American Petroleum Institute

STEP Strategies for Today's Environmental Partnership

Health and Environmental Sciences Department

Closed-Patch Repeated Insult Dermal Sensitization Study of Tertiary Amyl Methyl Ether (TAME) in Guinea Pigs

(Buehler Method)

FEBRUARY 1995

TOXICOLOGY REPORT NUMBER 403
CAIS ABSTRACT NO. 41-5416

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

API ENVIRONMENTAL MISSION AND GUIDING ENVIRONMENTAL PRINCIPLES

The members of the American Petroleum Institute are dedicated to continuous efforts to improve the compatibility of our operations with the environment while economically developing energy resources and supplying high quality products and services to consumers. The members recognize the importance of efficiently meeting society's needs and our responsibility to work with the public, the government, and others to develop and to use natural resources in an environmentally sound manner while protecting the health and safety of our employees and the public. To meet these responsibilities, API members pledge to manage our businesses according to these principles:

- To recognize and to respond to community concerns about our raw materials, products and operations.
- To operate our plants and facilities, and to handle our raw materials and products in a manner that protects the environment, and the safety and health of our employees and the public.
- To make safety, health and environmental considerations a priority in our planning, and our development of new products and processes.
- To advise promptly, appropriate officials, employees, customers and the public of information on significant industry-related safety, health and environmental hazards, and to recommend protective measures.
- To counsel customers, transporters and others in the safe use, transportation and disposal of our raw materials, products and waste materials.
- To economically develop and produce natural resources and to conserve those resources by using energy efficiently.
- To extend knowledge by conducting or supporting research on the safety, health and environmental effects of our raw materials, products, processes and waste materials.
- To commit to reduce overall emission and waste generation.
- To work with others to resolve problems created by handling and disposal of hazardous substances from our operations.
- To participate with government and others in creating responsible laws, regulations and standards to safeguard the community, workplace and environment.
- To promote these principles and practices by sharing experiences and offering assistance to others who produce, handle, use, transport or dispose of similar raw materials, petroleum products and wastes.

FOREWORD

API PUBLICATIONS NECESSARILY ADDRESS PROBLEMS OF A GENERAL NATURE. WITH RESPECT TO PARTICULAR CIRCUMSTANCES, LOCAL, STATE, AND FEDERAL LAWS AND REGULATIONS SHOULD BE REVIEWED.

API IS NOT UNDERTAKING TO MEET THE DUTIES OF EMPLOYERS, MANUFACTURERS, OR SUPPLIERS TO WARN AND PROPERLY TRAIN AND EQUIP THEIR EMPLOYEES, AND OTHERS EXPOSED, CONCERNING HEALTH AND SAFETY RISKS AND PRECAUTIONS, NOR UNDERTAKING THEIR OBLIGATIONS UNDER LOCAL, STATE, OR FEDERAL LAWS.

NOTHING CONTAINED IN ANY API PUBLICATION IS TO BE CONSTRUED AS GRANTING ANY RIGHT, BY IMPLICATION OR OTHERWISE, FOR THE MANUFACTURE, SALE, OR USE OF ANY METHOD, APPARATUS, OR PRODUCT COVERED BY LETTERS PATENT. NEITHER SHOULD ANYTHING CONTAINED IN THE PUBLICATION BE CONSTRUED AS INSURING ANYONE AGAINST LIABILITY FOR INFRINGEMENT OF LETTERS PATENT.

ACKNOWLEDGMENTS

THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

API STAFF CONTACT

Richard Rhoden, Ph.D., Health and Environmental Sciences Department

MEMBERS OF THE AIR/FUELS TOXICOLOGY HEALTH EFFECTS WORKGROUP

Russell D. White, Ph.D., *Chairman,* Chevron Research & Technology, Inc.
Rudolph J. Breglia, Ph.D., BP America, Inc.
Charles R. Clark, Ph.D., Unocal
Wayne Daughtrey, Ph.D., Exxon Biomedical Sciences, Inc.
David Duncan, Texaco, Inc.
Francis J. Koschier, Ph.D., ARCO
Charles A. Lapin, Ph.D., ARCO
David C. Logan, M.D., Mobil Corporation
John H. Mitchell, M.D., Amoco Corporation
Susan A. Rodney, Ph.D., Texaco, Inc.
Ceinwen A. Schreiner, Ph.D., Stonybrook Laboratories
David R. Steup, Ph.D., Shell Oil Company
Michael S. Wells, Ph.D., Amoco Corporation

American Petroleum Institute

Health and Environmental Sciences Department

QUALITY ASSURANCE STATEMENT

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

Testing Facility: Bio/dynamics, Now Pharmaco LSR

Testing Facility Number: 92-6222

This study was reviewed by API Quality Assurance personnel under the direction of API Management on the dates indicated below. This study was not required or intended to be in strict compliance with GLP Regulations.

Date(s) of Inspection/Review	Type of Inspection	Date of Report to Management
8/92	Protocol Evaluation	8/92
10/26/92	Laboratory Inspection and Data Audit	10/27/92
2/23/93	Draft Report Audit	2/23/93
9/12/94	Amended Final Report Review	9/12/94
9/19/94	Final Report Acceptance	9/19/94

Christine Sexsmith
Christine Sexsmith
Quality Assurance Coordinator

9/19/94
Date

PHARMACO :: LSR

Pharmaco LSR Study No.: 92-6222

Closed-Patch Repeated Insult Dermal Sensitization Study of Tertiary Amyl Methyl Ethyl (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 Pharmaco LSR Inc., Toxicology Service North America, 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 (5/sex/control material) were similarly treated with Light Mineral Oil (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 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 mineral oil was 0%. The Severity Indices at 24 and 48 hours were 0, for both mineral oil-treated animals and irritation control animals.

PHARMACO LSR INC. • P.O. BOX 2360 • METTLERS ROAD • EAST MILLSTONE, NEW JERSEY 08875-2360 • U.S.A.
TEL: 908.873.2550 FAX 908.873.3992

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.

TABLE OF CONTENTS

PHARMACO :: LSR

PHARMACO LSR STUDY NO.: 92-6222

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

Performed by: Pharmaco LSR Inc.
Toxicology Services North America
P.O. Box 2360, Mettlers Road
East Millstone, New Jersey 08875-2360

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

Attn: Robert T. Drew, Ph.D.

Date: October 8, 1993

Amended Report Date: September 8, 1994

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 Pharmaco LSR Inc., Toxicology Services North America, 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 Pharmaco LSR, 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 archives of the Testing Facility.

II. EXPERIMENTAL DESIGN

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

[a]Since TAME was administered at 100%, a sham control would have been adequate. However, because the protocol specified a vehicle control group, the vehicle used for the range-finding study was also used for the main study.
[b]Vehicle: 80% ethanol.
[c]Vehicle: acetone.
[d]Irritation control groups were treated at challenge only. The same ten animals served as irritation controls for all three materials.

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. Per sponsor request, refrigerated after 2 November 1992.
Sampling:	An archival sample of approximately 10 mls of the test material is stored in the archives of the Testing Facility.

2. Positive Control Material: 1-chloro, 2,4-dinitrobenzene (DNCB)

Lot Number:	A11T
Date of Receipt:	7 December 1989
Expiration Date:	December 1994
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 the Testing Facility.

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
Expiration Date:	June 1997
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 the Testing Facility.

4. Vehicle: Reagent Alcohol (Induction)

Lot Number:	7006 KHNE
Date of Receipt:	13 December 1991
Expiration Date:	December 1996
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
Expiration Date:	December 1994
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 (Sensitization Animals):	3-4 weeks at receipt. 6-7 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)

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 contact 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 placed on the test site, 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.

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 or control materials were applied to saturation (approximately 0.3 mls) beneath a 25 mm Hilltop Chamber® which was then 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.

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). This macroscopic change and the lack of additional deaths in the treatment group suggests that this death was not 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 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 mineral oil was 0%. The Severity Indices at 24 and 48 hours were 0, for both mineral oil-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)

X. RESULTS AND DISCUSSION (cont.)

C. Dermal Responses (cont.)

2. Challenge (cont.)

treated with 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

9/8/94
Date

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

9/8/94
Date

TABLE I

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY OF TERTIARY AMYL METHYL ETHER (TAME) IN GUINEA PIGS

BODY WEIGHTS (GRAMS)

	Animal No. and Sex	Pretest	Terminal	Weight Gain
Group IA	8106 M	490	642	152
Mineral Oil	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	8079 M	457	677	220
DNCB	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	8081 M	417	633	216
TAME	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	8080 M	439	672	233
	8093 M	460	704	244
Challenge	8132 M	426	618	192
Irritation	8085 M	399	626	227
Controls	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.

TABLE II

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION
STUDY OF TERTIARY AMYL METHYL ETHER (TAME) IN GUINEA PIGS

Individual Dermal Scores at First Induction[a]

Group	Material	Ind. Conc.	Animal No. and Sex	Interval 24 Hrs	Interval 48 Hrs	Animal No. and Sex	Interval 24 Hrs	Interval 48 Hrs
IA	Light Mineral Oil	100%	8106 M	0	0	8282 F	0	0
			8113 M	0	0	8257 F	0	0
			8083 M	0	0	8259 F	0	0
			8123 M	0	0	8308 F	0	0
			8089 M	0	0	8267 F	0	0
IIA	DNCB	0.5%	8079 M	0.5 Ed	0.5 Ed	8303 F	1	1 Ed
			8116 M	0.5 Ed	3 N,Ed	8314 F	1 Ed	1 Ed
			8100 M	0.5	1 Ed	8299 F	1 Ed	0.5 Ed
			8110 M	1 Ed	1 Ed	8265 F	0.5 Ed	0.5 Ed
			8137 M	1 Ed	0.5 Ed	8297 F	1 Ed	0.5 Ed
IIIA	TAME	100%	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

[a]Scored using the scoring system presented in Appendix B.

M=Male; F=Females; Ed=Edema.

TABLE III

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION
STUDY OF TERTIARY AMYL METHYL ETHER (TAME) IN GUINEA PIGS

INCIDENCE OF DERMAL RESPONSES AT CHALLENGE

Group	Material	Conc.[c]	Interval Hrs	Dermal Scores[a] 0	0.5	1	2	3	Ed	N	E	P[b]	Total No. of Animals
IA	Light Mineral Oil	100%	24	9	1	0	0	0	0	0	0	0	10
			48	10	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	10
			48	10	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

[a]Scored using the scoring system presented in Appendix B.
[b]P=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.
[c]Conc.=Concentration administered at challenge.
[d]Irritation control groups were treated at challenge only.

TABLE IV

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION
STUDY OF TERTIARY AMYL METHYL ETHER (TAME) IN GUINEA PIGS

INDIVIDUAL DERMAL SCORES[a] AT CHALLENGE

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

Group IA Animals Treated During Induction			Group IB Irritation Control Animals[b]		
Animal No. and Sex	Interval 24 Hrs	48 Hrs	Animal No. and Sex	Interval 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

[a]Scored using the scoring system presented in Appendix B.
[b]Irritation control animals were treated at challenge only.
[c]Mean=Severity Index.
M=Male; F=Female.

TABLE IV (cont.)

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION
STUDY OF TERTIARY AMYL METHYL ETHER (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 Animals Treated During Induction			Group IIB Irritation Control Animals[b]		
Animal No. and Sex	Interval 24 Hrs	48 Hrs	Animal No. and Sex	Interval 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

[a]Scored using the scoring system presented in Appendix B.
[b]Irritation control animals were treated at challenge only.
[c]Mean=Severity Index.
M=Male; F=Female; N=Necrosis; Ed=Edema.

TABLE IV (cont.)

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION
STUDY OF TERTIARY AMYL METHYL ETHER (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 24 Hrs	Challenge 48 Hrs	Animal No. and Sex	Challenge 24 Hrs	Challenge 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 24 Hrs	Interval 48 Hrs	Animal No. and Sex	Interval 24 Hrs	Interval 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

[a]Scored using the scoring system presented in Appendix B.
[b]Irritation control animals were treated at challenge only.
[c]Mean=Severity Index.
M=Male; F=Female.

Appendix A

Closed-Patch Repeated Insult Dermal Sensitization
Study of Tertiary Amyl Methyl Ethyl (TAME) in Guinea Pigs

Range-Finding Study - Individual Dermal Scores[a]

Animal No. and Sex	Concentration: Interval:	100% 24 Hours	100% 48 Hours	50%[b] 24 Hours	50%[b] 48 Hours	25%[b] 24 Hours	25%[b] 48 Hours	10%[b] 24 Hours	10%[b] 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

[a]Scored using scoring system presented in Appendix B.
[b]Vehicle: Light mineral oil.
F=Female

Appendix B

Closed-Patch Repeated Insult Dermal Sensitization
Study of Tertiary Amyl Methyl Ethyl (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

Appendix C

Closed-Patch Repeated Insult Dermal Sensitization
Study of Tertiary Amyl Methyl Ethyl (TAME) in Guinea Pigs

Quality Assurance Statement[a]

Listed below are dates that this study was inspected by the Quality Assurance Unit of Pharmaco LSR Inc., Toxicology Services North America, and the dates findings were reported to the Study Director and Management.

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

Jane Pasquito

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

9/7/94
Date

---

[a]Quality Assurance statment was originally signed January 26, 1993. Statement was re-signed because of change in name of Testing Facility and study title.

Appendix D

Closed-Patch Repeated Insult Dermal Sensitization Study of Tertiary Amyl Methyl Ethyl (TAME) in Guinea Pigs

Statement of Compliance[a]

This study was conducted in compliance with the United States Environmental Protection Agency's Good Laboratory Practice Standards 40 CFR Part 160 and applicable Standard Operating Procedures with the following exceptions:

1) Test material identity and stability testing was not performed at this laboratory and is the responsibility of the Sponsor.

2) Analyses to determine control material uniformity, stability and concentration in the vehicle were not performed.

Donna L. Blaszcak, B.S., AALAS LATG — Date 9/8/94

[a]Statement of Compliance was originally signed on October 8, 1993. Statement was re-signed because of change in the study title and to list areas of non-compliance.

Appendix E
Closed-Patch Repeated Insult Dermal Sensitization Study of Tertiary Amyl Methyl Ethyl (TAME) in Guinea Pigs

Report Amendments

The following is a list of changes made to the final report.

Page No./ Section	Change	Reason for Change
Cover page, Abstract and pages 11 through 19	Corrected study title to included full test material name.	Sponsor request.
Abstract	Changed "10/sex/control material" to "5/sex/control material".	Typographical error.
	Changed "vehicle control" to "control" and "vehicle" to "mineral oil".	Sponsor request.
Table of Contents	Added Appendix E, Report Amendments.	Addition of Report Amendments to document changes to final report.
2 and 3/ Section V.A.	Added expiration date for control materials and vehicles.	Sponsor request.
4/Section V.B.	Added "(Sensitization Animals)" to "Age".	Sponsor request.
	Changed "5-6" to "6-7".	Typographical error.
5/Section VI.B.	Changed "content" to "contact".	Typographical error.
6/Section VI.G.	Added "or control".	Typographical error.
9/Section X.A.	Revised mortality discussion.	Sponsor request.
9/Section X.C.2.	Changed "vehicle control" to "control" and "vehicle" to "mineral oil".	Sponsor request.

Appendix E (cont.)
Closed-Patch Repeated Insult Dermal Sensitization
Study of Tertiary Amyl Methyl Ethyl (TAME) in Guinea Pigs

Report Amendments (cont.)

Page No./ Section	Change	Reason for Change
10/Section X.C.2.	Changed "to" to "treated with". (Report was originally signed on October 8, 1993.)	Sponsor request.
12	Added Table II, "Individual Dermal Scores at First Induction".	Sponsor request.
20	Changed Compliance Statement to list areas of non-compliance.	To comply with GLP regulations.

Donna L. Blaszcak, B.S.
Study Director

9/8/94
Date

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

Order No. 848-00403