductance, 400 to 600 μ mhos/cm; and pH, 7.9 - 8.3.

4. **Diluter.** A 200-mL proportional or serial diluter (e.g., Mount and Brungs, 1967) is employed to deliver five toxicant concentrations, a control, and a solvent control, if necessary, to two replicate jars. Each dose level is at least 60% of the the next higher concentration of the test material. The exposure system is constructed entirely of glass, silicone, and nylon.

Based on the solubility of the compound, the stock solution stability and the range of test concentrations, one of the following toxicant delivery systems may be used: the gas-tight syringe injector metering device (most frequently used); the tube siphon delivery system; and the metering pump/predilution chamber system. Factors considered in the selection of the appropriate toxicant delivery system are the solubility of the compound in water under test conditions, and the range of concentrations to be tested.

A flow-splitting chamber is used between the diluter cells and the two replicate jars to promote mixing of the toxicant solution and diluent water and to equally split the test solution between the test jars. Separate 1 mm (I.D.) glass capillary tubes exit each splitter cell and enter individual delivery tubes which transfer the test solution to each replicate vessel. The capillary tubes baffle the flow of the test solution and minimize turbulence in the aquaria.

The calibration of the diluter system is checked prior to test initiation and at test termination. If there is any indication during the test that the diluter calibration has changed (e.g., diluter malfunction or unexplained differences in dissolved oxygen concentration or temperature in the jars), calibration of the necessary diluter components is checked. A complete check of diluter functioning is made at least twice daily. A test is not started until the diluter and toxicant delivery device have been observed to be properly functioning for 24 hours prior to the test. During a test, the flow rates vary no more than 10% from one replicate test chamber to another.

5. **Flow Rate.** Delivery rates of the test material to each of the 2-L battery jars is equal to approximately 6 vessel volumes per day. This flow rate is adequate to maintain good water quality and does not stress the organisms due to excessive turbulence.
6. **Replication.** Two replicates are included with each test concentration and control. Each replicate jar contains ten individuals, a total of 20 daphnids per concentration or control.

CHEMICAL SYSTEM:

1. **Test Material.** Upon arrival at Springborn Laboratories, Inc., the external packaging of the test material is inspected for damage. The packaging is removed and the primary storage

container is also inspected for leakage or damage. The sample identity is recorded and the material is stored in the dark at approximately 20°C until used, unless specified differently by the test sponsor.

2. **Toxicant Concentration Selection.** Toxicant concentrations for the acute toxicity test are selected based on information provided by the sponsor or obtained from a preliminary range-finder test with *D. magna*. 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 compounds, one or both levels may not be observed. Five concentrations and one dilution water control are used for each definitive test, each concentration consisting of 20 test daphnids.
3. **Stock Preparation.** Test material is weighed on an analytical balance for which a calibration log is maintained. A chemical usage Log is also maintained in which the amount, the date, the intended use and the user's initials are recorded each time test material is used. The frequency of the stock preparation will be based on consultation with the study sponsor, and will be specified in the study report. The stock solution is prepared according to the following formula:

$$\text{Stock concentration} = \frac{\text{H.C.} \times \text{M.C.}}{\text{B.D.} \times \% \text{ A.I.}}$$

where:

H.C. = high concentration (mg/L)

M.C. = mixing chamber volume (L)

B.D. = bird or syringe delivery (mL)

A.I. = % active ingredient

4. **Solvent Control.** If a solvent is used, a solvent control is established which contains a concentration of solvent equal to the amount present in the highest test concentration, but not to exceed 0.1 mL/L. Reagent grade or highest quality triethylene glycol (TEG), acetone, methanol or ethanol, or dimethyl formamide (DMF) is used.

EXPERIMENTAL PROCEDURE:

1. **Test Initiation.** The experimental design of this test incorporates two replicate 2-L glass jars per treatment. Each jar contains approximately 1.8-L of test solution and ten impartially-selected daphnids. The two vessels for each treatment are arranged in rows, with randomization of the position of each vessel within the row. Positions of rows are also randomly assigned. Daphnids are exposed to five concentrations of the test material.

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Additionally, a set of control beakers consisting of dilution water containing no test material is maintained. If a carrier solvent is used to prepare the concentrations of the test material, a solvent control is also tested which contains the highest concentration of solvent present in any test solution. At the initiation of the study, each test concentration is prepared as outlined above (see Chemical System). Daphnids are impartially selected and distributed to each beaker, and the test is initiated.

2. **Sampling and Measurement of Toxicant Concentrations.** Unless specified differently by the sponsor water samples of an appropriate volume are taken from one replicate of the high, middle and low test concentrations at least once during the pre-exposure period to document water concentrations and the proper functioning of the diluter. Water samples are taken from a point approximately midway between the surface, bottom and sides of each jar and either extracted immediately after sampling or appropriately preserved and stored until analysis can be performed. During the in-life phase, samples from one replicate jar of each concentration and control(s) are taken at the initiation of the test and at test termination for determination of toxicant concentrations. Replicates are alternated to ensure that each replicate has been sampled once during the test. Immediately after sampling, samples are passed through a 0.45 μm filter to remove any material which may be associated with particulate matter. Three quality control samples are prepared at each sampling interval and remain with the set of samples through extraction, storage and analysis. These samples are prepared in diluent water at test material concentrations similar to the treatment level range. Results of these analyses indicate the relative accuracy of the analytical methodologies for each sampling period.
3. **Photoperiod.** All tests are conducted in a temperature and light-controlled laboratory. The tests are illuminated to a light intensity of 20 - 100 footcandles using a combination of fluorescent bulbs. A 16-hour light, 8-hour dark photoperiod is maintained with an automatic timer.
4. **Measurement of Water Quality Variables.** At test initiation and daily thereafter, temperature, dissolved oxygen (DO) concentration and pH are measured and recorded in each test vessel. Total hardness, alkalinity, specific conductance are determined at test initiation in one replicate of each concentration and control. Temperature is monitored continuously in one test solution by using a minimum-maximum thermometer. Readings of temperature extremes are recorded daily.
5. **Dissolved Oxygen.** Total dissolved oxygen is not allowed to drop below 60% or exceed 105% of saturation for the duration of the test. Aeration (with oil free air) would be initiated as a last resort to raise and maintain the dissolved oxygen concentration at or above 60% of saturation.
6. **Temperature.** Water temperature of the test solutions is maintained at 20 ± 2 °C by conducting the test in a temperature-controlled room maintained at the appropriate test temperature, or in a temperature-controlled water bath.
7. **Biological Data.** The number of immobilized daphnids in each test vessel is recorded 3, 6, 24 and 48 hours after test initiation. The test is terminated following 48 hours of exposure at which time no more than 10% of the control organisms can appear immobilized or the test will

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be considered unacceptable. In addition, whenever test organisms are observed, characteristics of the test solutions are also observed and recorded, e.g., precipitated materials, cloudiness, etc.

STATISTICAL ANALYSES:

Test results derived from the acute test are used to statistically estimate a median effective concentration (EC50) and its 95% confidence interval after 3, 6, 24 and 48 hours of exposure. The EC50 is the estimated nominal or measured concentration of the test material in dilution water which produces 50% immobility in the test populations of daphnids at the stated times of exposure.

The computer program utilized estimates EC50 values using three statistical methods: probit analysis, moving average method, and binomial probability. The method selected and reported is determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.). An EC50 value cannot be calculated if the data derived is insufficient according to any of the three statistical methods. The method provides values of the slope, including 95% confidence intervals, for the probit analysis, as well as appropriate statistical tests to evaluate goodness-of-fit. In addition, the highest test concentration that shows no statistically significant difference from the control (No Observed Effect Concentration (NOEC) is determined and reported.

REPORTING

The raw data and final draft of the report are reviewed by the Quality Assurance Unit and Study Director. Upon request, a single copy of the draft report will initially be submitted to the study sponsor for review. Upon acceptance by the sponsor, three copies of the final report will be submitted. All reports include, but are not limited to, the following information:

- Springborn Laboratories, Inc., report and project numbers.
- Laboratory and site, the dates of testing and personnel involved in the study, i.e., Quality Assurance Unit, Program Coordinator (if applicable), Study Director, Principal Investigator.
- All information pertaining to the test material, e.g., its source, percent active ingredient, physical properties, sponsor's test material I.D., and sample number.
- Characterization and origin of the dilution water.
- Scientific name of the test organisms, source, and culturing information.
- Test container volume, dilution water volume, number of replicates used per concentration, and number of daphnids used per treatment.

- * Definition of criteria used to determine the sublethal effects, and general observations on nonquantifiable effects.
- * Description of diluter system, exposure system and stock preparation.
- * Test temperatures, dissolved oxygen concentration, and pH; and photoperiod and light intensity used, as well as specific conductance, total alkalinity and total hardness measured.
- * If applicable, means and standard deviations of measured concentrations of the test compound, as well as nominal test concentrations.
- * Description of, or reference to chemical and statistical procedures applied.
- * Percentage of daphnids that were immobilized in the controls and in each treatment at each observation period, in tabular form.
- * The 3-, 6-, 24- and 48-hour EC50's with 95 percent confidence limits, and the No Observed Effect Concentration (NOEC).
- * Deviations from the protocol not addressed in protocol amendments will listed, together with a discussion of the impact on the study and signed by the Study Director.
- * Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- * Dates of Quality Assurance reviews, signed by the QA Unit.

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 Substances Control Act (FEDERAL REGISTER, Part III, 40 CFR Part 792; August 17, 1989).

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final test report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

REFERENCES

APHA, AWWA, WPCF. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition, Washington, DC. 2168 pp.

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Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. Water Research 1: 20-29.

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

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: 15 December 1992
 PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Acute Toxicity Test with *Daphnia magna* Following TSCA § 797.1300."
 SPECIES: *Daphnia magna*
 STUDY SPONSOR: American Petroleum Institute
 TEST MATERIAL: Tert-Amyl Methyl Ether (TAME)
 SLI STUDY NO: 12827.0592.6102.115

AMENDMENT(S):

1. The study protocol cover identifies the purity and lot # of the test material as 94% and 02814BZ, respectively. During this study the purity and lot # of the test material used is 98.7% and lot # 07905KZ, respectively.
2. The study protocol states that the culture organisms are fed a combination of yeast suspension (5 mg/mL) and a unicellular green algae, *Ankistrodesmus falcatus*, (4×10^7 cells/mL), supplemented twice weekly with a Selco® suspension (0.6 mg/mL). During this study the culture organisms are fed a combination of trout food suspension (5 mg/mL) and *Ankistrodesmus falcatus* (4×10^7 cells/mL) once daily at rates of 0.5 mL and 2.0 mL, respectively.
3. The study protocol states that TOC, COD, particulate matter, unionized ammonia and organic chlorine analyses are conducted at least twice each year in the dilution water. During this study these analyses will be conducted on the dilution water source, not the dilution water.
4. The study protocol states that water samples of an appropriate volume are taken from one replicate of the high, middle and low test concentrations at least once during the pre-exposure period to document water concentrations and the proper functioning of the diluter. During the in-life phase, samples from one replicate jar of each concentration and control(s) are taken at the initiation of the test and at test termination of determination of toxicant concentrations. Replicates are alternated to insure that each replicate has been sampled once during the test. During this study, water samples of an appropriate volume are taken from both replicates of the high, middle and low test

Springborn Laboratories, Inc. Protocol # 92-12-4545 (TSCA § 797.1300) DM-F-1



LETTERS AND REPORTS: Springborn Laboratories, Inc. letters and reports are issued for the primary use of the client. The client is responsible for the accuracy of the data and the quality of the information presented. Springborn Laboratories, Inc. makes no warranty, expressed or implied, as to the accuracy or completeness of the data or the quality of the information presented. The client is responsible for the accuracy of the data and the quality of the information presented. The client is responsible for the accuracy of the data and the quality of the information presented.

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concentrations to document water concentrations and proper functioning of the diluter. During the in-life phase, samples from both replicate vessels are taken at test initiation and test termination for determination of toxicant concentrations.

- 5. The study protocol states that immediately after sampling, samples are passed through a 0.45 μm filter to remove any material which may be associated with particulate matter. During this study, samples are not passed through a 0.45 μm filter after sampling, due to the absence of particulate matter on the test and control solutions.

Approval Signatures: Arthur E. Putt 15 December 1992
Arthur E. Putt Date
SLI Study Director

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

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7.0 APPENDIX II - CERTIFICATE OF ANALYSIS

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chemists helping chemists in research & industry

SPRINGBORN LABORATORIES
508 295 8107
PAULA LECONTE

PO NBR:

PRODUCT INFORMATION

PRODUCT NUMBER: 28309-6

LOT NUMBER: 028148Z

PRODUCT NAME: TERT-AMYL METHYL ETHER, 94%

FORMULA: C6H14O

FORMULA WEIGHT: 102.18

APPEARANCE

COLORLESS LIQUID

REFRACTIVE INDEX AT
20 DEG C

1.3885

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 warrants that its products conform to the information contained in this and other Aldrich publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Aldrich Chemical Company
DAVID SWESSEL
NOVEMBER 11, 1992



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

Springborn Laboratories, Inc.

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chemists helping chemists in research & industry

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AMER PETRO INST
202 682 8270
DR DICK RHODEN

PD NBR: 820003008A13

12827.0592.652.45

PRODUCT INFORMATION

PRODUCT NUMBER: 28309-6

LOT NUMBER: 07905KZ

PRODUCT NAME: TERT-AMYL METHYL ETHER, 94X

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 I,
VOLUME 3 OF "THE ALDRICH LIBRARY OF FT-IR
SPECTRA".GAS LIQUID
CHROMATOGRAPHY

98.7 X

ALDRICH warrants that its products conform to the information contained in this and other Aldrich publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Aldrich Chemical Company
DAVID GWESSEL
NOVEMBER 6, 1992



aldrich chemical co.

P.O. Box 356, Milwaukee, Wisconsin 53201 USA • (414) 273-3850

Springborn Laboratories, Inc.

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8.0 APPENDIX III - CULTURE FOOD ANALYSIS

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Ank Suspension Grab Liquid Sample*		
Date Submitted: 4/29/92 Date Reported: 5/11/92		
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	< 0.05 µg/l	0.05
Toxaphene	< 1. µg/l	1.
PCB's	< 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.

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Ank Suspension Grab Liquid Sample*		
Date Submitted: 4/29/92 Date Reported: 5/11/92		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen I, II, III	attached	
Arsenic	< 0.1 mg/l	0.1
Cadmium	< 0.005 mg/l	0.005
Lead	< 0.05 mg/l	0.05
Mercury	0.0004 mg/l	0.0002
* Analyzed by Lancaster Laboratories, Inc.		

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Zeigler Brothers, Inc. Salmon Starter #1*		
Date Submitted: 12/04/91 Date Reported: 12/19/91		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen I;II;III	attached	
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
* Analyzed by Lancaster Laboratories, Inc.		

Zeigler Brothers Inc. Salmon Starter #1*		
Date Submitted:12/04/91 Date Reported:12/19/91		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen I;II;III	attached	
Arsenic	2.1 ppm	0.1
Cadmium	0.4 ppm	0.2
Lead	0.5 ppm	0.2
Mercury	0.06 ppm	0.02
Selenium (fluorometric)	1.0 ppm	0.1

* Analyzed by Lancaster Laboratories, Inc.

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9.0 APPENDIX IV - DILUTION WATER ANALYSIS

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GFT Grab Water Sample*		
Date Collected: 6/23/92 Date Reported: 7/9/92		
Analysis -	Result As Received	Limit of Quantitation
Pesticide screen (I, II, III)	attached	
Mercury	< 0.0002 mg/l	0.0002
Arsenic	< 0.05 mg/l	0.05
Selenium	< 0.05 mg/l	0.05
Boron	< 0.05 mg/l	0.05
Thallium	< 0.1 mg/l	0.1
Aluminum	< 0.2 mg/l	0.2
Antimony	< 0.05 mg/l	0.05
Barium	< 0.2 mg/l	0.2
Beryllium	< 0.005 mg/l	0.005
Cadmium	< 0.005 mg/l	0.005
Calcium	7.4 mg/l	0.5
Chromium	< 0.05 mg/l	0.05
Cobalt	< 0.05 mg/l	0.05
Copper	< 0.02 mg/l	0.02
Iron	< 0.1 mg/l	0.1
Lead	< 0.05 mg/l	0.05
Magnesium	2.2 mg/l	0.5
Manganese	< 0.01 mg/l	0.01
Molybdenum	< 0.1 mg/l	0.1
Nickel	< 0.04 mg/l	0.04
Potassium	1.0 mg/l	0.5
Silver	< 0.01 mg/l	0.01
Sodium	13.3 mg/l	0.5
Titanium	< 0.05 mg/l	0.05
Vanadium	< 0.05 mg/l	0.05
Zinc	< 0.02 mg/l	0.02

* Analyzed by Lancaster Laboratories, Inc.

GFT Grab Water Sample*		
Date Collected: 6/23/92 Date reported: 7/9/92		
Analysis	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	< 0.05 µg/l	0.05
Toxaphene	< 1. µg/l	1.
PCB's	< 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.		

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10.0 APPENDIX V - ANALYTICAL METHODOLOGY

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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 material. 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 $89.7 \pm 2.3\%$, $104 \pm 11\%$ for filtered seawater and 102 ± 10 for freshwater. 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 μ m 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 Sponsor 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 material 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 (μ L)	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 μ m film
Temperature ($^{\circ}$ C): Injector: 200
column temperature programmed: 40 - 250
Rate: 10 $^{\circ}$ C per minute from 40 to 70 $^{\circ}$ C
25 $^{\circ}$ C per minute from 70 - 250 $^{\circ}$ 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 through 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: $89.7 \pm 2.3\%$

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 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

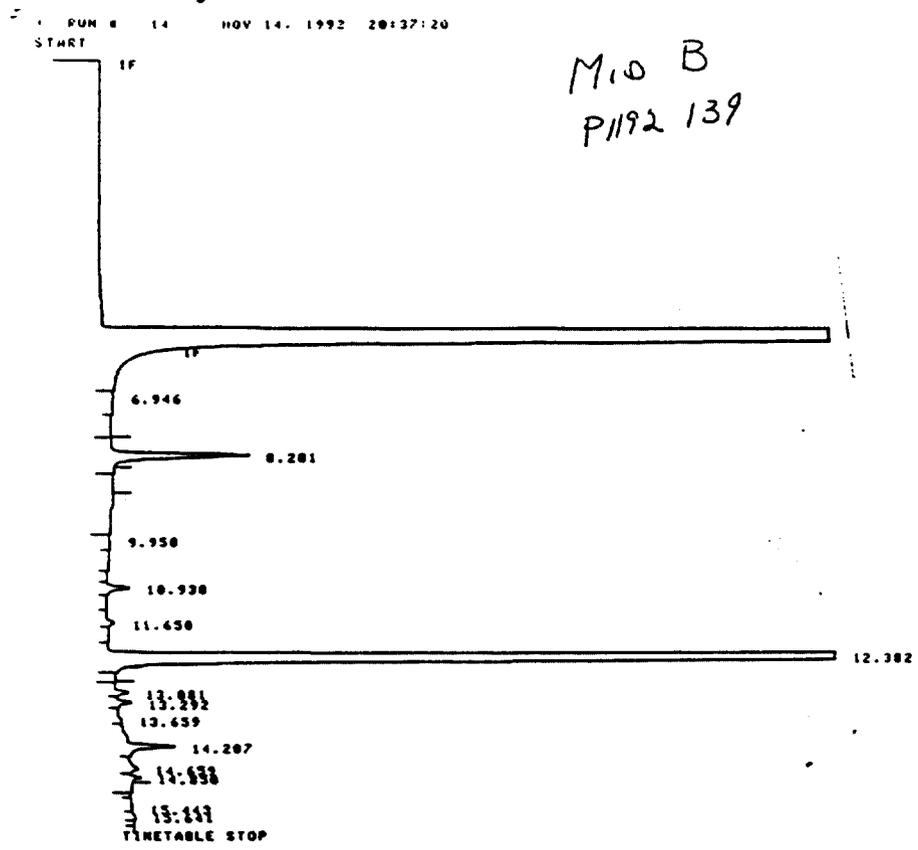
Mean Recovery: $102 \pm 10\%$

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.

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: N-TAMEPCTP.MET

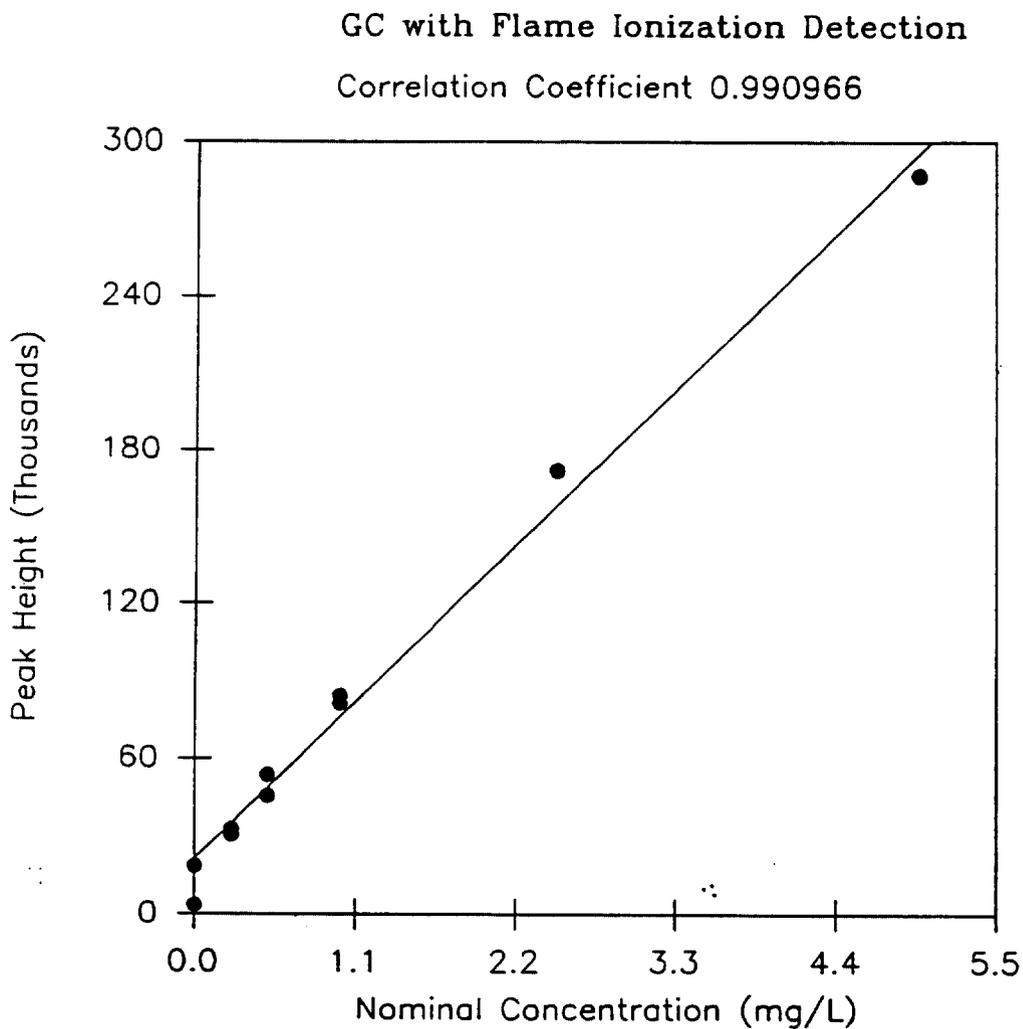
IDENTIFIER: 13140039449

PERMISSION: TAME
 ER 18/10/92

ESTD-HEIGHT	RT	TYPE	AREA	WIDTH	HEIGHT	CALC	AS/L	NAME
	8.201	PP	156375	.107	24263			.000
	10.930	PP	10711	.082	3017			.000
	12.382	PP	3787170	.103	612042	1R	3.596	TAME
	13.292	VP	11101	.071	2610			.000
	14.207	VV	69006	.129	8923			.000
	14.659	VV	17942	.164	1025			.000

TOTAL HEIGHT= 654200
 MUL FACTOR=1.0000E+00

Figure 2A. ¹/₂ A representative linear regression analysis from standard TAME analysis.



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

A.PI
TAME

12827-0692-6100-250 PAGE 6
METHOD VALIDATION

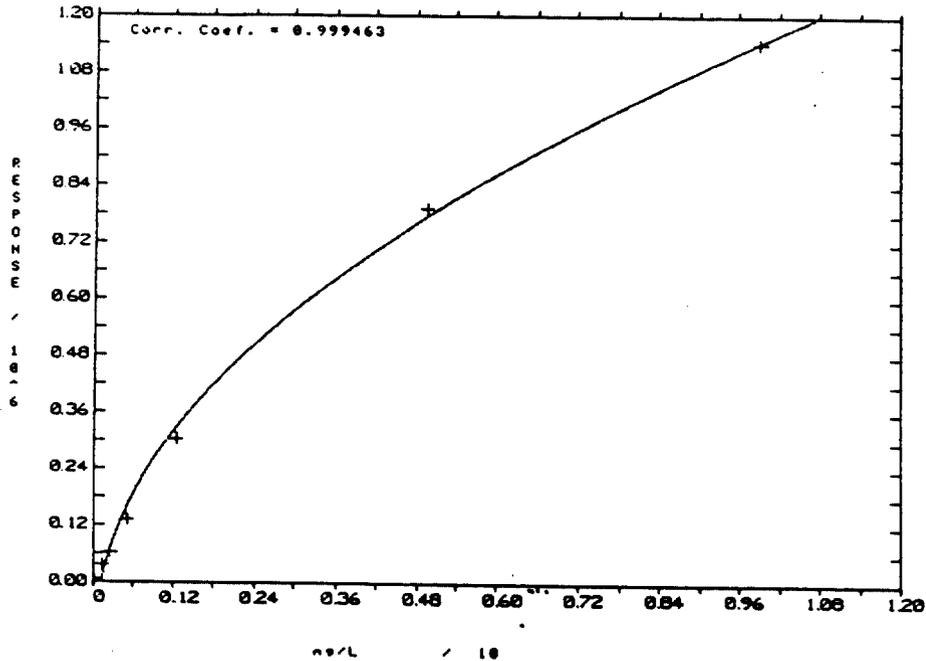
WELCOME TO THE HP3396 CALIBRATION CURVE PLOTTING PROGRAM REV. 8.02.00

At any prompt: '0' [ENTER] Quits
'5' [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/N]: N

* EDIT CALIB #

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

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
pH										
950	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
570	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
340	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
210	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
120	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Control	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Dissolved Oxygen Concentration, mg/L (% Saturation)										
950	9.8 (91)	9.6 (89)	9.6 (87)	9.4 (85)	9.0 (83)	9.3 (86)	9.8 (89)	9.6 (87)	9.0 (83)	9.1 (84)
570	9.6 (89)	9.7 (90)	9.6 (87)	9.6 (87)	9.2 (85)	9.0 (83)	9.7 (88)	9.4 (85)	9.1 (82)	8.9 (82)
340	9.8 (91)	9.8 (91)	9.6 (87)	9.4 (85)	9.3 (86)	9.4 (87)	9.6 (87)	9.6 (87)	9.1 (82)	9.1 (82)
210	9.8 (91)	9.7 (90)	9.5 (86)	9.4 (85)	9.4 (87)	9.4 (87)	9.8 (89)	9.7 (88)	9.6 (87)	9.0 (81)
120	9.8 (91)	9.8 (91)	9.4 (85)	9.6 (87)	9.0 (83)	9.2 (83)	9.6 (87)	9.7 (88)	8.9 (81)	9.0 (81)
Control	9.6 (89)	9.7 (90)	9.5 (86)	9.6 (87)	9.1 (84)	9.2 (83)	9.5 (86)	9.6 (87)	8.9 (82)	8.9 (81)
Temperature (°C)^a										
	12		11		11 - 12		11		11 - 12	

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

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

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

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

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

^c N = 3

^d QC = Quality Control sample.

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

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

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

^a All of the surviving fish exhibited complete loss of equilibrium,

^b Several of the surviving fish exhibited complete loss of equilibrium.

^c Several of the surviving fish were observed to be lethargic.

^d All of the surviving fish exhibited darkened pigmentation and complete loss of equilibrium.

^e Several of the surviving fish exhibited darkened pigmentation and complete loss of equilibrium.

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

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

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

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

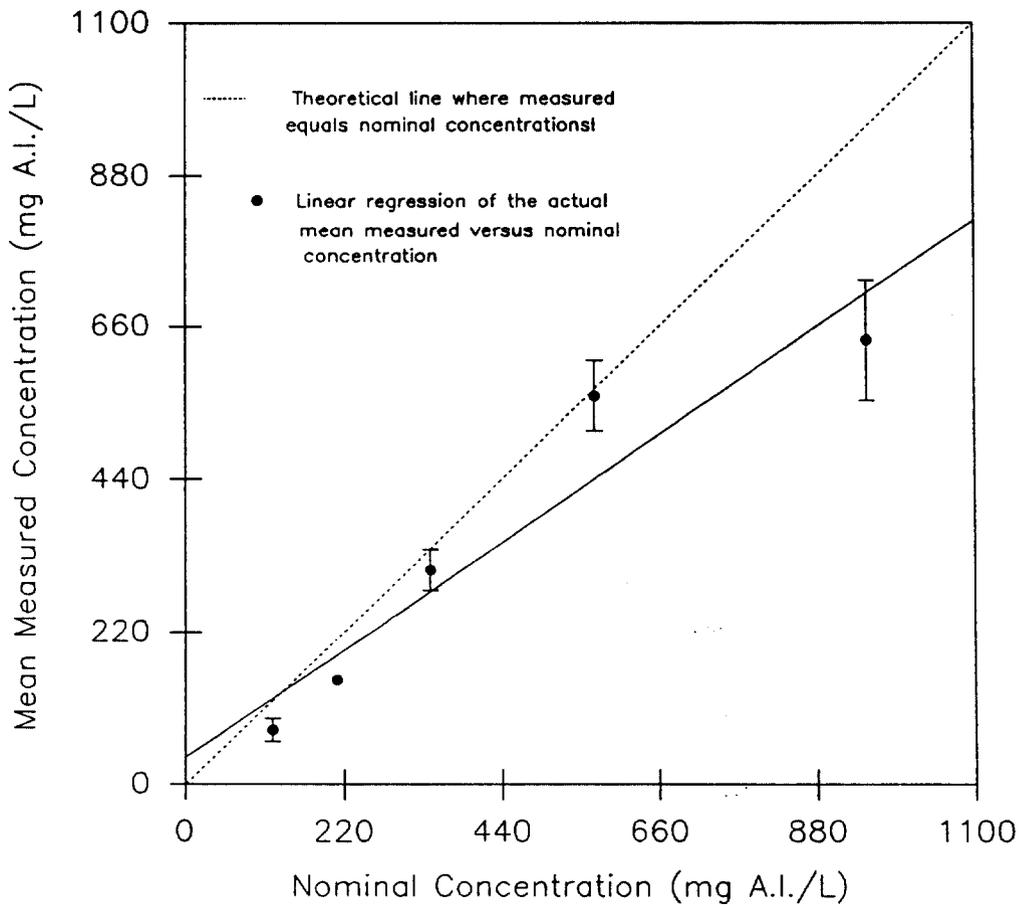
^c LC50 value (95% confidence interval) calculated by probit analysis.

^d LC50 value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability.

^e Since the 96-hour LC50 value was not less than 50% of the 48-hour value, the study was not extended beyond 96-hours to determine the incipient LC50.

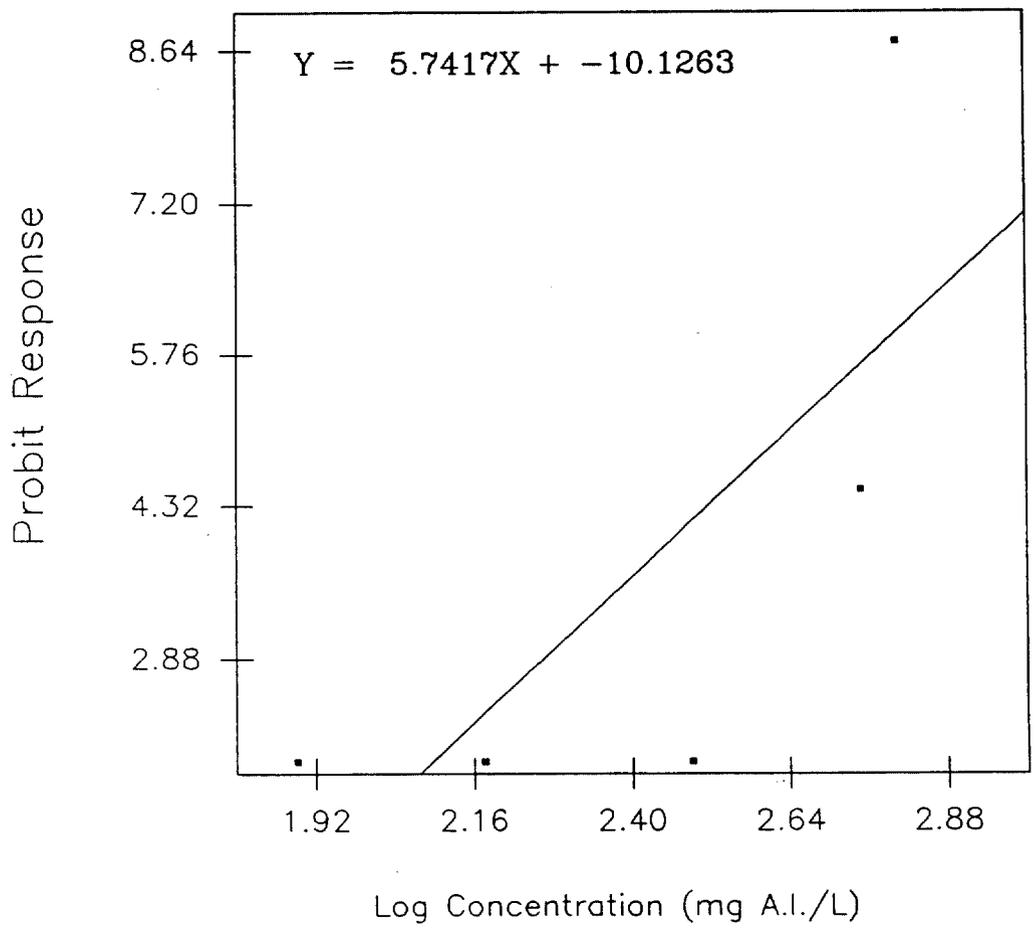
FIGURES

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



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



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6.0 APPENDIX I - STUDY PROTOCOL

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Springborn Laboratories, Inc.

Environmental Sciences Division

790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

TEST PROTOCOL

PROTOCOL TITLE: Protocol for Conducting a Flow-Through Acute Toxicity Test with Rainbow Trout following TSCA 797.1400.

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#: 02814BZ

Additional Comments and/or Modifications: _____

 Sponsor Approval Date: 10/6/92

TO BE COMPLETED BY SLI PRIOR TO TEST INITIATION:

Testing Facility: Springborn Laboratories, Inc. Project #: 12827-0692-6104-108

Study Director: MARK W. MACUADO

Test Concentrations: 950, 570, 340, 210, 120 mg A.F.I. plus control

Solvent Used: NA CAS# or LOT#: NA

Proposed Schedule: (Start) 2/27/93 (Completion) 3/3/93

Additional Comments and/or Modifications: _____

 Study Director Date: 10/14/92

Springborn Laboratories Protocol #: 091192/TSCA 797.1400

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PROTOCOL FOR CONDUCTING A FLOW-THROUGH ACUTE TOXICITY TEST WITH
RAINBOW TROUT FOLLOWING TSCA 797.1400

OBJECTIVE

The purpose of this test is to determine the acute lethal effects of a test material on a representative cold water fish species under flow-through conditions. The methods described in this protocol generally follow the standard procedures described in the EPA/OTS guidelines for testing the effects of chemicals on fish (U.S. EPA, 1985, amended 1987). Test results are reported as LC50 values together with 95% confidence limits, as No Observed Effect Level (NOEL), and as the incipient LC50, i.e., that value which indicates an LC50 when exposure to the test substance is continued until the mean increase in mortality does not exceed ten percent in any concentration over a 24-hour period.

MATERIALS AND METHODS

TEST ORGANISMS:

1. Species. Juvenile rainbow trout *Salmo gairdneri*, are used to conduct the dynamic acute toxicity test with cold water fish. The fish are of approximately the same size and age, i.e., the length of the largest fish does not exceed the length of the smallest fish by more than two-fold.
2. Origin and Acclimation. The fish are obtained from a reliable commercial supplier and are gradually acclimated to the test conditions. They are held for at least an additional 14 days in the dilution water prior to testing, a minimum of 7 days of which at the required test temperature. During the final 48 hours of fish holding, total mortality must not exceed three percent, or the batch will not be used.
3. Feeding. The fish are fed at least once daily prior to the test, but are not fed during the final 48 hours before the test, nor during the first 96-hours of the in-life test.
4. Handling. Fine-mesh dip nets are used to transfer the fish, taking care to minimize possible stress due to handling. Fish that are damaged or dropped during transfer are not used.
5. Loading. Fish biomass to solution ratio ("loading") does not exceed 0.5 grams per liter per 24 hours.

PHYSICAL SYSTEM:

1. **Test Containers.** The test chambers used in the flow-through acute bioassay are 19-L clear glass aquaria which are chemically clean. Each aquarium maintains a consistent solution volume (11 or 15 liters of test medium). This size is adequate to meet the maximum allowable loading requirements (see above).
2. **Cleaning.** The test aquaria are chemically cleaned before the test is started following standard laboratory procedures.
3. **Dilution Water.** Water from a 100 meter bedrock well is pumped to a concrete reservoir where it is supplemented on demand with untreated, unchlorinated, Town of Wareham well water and aerated before flowing to the exposure system through aged PVC pipe. The pH, total hardness, alkalinity, and specific conductance of this water are measured and recorded weekly in Springborn Laboratories' GFT Laboratory Notebook. The water is characterized as being "soft" with a pH range of 6.9 - 7.2, a total hardness of 25 - 40 mg/L and a specific conductance of 80 - 150 μ mhos/cm. During any one month, weekly analysis of the dilution water should show that the water quality characteristics of hardness, alkalinity and specific conductance do not vary by more than 10% from the respective monthly average and the monthly pH range should be less than 0.4 pH units. At least twice a year, analyses of representative samples of dilution water are conducted to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the fish. None of these compounds have been detected at concentrations which may be harmful to the fish. None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with US EPA and ASTM standard practices. In addition, TOC, COD, particulate matter, unionized ammonia and organic chlorine analyses are conducted at least twice each year in the dilution water.
4. **Replication.** Two replicates are included with each test concentration and control. Test aquaria are positioned inside the water bath by stratified random design, and labeled by replicate and concentration (or control). Fish are added impartially to the test aquaria by adding no more than two fish to each replicate until all aquaria contain two fish. This procedure is repeated until each aquarium contains ten fish (20 fish per concentration or control).

CHEMICAL SYSTEM:

1. **Test Material.** Upon arrival at Springborn Life Sciences, the external packaging of the test material is inspected for damage. The packaging is removed and the primary storage container is also inspected for leakage or damage. The sample identity and percent active ingredient are recorded and, unless different arrangements are made with the study Sponsor, the material is stored in the dark at approximately 20°C until used.

2. **Toxicant Concentration Selection.** Toxicant concentrations for the acute toxicity test are selected based on information provided by the Sponsor or obtained from a preliminary static or flow-through range-finding test. The preliminary test consists of three widely spaced concentrations, usually of 3-L volume, each containing at least three fish. A geometric series of five concentrations and one control are used for each definitive test, each concentration consisting of twenty test fish (see Section "Replication", above). Each dose level is 60% of the next higher concentration of the test material. 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 materials, one or both levels may not be observed.
3. **Diluter.** A proportional or serial diluter (e.g., Mount and Brungs, 1967) is employed to deliver five toxicant concentrations, a control, and a solvent control, if necessary, to duplicate aquaria. If no solvent control is required, a sixth toxicant concentration may be added. Based on the solubility of the test material, the stock solution stability and the range of test concentrations, one of the following toxicant delivery systems is used: the gas-tight syringe injector metering device; the Mariotte bottle/"dipping bird" system, or the metering pump/predilution chamber system.

A flow-splitting chamber is used between the diluter cells and the aquaria to promote mixing of the toxicant solution and diluent water. In each chamber, two separate standpipes are employed to equally split the test solution between the A and B duplicate test aquaria.

The calibration of the diluter system is checked prior to test initiation. During the test, the diluter is visually inspected twice daily. If there is any indication during the test that the diluter calibration has changed (e.g., diluter malfunction or unexplained differences in dissolved oxygen concentration or temperature in the aquaria), calibration of the necessary diluter components is checked. A test is not started until the diluter and toxicant delivery device have been observed to be properly functioning for at least 24 hours prior to the test. During a test, the flow rates shall not vary by more than 10% from one replicate test chamber to another.

4. **Stock Preparation.** The stock solution is prepared according to the following formula:

$$\text{Stock concentration} = \frac{\text{H.C.} \times \text{M.C.}}{\text{B.D.} \times \% \text{ A.I.}}$$

where:

H.C. = high concentration (mg/L)

M.C. = mixing chamber volume (L)

B.D. = bird or syringe delivery (mL)

A.I. = % active ingredient

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

5. **Solvent Control.** If a solvent is used, a solvent control is established which contains a concentration of solvent equal to the amount present in the test concentrations, but not to exceed 0.1 mL/L. The solvent concentration is kept as low as possible. If >0.1 mL/L solvent is needed to solubilize a required quantity of the test material, the Sponsor will be notified, and the solvent concentration used will be identified on the cover page of the protocol. Reagent grade or highest quality triethylene glycol (TEG), acetone, ethanol, or dimethyl formamide (DMF), in this order of preference, is used.

SAMPLING AND OBSERVATIONS:

1. **Sampling.** Unless specified differently by the Sponsor, water samples of an appropriate volume are taken from one replicate of the high, middle and low test concentrations at least once during the pre-exposure period to document water concentrations and the proper functioning of the diluter. Samples from both replicate aquarium of each concentration and control(s) are taken at the initiation, mid-term (48 hours) and termination of the test (96 hours) for determination of toxicant concentrations. The test solution of at least one appropriate test aquarium is measured whenever a malfunction is detected in any part of the test delivery system. Prior to analysis, and, if possible, within 30 minutes of sampling, samples are passed through a 0.45 μ m filter to remove any material which may be associated with particulate matter. The filters and filter holders are pre-rinsed with distilled water and finally with test solution prior to use. Three quality control samples are prepared at each sampling interval and remain with the set of samples through extraction, storage and analysis. These samples are prepared in diluent water at test material concentrations similar to the treatment level range. Results of these analyses indicate the relative accuracy of the analytical methodologies for each sampling period. Water samples are taken from a point approximately midway between the surface, bottom and sides of each aquarium and either extracted immediately after sampling or appropriately preserved and stored until analysis can be performed.
2. **Measurement of Water Quality Variables.** At test initiation and every 24 hours thereafter, water quality variables (temperature, pH, and dissolved oxygen concentrations) are recorded in each test aquarium. The following water quality conditions are maintained during the test:

Dissolved Oxygen. Total dissolved oxygen exceeds 90% of saturation at the initiation of the test, and is maintained at ≥ 8.2 mg/L for the duration of the test. Aeration (with oil free air) would be initiated as a last resort to raise and maintain the dissolved oxygen concentration at acceptable levels.

Temperature. Water temperature of the test solutions is maintained at 12 ± 2 °C by maintaining the aquaria in a water bath at the appropriate test temperature. Temperature is monitored continuously in one aquarium by using a minimum-maximum thermometer which is read and recorded daily.

Lighting. A combination of fluorescent bulbs is used to illuminate the aquaria which provides a wide spectrum of light, simulating the spectrum of natural sunlight. Light intensity at the water surface is within the range of 20-100 foot candles. An 8-hours dark and 16-hours light photoperiod is maintained during the test.

3. **Biological Data.** Observations of stress, abnormal behavioral activity and mortality are made daily. Dead fish are removed from test solutions at these intervals. In addition, characteristics of the test solutions are also observed and recorded, e.g., precipitated materials, cloudiness, etc.
4. **Acceptability Criteria.** The test is unacceptable if more than 10 percent of the control fish die in 96 hours.

STATISTICS

Mortality data derived from the acute test is 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 estimated nominal or measured concentration of the test material in dilution water which produces 50% mortality in the test fish population at the stated times of exposure. The incipient LC50 is determined also, i.e., that value which indicates an LC50 when exposure to the test substance is continued until the mean increase in mortality does not exceed ten percent in any concentration over a 24-hour period. LC50 values are computed using measured concentrations, if available.

The computer program utilized estimates 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 method provides values of the slope, including 95% confidence intervals, for the probit analysis, as well as appropriate statistical tests to evaluate goodness-of-fit.

In addition, the highest test concentration that shows no statistically significant difference from the control (No Observed Effect Concentration, NOEC) is determined and reported.

REPORTING

The raw data and final drafts of the report are reviewed by the Quality Assurance Unit and Study Director. All values of chemical and water quality measurements are 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 initially be submitted to the study Sponsor for review. Upon acceptance by the Sponsor, three copies of the final report will be submitted. All reports include, but are not 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 material which appears on the sample bottle, e.g., its source and percent activity, if available.
- * Characterization and origin of the dilution water.
- * Scientific name of the test organism, source, percent mortality of the fish population 48 hours prior to testing, and acclimation temperature, pH, and DO range.
- * Description of stock preparation.
- * Information regarding test temperatures, dissolved oxygen concentration, pH and photoperiod.
- * Observations of insolubility of the test material, including the test levels and when observed.
- * Number of fish that showed lethality in the controls and in each treatment at each observation period, as well as percent mortality at test termination, in tabular form.
- * Description or reference (or inclusion as an appendix) to chemical and statistical procedures applied.
- * The LC50 value for each day it can be calculated, with 95 percent confidence limits, the incipient LC50, and the No Observed Effect Level (NOEL).
- * Analytical results of test concentration measurements and QC samples.
- * Deviations from the protocol not addressed in protocol amendments will be listed, together with a discussion of the impact on the study and signed by the Study Director.
- * Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- * Dates of Quality Assurance reviews, signed by the QA Unit.

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 Substances Control Act (*FEDERAL REGISTER*, Part III, August 17, 1989)

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final test report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

REFERENCES

- APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, Washington, DC. 2168 pp.
- Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. *Water Research* 1: 21-39.
- U.S. Environmental Protection Agency. 1985. *Toxic Substances Control Act Test Guidelines*. Federal Register 50(188):39252-39516, September 27, 1985. Amended in Fed. Reg. 52:19062 (1987).

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: 10 March, 1993
PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Acute Toxicity Test with Rainbow Trout following TSCA 797.1400."
SPECIES: *Oncorhynchus mykiss*
STUDY SPONSOR: American Petroleum Institute
TEST MATERIAL: Tert-Amyl Methyl Ether, ("TAME")
SLI STUDY NO: 12827.0692.6104.108

AMENDMENT(S):

1. The protocol states that a proportional or a serial diluter is employed to deliver five toxicant concentrations, a control and solvent control, if necessary, to duplicate aquaria and that a flow-splitting chamber is used between the diluter cells and aquaria to promote mixing of the toxicant solution and diluent water.

During the conduct of this study, however, it became necessary to modify the serial diluter and toxicant delivery method in order to compensate for the volatile nature of the test material and to maximize the concentration of test material in solution. This modification consisted of circumventing the mixing chamber and chemical cells of the diluter and changing the point of entry of the test material stock solution such that the toxicant pumps delivered the solution directly into the individual delivery tubes exiting each of the splitter cell compartments. This modification effectively eliminated the necessity for the mixing chamber and subsequent dilution accomplished in the chemical cells and splitters. The 60% dilution series necessary to provide the five treatment levels to conduct the study was accomplished by adjusting the delivery of each toxicant pump.

2. The protocol states that samples from both replicate aquaria of each concentration and control(s) are taken at the initiation, mid-term (48 hours) and termination of the test (96 hours) for determination of toxicant concentrations.

During this study the mid-term sampling interval was eliminated. A 48-hour sampling interval was never intended for this study, but was inadvertently included in the study protocol.

Springborn Laboratories Protocol #091192/TSCA 797.1400

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LABORATORIES

- 3. The protocol incorrectly states that juvenile rainbow trout, *Salmo gairdneri*, are used in the test. The scientific name for rainbow trout has been changed to *Oncorhynchus mykiss*.

Approval Signatures: Mark W. Machado 3/22/93
Mark W. Machado Date
SLI Study Director

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

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Environmental Sciences Division
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PROTOCOL AMENDMENT

AMENDMENT #: 2

DATE: 24 March, 1993

PROTOCOL TITLE: "Protocol for Conducting a Flow-Through Acute Toxicity Test with Rainbow Trout following TSCA 797.1400."

SPECIES: *Oncorhynchus mykiss*

STUDY SPONSOR: American Petroleum Institute

TEST MATERIAL: Tert-Amyl Methyl Ether, ("TAME")

SLI STUDY NO: 12827.0692.6104.108

AMENDMENT(S):

1. The protocol incorrectly states that the test chambers used in the flow-through acute bioassay are 19-L clear glass aquaria. Test chambers used in this study were 19.5 L all glass aquaria.
2. The protocol states that test results are reported as LC 50 values together with 95% confidence limits, along with a No Observed Effect Level (NOEL) and the incipient LC50, i.e., that value which indicates on LC50 when exposure to the test substance is continued until the mean increase in mortality does not exceed ten percent in any concentration over a 24-hour period. Based on the criteria established in the Environmental Effects Testing Guidelines under TSCA (40 CFR, Part 797, § 797-1400) an incipient LC50 was not calculated because the 96-hour LC50 value for this study was not less than 50% of the 48-hour LC50 value and the test was not continued beyond the 96-hour interval.

Springborn Laboratories Protocol #091192/TSCA 797.1400

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LABORATORIES

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3. The protocol states that samples will be passed through a 0.45 μm filter to remove any material which may be associated with particulate matter. Due to the test material characteristics, use of a filtration apparatus was anticipated to cause significant test material loss and deterioration of membrane filters. For these reasons, filtration was not utilized.

Approval Signatures: Mark W. Machado 3/24/93
Mark W. Machado Date
SLI Study Director

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

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7.0 APPENDIX II - CERTIFICATE OF ANALYSIS

aldrich chemical co.

chemists helping chemists in research & industry

SPRINGBURN LABORATORIES
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

1.3885

INFRARED SPECTRUM

CONFORMS TO STRUCTURE AND STANDARD AS
ILLUSTRATED ON PAGE 268A OF EDITION I,
VOLUME 3 OF "THE ALDRICH LIBRARY OF FT-IR
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CHROMATOGRAPHY

98.8 %

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Aldrich Chemical Company
DAVID SWESSEL
NOVEMBER 11, 1992



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928

AMER PETRO INST
202 682 8270
DR DICK RHODEN

PO NBR: 820003008A13

12827.0592.662.45

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 I,
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SPECTRA".

GAS LIQUID
CHROMATOGRAPHY

98.7 %

ALDRICH warrants that its products conform to the information contained in this and other Aldrich publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Aldrich Chemical Company
DAVID GWESSEL
NOVEMBER 6, 1992



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Springborn Laboratories, Inc.

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8.0 APPENDIX III - CULTURE FOOD ANALYSIS

Zeigler Brothers, Inc. Salmon Starter Feed Sample*		
Date Submitted: 11/13/92 Date Reported: 12/1/92		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen I;II,III	Result as Received	
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.

Zelgler Brothers Inc. Salmon Starter Feed Sample*		
Date Submitted:11/13/92 Date Reported:12/1/92		
Analysis	Final Result	Limit of Quantitation
Pesticide Screen b1;ll	attached	
Arsenic	2.1 ppm	0.1
Cadmium	0.4 ppm	0.1
Copper	2.1 mg/100g	0.2
Lead	0.4 ppm	0.2
Mercury	0.10 ppm	0.02
Zinc	29.4 mg/100g	0.2
Selenium (fluorometric)	1.6 ppm	0.1
* Analyzed by Lancaster Laboratories, Inc.		

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9.0 APPENDIX IV - DILUTION WATER ANALYSIS

GFT Grab Water Sample*		
Date Sampled: 1/28/93 Date Reported: 2/12/93		
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.		

GFT Grab Water Sample*		
Date Sampled: 1/28/93 Date Reported: 2/12/93		
Analysis	Result As Received	Limit of Quantitation
Pesticide Screen (I,II,III)	attached	
Mercury	< 0.0002 mg/l	0.0002
Arsenic	< 0.2 mg/l	0.2
Selenium	< 0.2 mg/l	0.2
Boron	< 0.04 mg/l	0.04
Thallium	< 0.3 mg/l	0.3
Aluminum	< 0.2 mg/l	0.2
Antimony	< 0.2 mg/l	0.2
Barium	< 0.1 mg/l	0.1
Beryllium	< 0.01 mg/l	0.01
Cadmium	< 0.01 mg/l	0.01
Calcium	7.8 mg/l	0.2
Chromium	< 0.05 mg/l	0.05
Cobalt	< 0.05 mg/l	0.05
Copper	< 0.02 mg/l	0.02
Iron	< 0.1 mg/l	0.1
Lead	< 0.1 mg/l	0.1
Magnesium	2.3 mg/l	0.1
Manganese	0.02 mg/l	0.01
Molybdenum	< 0.1 mg/l	0.1
Nickel	< 0.05 mg/l	0.05
Potassium	1.0 mg/l	0.5
Silver	< 0.02 mg/l	0.02
Sodium	14.9 mg/l	0.4
Titanium	< 0.01 mg/l	0.01
Vanadium	< 0.01 mg/l	0.01
Zinc	< 0.04 mg/l	0.04

* Analyzed by Lancaster Laboratories, Inc.

10.0 APPENDIX V - ANALYTICAL METHODOLOGY

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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 material. 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 $89.7 \pm 2.3\%$, $104 \pm 11\%$ for filtered seawater and 102 ± 10 for freshwater. 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 μ m 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 Sponsor 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 material 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 (μ L)	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 μ m film
Temperature ($^{\circ}$ C): Injector: 200
column temperature programmed: 40 - 250
Rate: 10 $^{\circ}$ C per minute from 40 to 70 $^{\circ}$ C
25 $^{\circ}$ C per minute from 70 - 250 $^{\circ}$ 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

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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 through 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: $89.7 \pm 2.3\%$

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 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

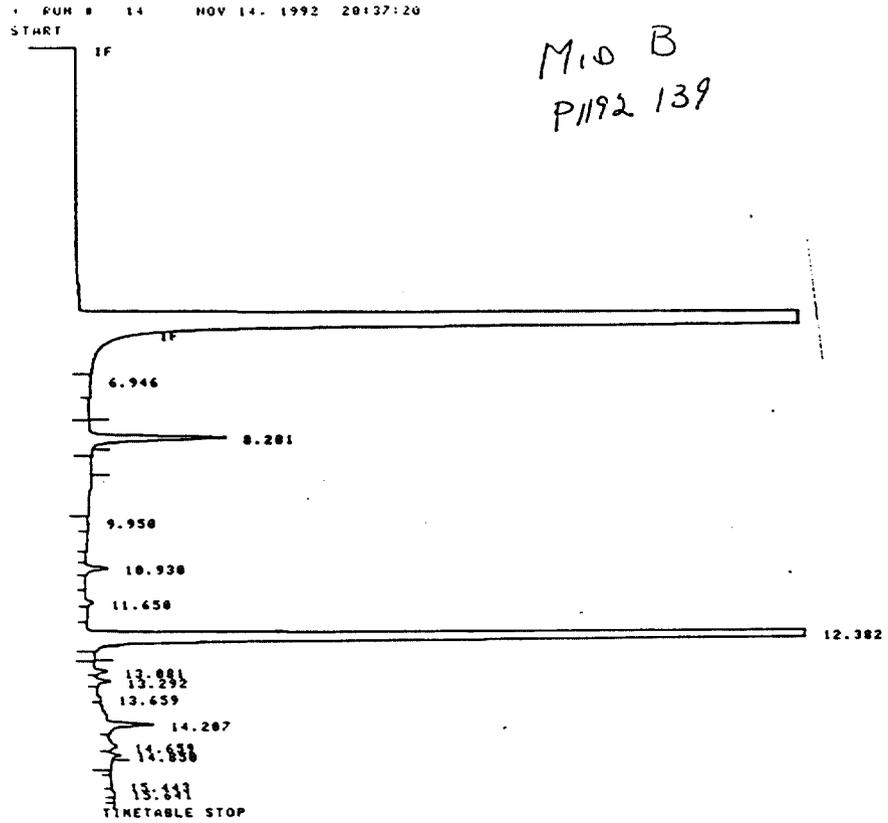
Mean Recovery: $102 \pm 10\%$

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.

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: H-TAMEPCTP.MET

IDENTIFIER : 3140A39449

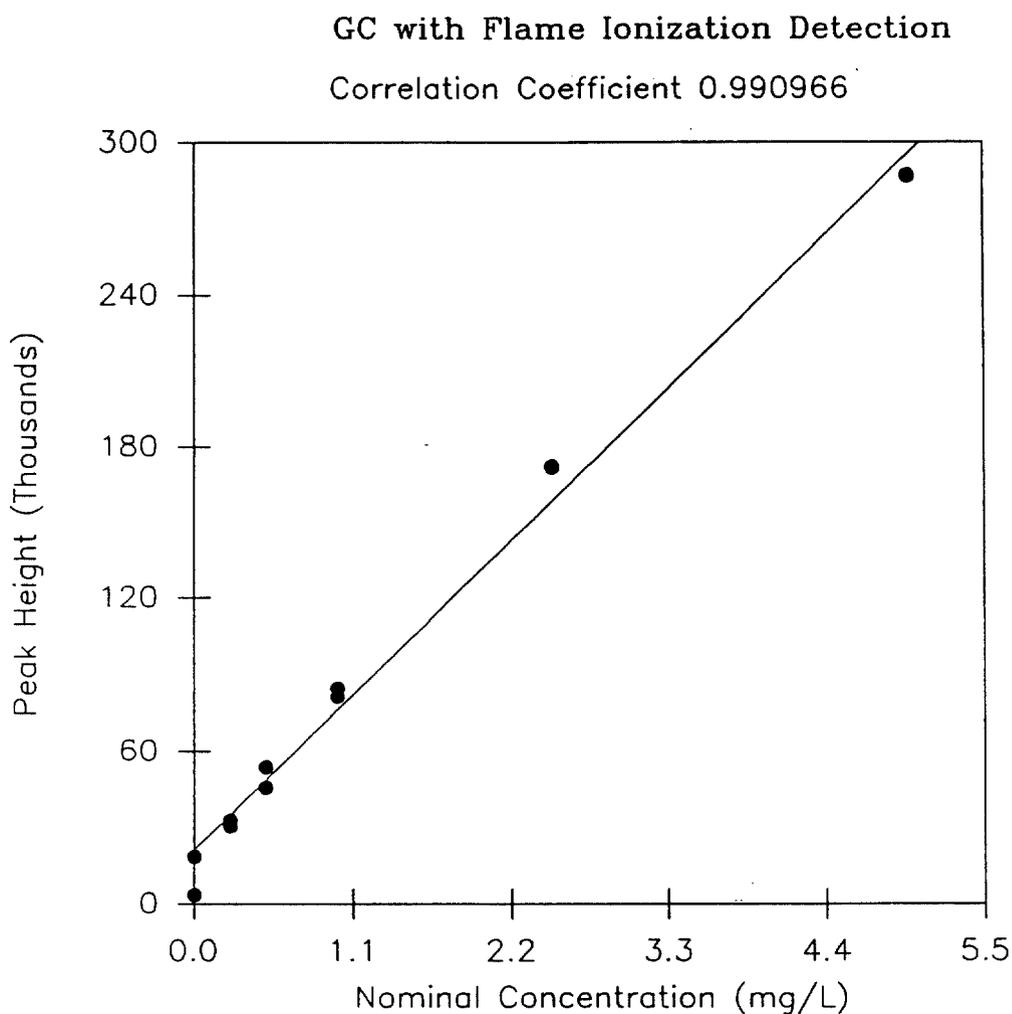
*PERFORM TAME
IR FOR 11/10/92*

ESTD-HEIGHT	RT	TYPE	AREA	WIDTH	HEIGHT	CALL	W/L	NAME
	8.201	PB	156375	.107	24263			.000
	10.930	PP	18711	.002	3817			.000
	12.382	PB	3787178	.103	612842	IR		3.596 TAME
	13.292	VF	11101	.071	2618			.000
	14.207	VV	69006	.129	8923			.000
	14.659	VV	17942	.164	1823			.000

TOTAL HEIGHT= 654288
MUL FACTOR=1.0000E+00

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



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

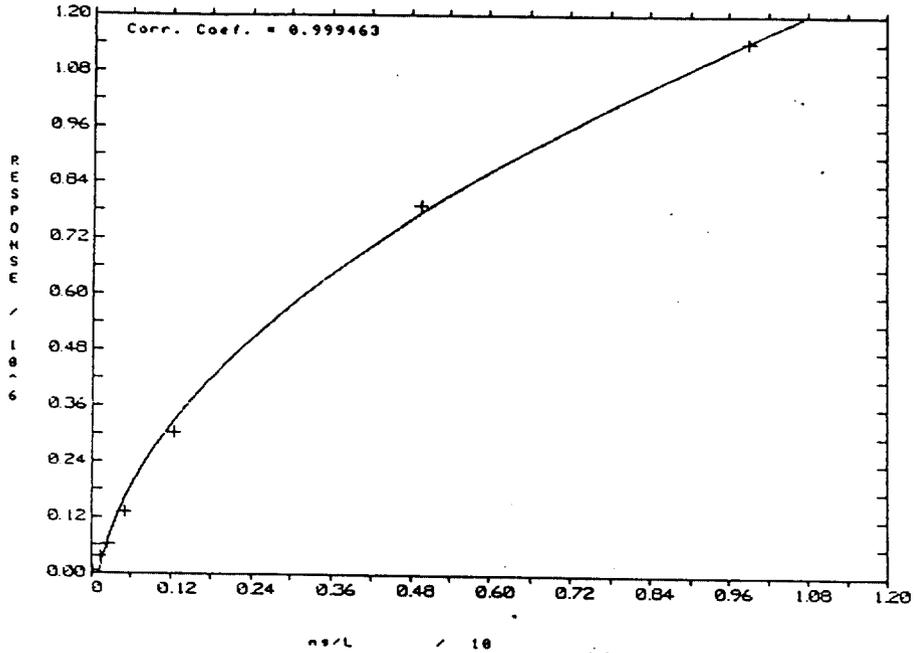
WELCOME TO THE HP3396 CALIBRATION CURVE PLOTTING PROGRAM Rev. 8.02.00

At any prompt: 'Q' (ENTER) Quits
'S' (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 peaks (Y*/N): N

* EDIT CALIB 2

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

11.0 APPENDIX VI - RAW DATA

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SPRINGBORN LABORATORIES, INC.
ACUTE TOXICITY TEST - BIOLOGICAL OBSERVATIONS AND WATER QUALITY FORM

SUBJECT: BIOLOGICAL OBSERVATIONS AND WATER QUALITY MEASUREMENTS (continued)

Test Conc.	Rep.	72 Hour				96 Hour				D.O.	pH	To	Sal.	Observation	#Dd/Cm
		Temp	pH	Sal.	Observations	Temp	pH	Sal.	Observations						
950	1A	9.0	7.2	11	NA	9.0	7.2	12	NA	9.0	7.2	12	NONE	10	
	1B	9.6	7.2	11	NONE	9.1	7.2	12	NONE	9.1	7.2	12	NONE	10	
	2A	9.7	7.2	11	DRY-CLE	9.1	7.2	11	DRY-CLE	9.1	7.2	11	DRY-CLE	4	
	2B	9.4	7.2	11	DRY-CLE	8.9	7.2	12	DRY-CLE	8.9	7.2	12	DRY-CLE	2	
340	3A	9.6	7.2	11	NONE	9.1	7.2	11	NONE	9.1	7.2	11	NONE	0	
	3B	7.6	7.2	11	NONE	9.1	7.2	11	NONE	9.1	7.2	11	NONE	0	
	4A	9.8	7.2	11	NONE	9.6	7.2	11	NONE	9.6	7.2	11	NONE	0	
	4B	9.7	7.2	11	NONE	9.0	7.2	11	NONE	9.0	7.2	11	NONE	0	
120	5A	9.6	7.2	11	NONE	8.9	7.2	11	NONE	8.9	7.2	11	NONE	0	
	5B	9.7	7.2	11	NONE	9.0	7.2	11	NONE	9.0	7.2	11	NONE	0	
	6A	9.5	7.2	11	NONE	8.9	7.2	12	NONE	8.9	7.2	12	NONE	0	
	6B	9.6	7.2	11	NONE	8.9	7.2	11	NONE	8.9	7.2	11	NONE	0	
S. Cont.	7A	-	-	-	-	-	-	-	-	-	-	-	-	-	
	7B	-	-	-	-	-	-	-	-	-	-	-	-	-	

6/14/93

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12827 0692 6104 108

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12827 0692 6104 108

SPRINGBORN LABORATORIES, INC.			
ACUTE TOXICITY TEST - BIOLOGICAL OBSERVATIONS AND WATER QUALITY FORM			
SUBJECT: BIOLOGICAL OBSERVATIONS AND WATER QUALITY MEASUREMENTS (continued)			
OBSERVATION KEY			
GENERAL BEHAVIOR	SWIMMING	RESPIRATION	
AS At the Surface	ERR Erratic	RA Rapid	
MSP Muscle Spasm	GY Gyrating	G Gulping	
CLE Complete Loss of Equilibrium	SK Skittering	SOLUTION	
PLE Partial Loss of Equilibrium		PRE Precipitate	
LETH Lethargic	DRK Dark	FOS Film on Surface	
PFAE Pect. Fins Anteriorly Extended		UC Undissolved Chemical	
EXO Exophthalmus	MS Mucous Shedding		
EA Extended Abdomen	EMP Excessive Mucous Production		
SU Surfacing	HEM Hemorrhagic		
HYP Hyperactive			
Additional Comments/Observations (Sign and Date each entry):			

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SPRINGBORN LABORATORIES, INC.

Page 3

RESULTS OF CHROMATOGRAPHIC ANALYSIS
MEAN MEASURED TABLE

Sponsor: AMERICAN PETROLEUM INC.
 Test Material: TAME
 Project No.: 12827-0692-6104-108
 Test Type: 96HR FIA W/ RT
 Data Entered By: MD *MD*
 Date Program Run: 22-Feb-93

Sample ID	Nominal Concentration (MG/L)	INTERVAL (HR)	Analytical Result (MG/L)	MEAN	N	STD.DEV.
2-93-1193CNT	0	OHR	< 5.2799	NA	4	NA
2-93-1194	0	OHR	< 5.2799			
3-93-115	0	96HR	< 5.2074			
3-93-116	0	96HR	< 5.2074			
2-93-1191	120	OHR	5.565E+01	78.2	4	16.7
2-93-1192	120	OHR	8.862E+01			
3-93-117	120	96HR	7.578E+01			
3-93-118	120	96HR	9.267E+01			
2-93-1189	210	OHR	1.483E+02	150	4	6.73
2-93-1190	210	OHR	1.534E+02			
3-93-119	210	96HR	1.566E+02			
3-93-120	210	96HR	1.411E+02			
2-93-1187	340	OHR	2.849E+02	305	4	28.6
2-93-1188	340	OHR	3.356E+02			
3-93-121	340	96HR	3.233E+02			
3-93-122	340	96HR	2.771E+02			
2-93-1185	570	OHR	5.068E+02	559	3	51.3
2-93-1186	570	OHR	1.110E+02 *			
3-93-123	570	96HR	5.618E+02			
3-93-124	570	96HR	6.094E+02			
2-93-1183	950	OHR	7.391E+02	644	4	87.0
2-93-1184	950	OHR	6.968E+02			
3-93-125	950	96HR	5.614E+02			
3-93-126	950	96HR	5.804E+02			
2-93-1195	760000	OHR	2.051E+05 *	962572	1	NA
3-93-130	760000	96HR	9.626E+05			

* SAMPLES ARE LOW DUE TO INJECTOR ERROR. A FULL SAMPLE WAS NOT INJECTED AND WILL NOT BE INCLUDED IN THE STATISTICAL ANALYSIS.

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SPRINGBORN LABORATORIES, INC.

Page 4

RESULTS OF CHROMATOGRAPHIC ANALYSIS
QA SUMMARY TABLE

Sponsor: AMERICAN PETROLEUM INC.
 Test Material: TAME
 Project No.: 12827-0692-6104-108
 Test Type: 96HR FTA W/ RT
 Data Entered By: MD *MD*
 Date Program Run: 22-Feb-93

Sample ID	Nominal Concentration (MG/L)	INTERVAL (HR)	Analytical Result (MG/L)	Percent of Nominal
2-93-1196	120	0HR	1.275E+02	106
2-93-1197	350	0HR	3.571E+02	102
2-93-1198	950	0HR	1.215E+03	128
3-93-127	120	96HR	1.182E+02	98.5
3-93-128	350	96HR	4.543E+02	130
3-93-129	950	96HR	8.977E+02	94.5

MEAN 110
 N= 6
 STD.DEV. 15

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