National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 98
1979

BIOASSAY OF dl-MENTHOL FOR POSSIBLE CARCINOGENICITY

CAS No. 89-78-1*

***The Chemical Abstracts Service (CAS) Registry Number used to track this bioassay is 15356-70-4 which is determined to best define the material used in the conduct of this bioassay.**

NCI-CG-TR-98

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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DHEW Publication No. (NIH) 79-1348

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FOREWORD: This report presents the results of the bioassay of dl-menthol conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of dl-menthol was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The principal investigators for the dl-menthol study were Drs. M. B. Powers[1] and R. W. Voelker[1]. Drs. Powers, C. Cueto, Jr.[2], and O. G. Fitzhugh[3] were responsible for the selection of the doses administered during the chronic study. Dr. Powers prepared a report of the methodology. Ms. K. J. Petrovics[1] was responsible for data management and Mr. G. Najarian[1] for animal care. Histopathologic examinations were performed by Drs. D. A. Banas[1] and R. H. Habermann[1] and reviewed by Dr. Voelker, and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[3] and Ms. P. L. Yong[3], using methods selected for the bioassay program by Dr. J. J. Gart[5]. Chemicals used in this bioassay were analyzed at Midwest Research Institute under the direction of Dr. E. Murrill[6], and feed mixtures containing the test chemical were analyzed at Hazleton Laboratories by Dr. C. L. Guyton[1] and Mr. E. Missaghi[1]. The results of these analyses were reviewed by Dr. S. S. Olin[3].

This report was prepared at Tracor Jitco[3] in collaboration with Hazleton Laboratories and NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following other scientists at NCI[2] were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman[7], Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[8], Dr. Sherman Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of dl-menthol for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered dl-menthol at one of the following doses, either 3,750 or 7,500 ppm for the rats and either 2,000 or 4,000 ppm for the mice, for 103 weeks, then observed for 1 or 2 additional weeks. Matched controls consisted of 50 untreated rats of each sex and 50 untreated mice of each sex. All surviving rats were killed at 105 weeks and all surviving mice at 104 weeks.

Mean body weights of dosed rats and mice were only slightly lower than those of corresponding controls. No other clinical signs related to administration of the dl-menthol were noted in the dosed groups of animals. A dose-related trend in mortality was observed only in the female mice. Survival at the end of the bioassay was at least 62% in all dosed and control groups of animals of each species, and sufficient numbers of animals were at risk for the development of late-appearing tumors.

In male rats, no tumors occurred at incidences which were considered to be related to the administration of dl-menthol.

In female rats, no tumors occurred at higher incidences in the dosed groups than in the control groups. Fibroadenomas of the mammary gland occurred at lower incidences in the low-dose (10/49) and high-dose (7/49) groups than in the control group (20/50), and alveolar/bronchiolar adenomas or carcinomas of the lung occurred only in the controls (3/50).

In mice of either sex, no tumors occurred in dosed groups at incidences that were significantly different from those for corresponding control groups.

It is concluded that under the conditions of this bioassay, dl-menthol was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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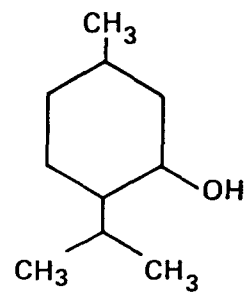
I. INTRODUCTION

Menthol (CAS 89-78-1; NCI C50000) is a naturally occurring monocyclic terpene found in the oils of the mint tree Mentha arvensis. This species is native to China, Japan, Brazil, and South Africa, and imports of natural menthol from these countries in 1976 amounted to 146 million pounds (USITC, 1977). An additional 285 thousand pounds of menthol were produced synthetically from either citronellal, thymol, or 3-menthene in the United States in 1974, the last year when production data were made public (USITC, 1975). Peppermint oil yields primarily the L isomer, as does synthesis from citronellal. The racemic mixture, DL- (or dl-)menthol, is the end result of synthesis from thymol and 3-menthene (Booth, 1965). USP standards exist for both the L isomer and racemic menthol (USP, 1974).



dl - Menthol

Menthol is well known for its cooling effects and its mint flavor and odor, which are the basis for the majority of its uses. The single largest use for menthol is probably in cigarettes (Stanford Research Institute, 1973). A survey of pharmaceutical products

indicates that menthol is formulated in over-the-counter rubs and liniments (2-10% concentration), antipruritic lotions, nasal sprays, expectorants, mouthwashes and sprays, cough drops, and foot powders (Kastrup, 1976; Billups, 1977). Menthol is used on inflamed or irritated skin and on oral mucosal membranes, since it is reported to have mild anesthetic, antiseptic, and counterirritant properties (Kastrup, 1976; Rosenthal, 1972), although it is possible that the only real effect it produces is a cooling sensation (AMA, 1977).

Mentholated cosmetics include preshave and aftershave creams, depilatories, cleansing creams, bath oils, perfumes, soaps, toothpastes, and baby powder (Rosenthal, 1972; Bell, 1972; Opdyke, 1976).

Menthol is generally recognized as safe for use in foods as a flavoring agent (FDA, 1976). It is used as a component of peppermint and lime flavors (Swaine, 1975) in products such as chewing gum (0.11%), baked goods (0.01%), candy (0.4%), ice cream (0.007%), and nonalcoholic beverages (0.004%) (Fenaroli, 1971). Menthol is also used as a denaturant in alcohol not intended for human consumption (Penty and Lescisin, 1965).

dl-Menthol was selected for study by the Carcinogenesis Testing Program because of its extensive use both as a medicinal and flavoring agent.

II. MATERIALS AND METHODS

A. Chemical

USP-grade dl-menthol was obtained from Glidden Organics International, Jacksonville, Florida (Lot No. 4-HTP-6), and from Norda, Inc., New York, New York (Lot No. N11-26-74-2054). Subchronic studies were conducted using Lot No. 4-HTP-6, and chronic studies were conducted with this lot (3 weeks only in rats) and the rest of the study with Lot No. N11-26-74-2054.

The identity of the chemical in both cases was established on the basis of elemental analyses (C, H) and infrared, ultraviolet, and nuclear magnetic resonance spectra. Gas-liquid chromatography of Lot No. 4-HTP-6 showed two impurities estimated at 0.3% each, which were very similar in volatility to the major component, and one less volatile impurity of about 1.3%. One minor impurity (0.2%) was detected in Lot No. N11-26-74-2054 by the same technique. No impurities were detectable in either lot by thin-layer chromatography. Karl Fischer analysis showed 0.22 $\pm$ 0.02% water in Lot No. 4-HTP-6, and less than 0.1% water in Lot No. N11-26-74-2054. Infrared and nuclear magnetic resonance spectra were consistent with spectra in the literature (Sadtler, 1965).

The bulk chemicals were stored at 1°C in their original containers.

B. Dietary Preparation

The appropriate weight of the dl-menthol required for each dietary concentration was dissolved in corn oil by stirring over a low heat. This solution was then added to the Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) and thoroughly mixed in a Patterson-Kelly twin-shell blender. The final concentration of corn oil in the test diets for the dosed groups of animals and in the basal diets for the untreated groups of controls was 2% by weight. The test diets and basal diets were prepared each week and used within 1 week of preparation. These diets were stored at room temperature.

As a quality control measure, samples of freshly mixed diets were analyzed periodically during the study. The results of these analyses are reported in Appendix G. At each dietary concentration, the mean of the analytical concentrations was within 10% of the theoretical concentration, and the coefficient of variation was 13-16%. The relatively large coefficient of variation was due to low recoveries obtained from samples that were analyzed several weeks after mixing and had lost a significant amount of the dl-menthol by vaporization. For

samples analyzed within 3 weeks of mixing (73% of the samples), the average coefficient of variation was 9.28%, which is more acceptable. Since the test diets were less than 1 week old when fed to the animals, accurate dietary concentrations of dl-menthol were maintained.

Temperature-dependent stability analyses conducted at Midwest Research Institute also confirmed the stability of dl-menthol in feed for 2 weeks at temperatures up to 45°C.

C. Animals

The Fischer 344 rats and the B6C3F1 hybrid mice of each sex were supplied by the Frederick Cancer Research Center, Frederick, Maryland, through contracts with the Division of Cancer Treatment, National Cancer Institute. On arrival at the laboratory, the rats were quarantined for approximately 4 weeks and the mice for approximately 2 weeks; they were determined to be free from observable disease or parasites and assigned to the various dosed or control groups on the basis of initial individual body weights so that a homogeneous distribution of mean weights and weight ranges was obtained between groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was generally maintained at 20-24°C and

the relative humidity at 45-55%. Incoming air was filtered through 2-inch-thick disposable fiberglass filters at a rate that allowed 12 changes of room air per hour. Fluorescent lighting was provided on a 12-hour-per-day cycle.

The rats and mice were each housed in polycarbonate cages covered with stainless steel cage lids and non-woven fiber filter bonnets (Filtek, Appleton, Wis.). The rats were initially housed five per cage; however, at week 48, the males were divided into groups of two or three per cage. The mice were housed five per cage throughout the study. The rats and the mice were housed in separate rooms.

All cages were furnished with heat-treated hardwood chip bedding (Sani-Chips®, Shurfire Products Corporation, Beltsville, Maryland); the bedding was changed twice per week. Diets and county supplied water were made available ad libitum. Food hoppers and water bottles were refilled twice per week.

Cages, water bottles and sipper tubes were sanitized at 81°C twice per week, feed hoppers once per week, and cage racks once per month. An industrial dish washer was used for the water bottles and sipper tubes; a cage and rack washer was used for the food hoppers, cages, and racks. The detergent used contained a phosphate base (Acclaim®, Economics Laboratory, St. Paul, Minn.).

When racks were changed, clean racks were randomly repositioned in the rooms.

The rats and mice were housed in separate rooms. Control animals were housed in the same room as the respective dosed animals. Rats administered dl-menthol in the diet were maintained in the same room as rats being administered the following chemicals:

Rats

Feed Studies

(CAS 13463-67-7) titanium dioxide
(CAS 119-53-9) benzoin
(CAS 120-61-6) dimethylterephthalate

Gavage Studies

(CAS 127-69-5) sulfisoxazole
(CAS 7488-56-4) selenium disulfide
(CAS 108-60-1) bischloroisopropyl ether

Drinking Water Studies

(CAS 108-95-2) phenol

At week 48, the rats fed dl-menthol, together with those fed titanium dioxide and those fed benzoin, were moved to a separate room for the remainder of the bioassay.

Mice administered dl-menthol in the diet were maintained in the same room as mice being administered the following chemicals:

Mice

Feed Studies

(CAS 13463-67-7) titanium dioxide
(CAS 119-53-9) benzoin
(CAS 120-61-6) dimethylterephthalate

Gavage Studies

(CAS 127-69-5) sulfisoxazole
(CAS 7488-56-4) selenium disulfide
(CAS 108-60-1) bischloroisophropyl ether

Drinking Water Studies

(CAS 108-95-2) phenol

The control groups of rats and mice used for the dl-menthol studies were used also for the titanium dioxide studies. The control groups were maintained in the same rooms with the dosed groups.

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of dl-menthol, on the basis of which two concentrations (hereinafter referred to as "low doses" and "high doses") were determined for use in the chronic studies. On the basis of the results of a 14-day range-finding study, doses of 930, 1,870, 3,750, 7,500, and 15,000 ppm were selected to be administered in the diet in the subchronic studies. At each dose, 10 males and 10 females of each species received the test diets 7 days per week for 13 weeks, and 10 males and 10 females

of each species were given basal diets for the same period of time. At termination of the subchronic studies, all animals were necropsied and histopathologic examination was made of tissues from controls, the highest-dose groups, and selected tissues from the second highest-dose groups.

There were no deaths among the rats, and the mean body weight gains in the dosed groups were comparable to those in the control groups at all doses. There was a slightly increased incidence of interstitial nephritis in the male rats in the highest-dose groups. In the mice, the six deaths occurring during the study could not be related to compound administration; however, females receiving 15,000 ppm gained 2 grams less than did the controls. There was a slightly increased incidence of perivascular lymphoid hyperplasia and interstitial nephritis among the female mice in the two highest-dose groups. The low and high doses for the chronic studies using rats were set at 3,750 and 7,500 ppm, and the low and high doses for the chronic studies using mice were set at 2,000 and 4,000 ppm.

F. Designs of Chronic Studies

The test groups, doses administered, and times on study of the chronic feeding studies are shown in tables 1 and 2.

Table 1. Design of dl-Menthol Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	dl-Menthol Doses[b] (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	50	0		105
Low-Dose	50	3,750	103	2
High-Dose	50	7,500	103	2
Female				
Matched-Control	50	0		105
Low-Dose	50	3,750	103	2
High-Dose	50	7,500	103	2

[a]Rats were 9 weeks of age when placed on study.

[b]dl-Menthol was administered in test diets containing 2% corn oil *ad libitum* 7 days per week. The control groups received a basal diet containing 2% corn oil.

Table 2. Design of dl-Menthol Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	dl-Menthol Doses[b] (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	50	0		104
Low-Dose	50	2,000	103	1
High-Dose	50	4,000	103	1
Female				
Matched-Control	50	0		104
Low-Dose	50	2,000	103	1
High-Dose	50	4,000	103	1

[a]Mice were 6 weeks of age when placed on study.

[b]dl-Menthol was administered in test diets containing 2% corn oil *ad libitum* 7 days per week. The control groups received a basal diet containing 2% corn oil.

G. Clinical and Pathological Examinations

All animals were observed twice daily for signs of toxicity. Clinical signs and the presence of palpable masses were recorded every week. Mean body weights and food consumption were recorded every 2 weeks for the first 12 weeks and every month thereafter.

Animals that were moribund and those that survived to the termination of the study were killed by exsanguination under sodium pentobarbital anesthesia (Diabutal®, Diamond Laboratories, Inc., Des Moines, Iowa).

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons), pituitary, spinal cord (if neurologic signs were present), eyes (if grossly abnormal), esophagus, trachea, salivary gland, mandibular lymph node, thyroid, parathyroid, heart, thymus, lungs and mainstem bronchi, liver, gallbladder (mice), pancreas, spleen, kidney, adrenal, stomach, small intestine, colon, urinary bladder, prostate or uterus, testes or ovaries, sternebrae,

femur, or vertebrae including marrow, mammary gland, tissue masses, and any macroscopic lesions.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals may have been missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental

results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have

appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relation-

ship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise

noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically

significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the dosed male and female rats were slightly lower than those of the corresponding controls throughout the bioassay (figure 1). No other clinical signs related to administration of the dl-menthol were noted. Clinical signs commonly observed among rats of this strain were noted at comparable rates in the control and dosed groups, particularly during the second year of the bioassay, and increased in incidence as the animals aged. These signs included eye changes (redness, paleness, cloudiness, lacrimination, a red discharge or bloody crust, and an enlarged or protruding eye), a hunched and/or thin appearance, urine stains on the abdominal fur, and occasionally, nasal discharge, sores on the body or the extremities, soft feces, and enlarged testes.

The incidence of palpable nodules and tissue masses in the dosed males was generally comparable to that in the control males, but was lower in the dosed females than in the control females.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered dl-menthol in the

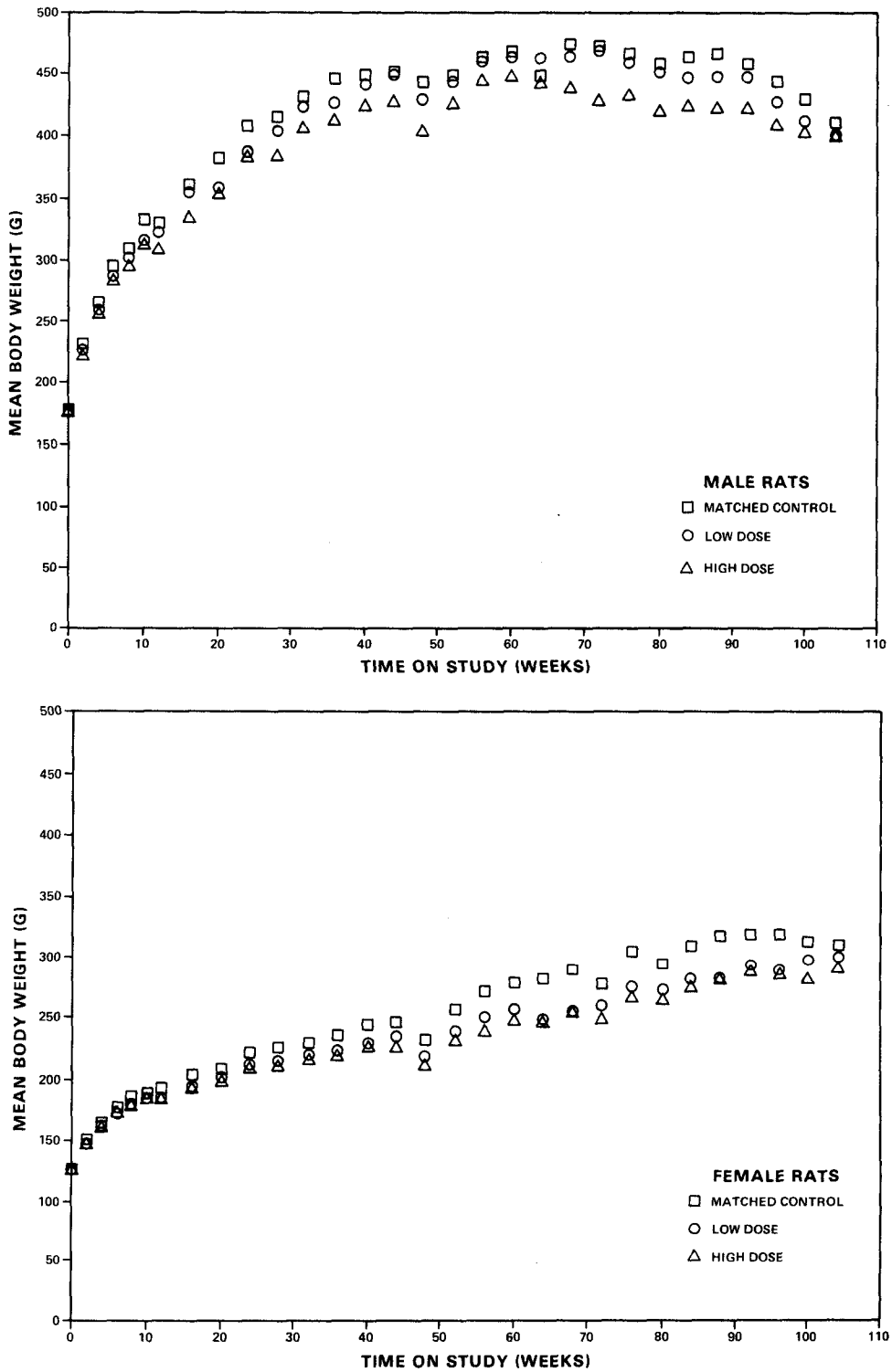


Figure 1. Growth Curves for Rats Administered dl-Menthol in the Diet

diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. The results of the Tarone test for dose-related trend in mortality and the results of the Cox test comparing the survival of the control group with each dosed group are not significant in either sex.

In male rats, 34/50 (68%) of the high-dose group, 33/50 (66%) of the low-dose group, and 31/50 (62%) of the controls were alive at week 105. In females, 38/50 (76%) of the high-dose group, 35/50 (70%) of the low-dose group, and 36/50 (72%) of the controls were alive at week 105. Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

Each of the tumor types observed has been encountered previously as a spontaneous lesion, and occurred with no appreciable differences in frequency between control and dosed rats with a few exceptions. In female rats, chromophobe adenomas of the pituitary gland and fibroadenomas of the mammary gland were observed with greater frequency in female control rats. Chromophobe adenomas occurred in 28/48 controls, 25/47 low-dose,

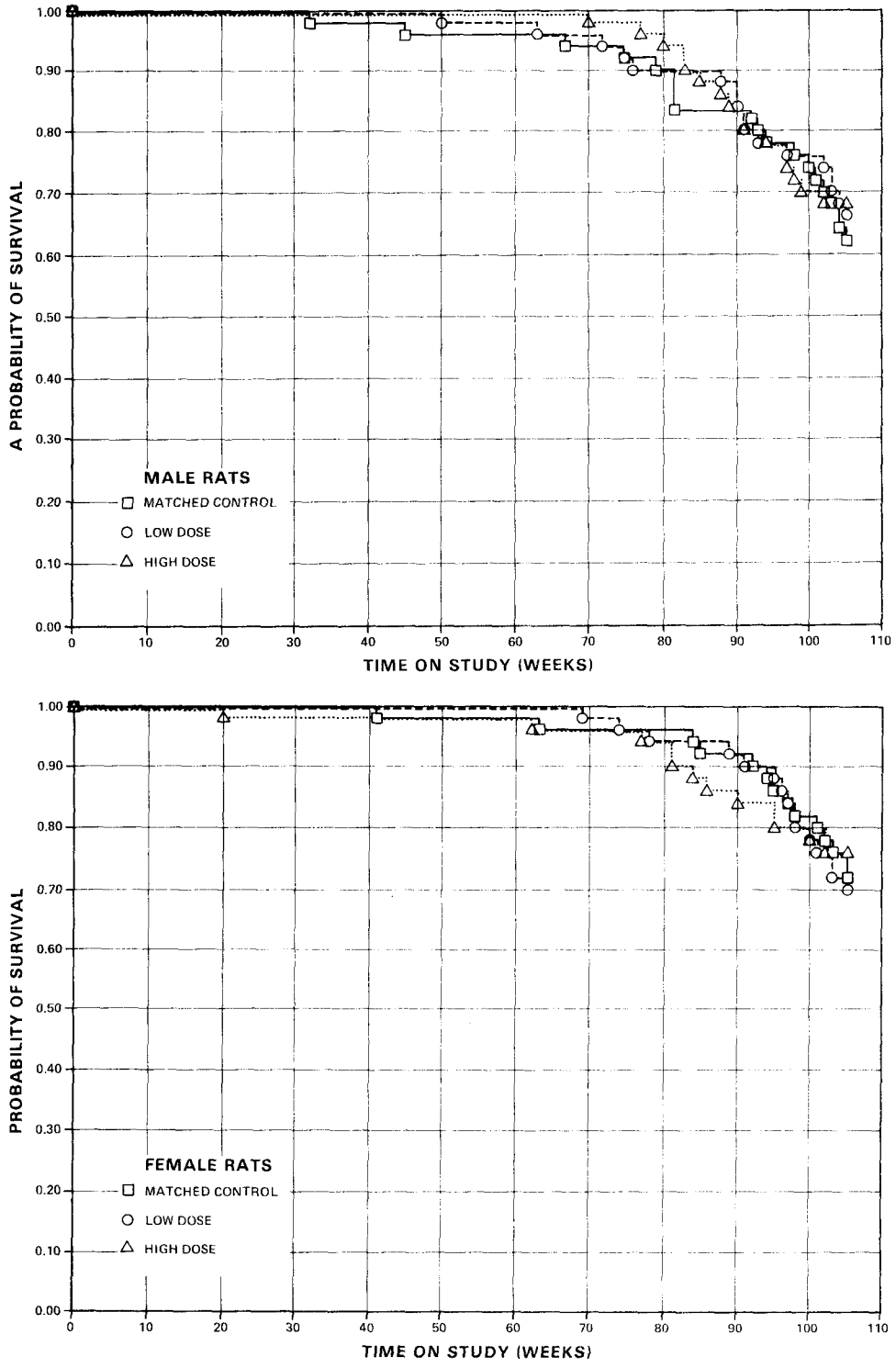


Figure 2. Survival Curves for Rats Administered dl-Menthol in the Diet

and 19/43 high-dose female rats. Mammary gland fibroadenomas were diagnosed in 20/50 female controls, 10/49 low-dose, and 7/49 high-dose rats. Mammary adenocarcinomas were seen in 1/50 controls, 3/49 low-dose, and 0/49 high-dose rats.

Chronic inflammation of the kidney was observed with greater frequency in the dosed males than in the control males (29/49 controls, 41/50 low-dose, 41/50 high-dose); however, this finding is of questionable importance, since such lesions are often found in aged male Fischer 344 rats.

All other inflammatory, degenerative, and hyperplastic lesions that occurred were similar in incidence and kind to those naturally occurring lesions found in aged Fischer 344 rats.

Based on the histopathologic examination, dl-menthol was neither toxic nor carcinogenic to Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Table E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In female rats, the results of the Cochran-Armitage test for

positive dose-related trend are not significant, and none of the results of the Fisher exact tests are significant in the positive direction. Significant results in the negative direction are observed, however, in the Cochran-Armitage test on the incidence of tumors of the lung in female rats and in all statistical tests on the incidences of fibroadenomas of the mammary gland in the females, due to lower incidences of those tumors in the dosed groups than in the control groups.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, except the incidence of fibroadenomas of the mammary gland in high-dose females, indicating the theoretical possibility of the induction of tumors by dl-menthol, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed male and female mice were slightly lower than those of the corresponding controls throughout the bioassay (figure 3). The appearance and behavior of the dosed and control groups of animals were generally similar, and clinical signs usually associated with aging were noted at comparable rates in the control and dosed groups. These signs included alopecia (generalized or localized), sores on the back and other parts of the body, particularly in the males, anal and/or penile irritation, a hunched and/or thin appearance, and occasional abdominal distention.

The incidences of palpable nodules and tissue masses in the dosed male or female groups were generally comparable to those of corresponding control groups.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered dl-menthol in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. In male mice, the result of the Tarone test for dose-related trend in mortality,

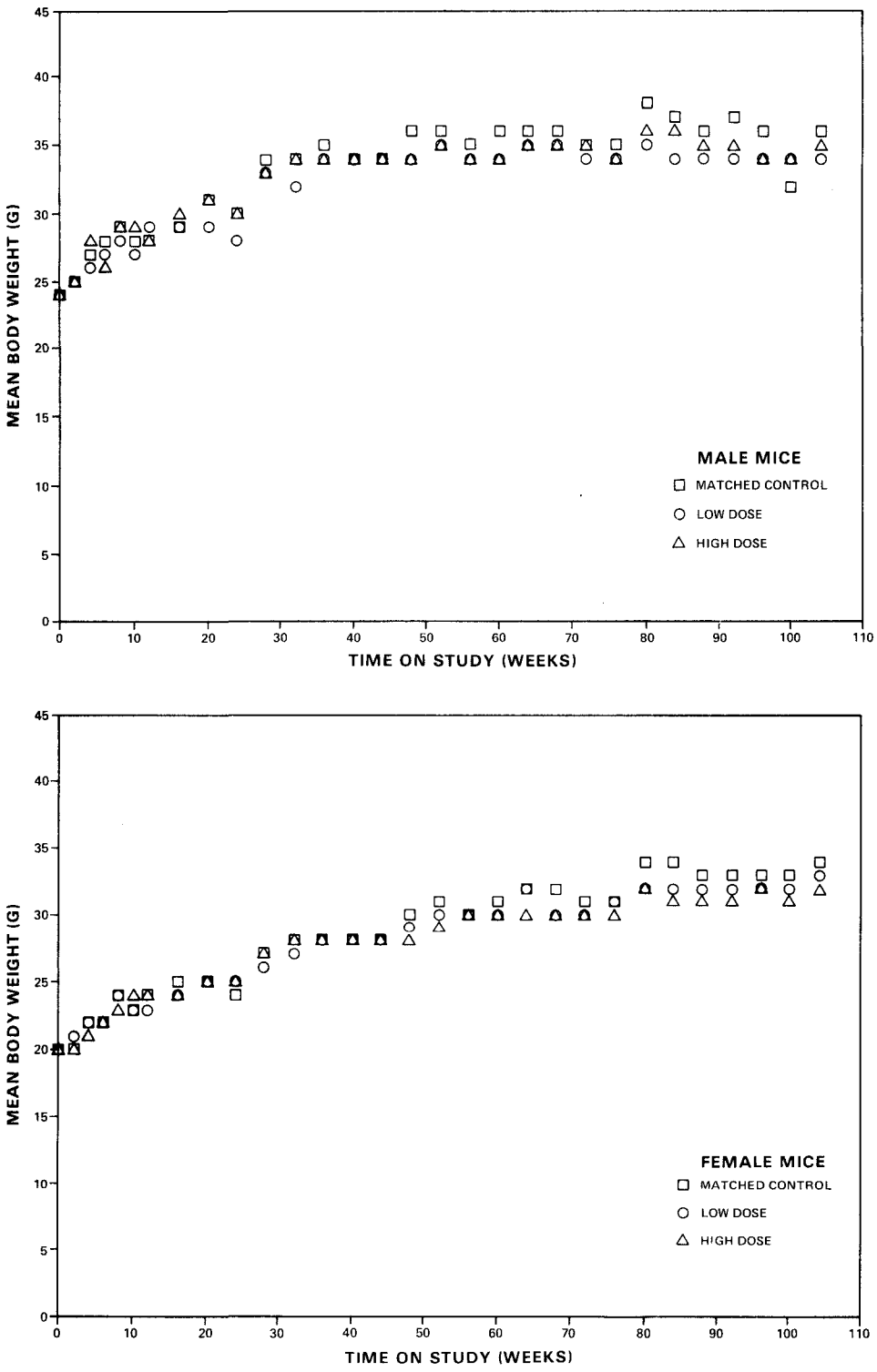


Figure 3. Growth Curves for Mice Adminstered dl-Menthol in the Diet

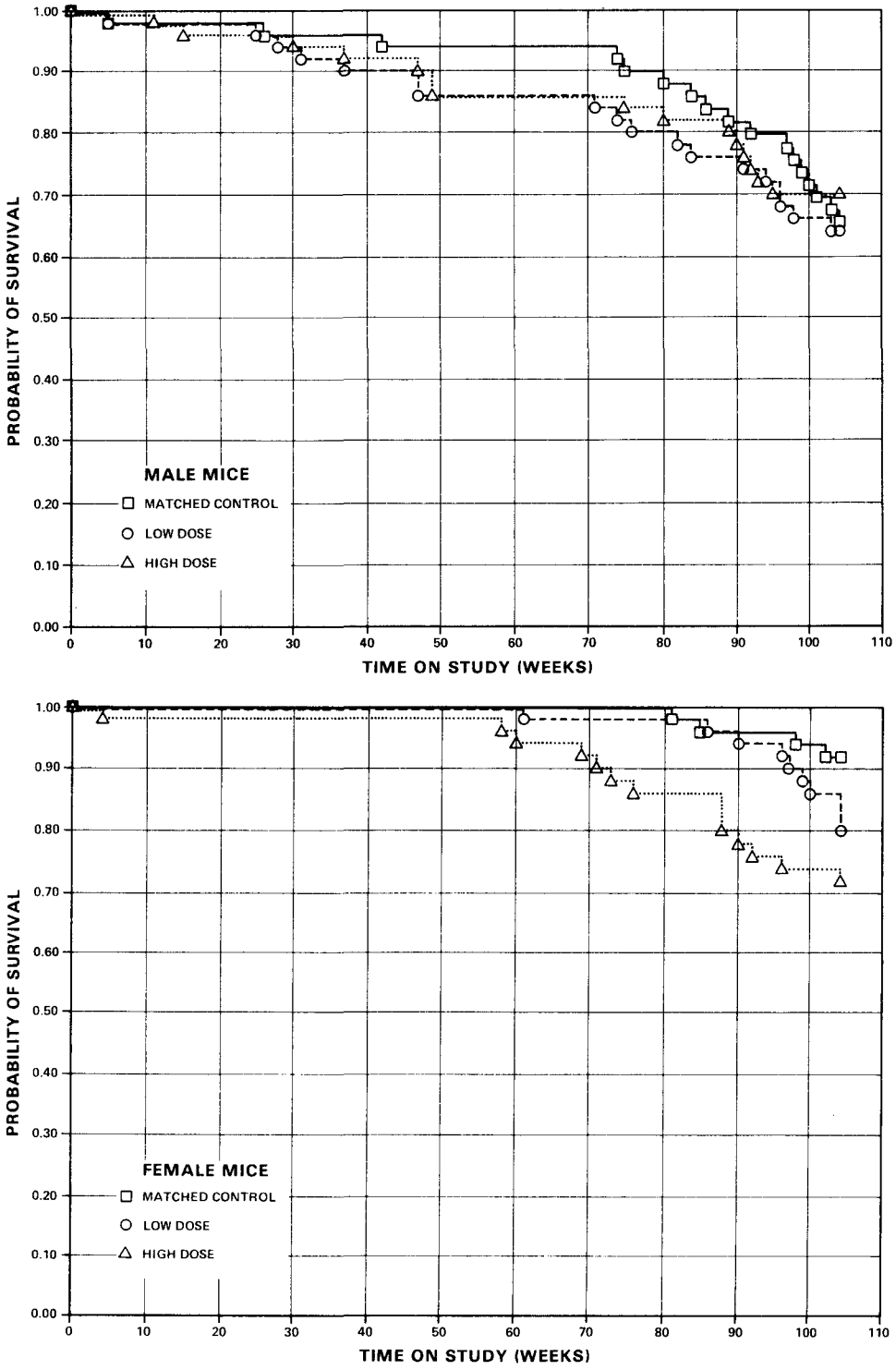


Figure 4. Survival Curves for Mice Administered dl-Menthol in the Diet

and the results of the Cox test comparing the survival of the control group with each dosed group, are not significant. In females, the result of the Tarone test is significant (P = 0.008). The result of the Cox test comparing the survival of the control group with the high-dose group is significant (P = 0.020), but the comparison between the control and low-dose groups is not significant.

In male mice, there were 35/50 (70%) of the high-dose group, 32/50 (64%) of the low-dose group, and 32/50 (64%) of the controls still alive at week 104. In female mice, there were 36/50 (72%) of the high-dose group, 40/50 (80%) of the low-dose group, and 45/50 (90%) of the controls still alive at week 104. Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

A low incidence of neoplasia was observed in both control and dosed groups of mice. These neoplasms were of the usual number and type observed in mice of this age and strain. A slightly increased incidence of hepatocellular carcinomas was observed in

the high-dose males (8/47 controls, 8/49 low-dose, 14/48 high-dose); however, the incidence was not increased over that observed occasionally in historical-control groups of mice of this age and strain. Alveolar/bronchiolar adenomas or carcinomas of the lung occurred primarily in the dosed females (1/49 controls, 3/47 low-dose, 5/48 high-dose). The incidence of lung neoplasms was not considered indicative of a carcinogenic effect, as this neoplasm has been commonly seen at a similar low incidence in historical-control groups.

Other degenerative, proliferative, and inflammatory lesions observed were also of the usual incidence and kind observed in aged B6C3F1 mice, and incidences in dosed groups were comparable with those in control groups.

Based on the histopathologic examination, dl-menthol was neither toxic nor carcinogenic to B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

The results of the Cochran-Armitage test for dose-related trend and those of the Fisher exact test comparing the incidence of tumors in the control group with that in each of the dosed groups are not significant in either sex.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by dl-menthol, which could not be detected under the conditions of this test.

V. DISCUSSION

Mean body weights of the dosed rats and mice were slightly lower than those of corresponding controls. No other clinical signs related to administration of dl-menthol were noted in any dosed groups of rats and mice. A dose-related trend in mortality was observed only in the female mice. Survival at the end of the bioassay was at least 62% in all dosed and control groups of animals of each species, and sufficient numbers of animals were at risk for the development of late-appearing tumors.

In male rats, no tumors occurred at incidences which were considered to be associated with the administration of dl-menthol.

In female rats, no tumors occurred at higher incidences in dosed groups than in control groups. Fibroadenomas of the mammary gland occurred at lower incidences in the low-dose (10/49) and high-dose (7/49) groups than in the control group (20/50), and alveolar/bronchiolar adenomas or carcinomas of the lung occurred only in the controls (3/50).

In mice of either sex, no tumors occurred in dosed groups at incidences that were significantly different from those for corresponding control groups.

The acute oral LD_{50} of menthol in Osborne-Mendel rats has been reported as 3,180 mg/kg body weight (Jenner et al., 1964) and as 2,900 mg/kg body weight (Herken, 1961). When administered in the diet to male and female rats for 5.5 weeks, d- or dl-menthol at 100 or 200 mg/kg body weight caused no adverse effects on gain in weight (Herken, 1961). No long-term studies have been reported previous to the present bioassay.

It is concluded that under the conditions of this bioassay, dl-menthol was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED dl-MENTHOL IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED *dl*-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		1 (2%)
BASAL-CELL CARCINOMA		1 (2%)	
ANGIOMA		1 (2%)	
*SUBCUT TISSUE	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)	1 (2%)	
FIBROMA	1 (2%)		2 (4%)
FIBROSARCOMA	1 (2%)	1 (2%)	
LIPOSARCOMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
HEMANGIOPERICYTOMA, MALIGNANT	1 (2%)		1 (2%)
OSTEOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
HEMANGIOPERICYTOMA, METASTATIC	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MONOCYTIC LEUKEMIA	14 (29%)	14 (28%)	11 (22%)
#SPLEEN	(49)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)		1 (2%)
#CERVICAL LYMPH NODE	(49)	(49)	(50)
HEMANGIOPERICYTOMA, METASTATIC			1 (2%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(49)	(50)	(50)
KUPFFER-CELL SARCOMA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(48)	(50)
HEMANGIOSARCOMA, INVASIVE		1 (2%)	
HEMANGIOPERICYTOMA, METASTATIC			1 (2%)
#LIVER	(49)	(50)	(50)
NEOPLASTIC NODULE	1 (2%)		
HEPATOCELLULAR CARCINOMA		3 (6%)	2 (4%)
#PANCREAS	(49)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
#SMALL INTESTINE	(49)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
TUBULAR-CELL ADENOMA		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(49)	(46)
CHROMOPHOBE ADENOMA	5 (10%)	7 (14%)	2 (4%)
#ADRENAL	(49)	(50)	(50)
PHEOCHROMOCYTOMA	7 (14%)	7 (14%)	7 (14%)
#THYROID	(49)	(48)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (2%)	1 (2%)	2 (4%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
C-CELL ADENOMA		1 (2%)	
C-CELL CARCINOMA	4 (8%)		1 (2%)
#PANCREATIC ISLETS	(49)	(50)	(50)
ISLET-CELL ADENOMA	1 (2%)	2 (4%)	1 (2%)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROADENOMA	1 (2%)	4 (8%)	2 (4%)
*PREPUTIAL GLAND	(49)	(50)	(50)
CARCINOMA,NOS	2 (4%)	2 (4%)	4 (8%)
#TESTIS	(49)	(50)	(50)
INTERSTITIAL-CELL TUMOR	44 (90%)	47 (94%)	50 (100%)
INTERSTITIAL-CELL TUMOR, MALIGNA	1 (2%)		
*EPIDIDYMIS	(49)	(50)	(50)
INTERSTITIAL-CELL TUMOR, INVASIV	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(50)
ASTROCYTOMA		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(49)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*BONE	(49)	(50)	(50)
OSTEOSARCOMA	1 (2%)		
*SKELETAL MUSCLE	(49)	(50)	(50)
OSTEOSARCOMA, INVASIVE	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
MESOTHELIOMA, NOS	2 (4%)	2 (4%)	
MESOTHELIOMA, MALIGNANT		2 (4%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	18	13	15
MORIBUND SACRIFICE	1	4	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	31	33	34
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	47	48	50
TOTAL PRIMARY TUMORS	90	106	97
TOTAL ANIMALS WITH BENIGN TUMORS	46	48	50
TOTAL BENIGN TUMORS	59	70	66
TOTAL ANIMALS WITH MALIGNANT TUMORS	24	28	26
TOTAL MALIGNANT TUMORS	28	33	30
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	2	1
TOTAL SECONDARY TUMORS	3	2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	3	1
TOTAL UNCERTAIN TUMORS	3	3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		
FIBROUS HISTIOCYTOMA, METASTATIC		2 (4%)	
*SUBCUT TISSUE	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		
FIBROMA	1 (2%)		
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		
FIBROUS HISTIOCYTOMA, METASTATIC		2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
MYELOMONOCYTIC LEUKEMIA		1 (2%)	
MONOCYTIC LEUKEMIA	10 (20%)	5 (10%)	6 (12%)
#SPLEEN	(50)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
#CERVICAL LYMPH NODE	(50)	(47)	(47)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
#BRONCHIAL LYMPH NODE	(50)	(47)	(47)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(50)	(47)	(47)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(50)	(49)	(48)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
NEOPLASTIC NODULE	1 (2%)	1 (2%)	1 (2%)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
#PANCREAS	(50)	(47)	(49)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
#RIGHT KIDNEY	(50)	(49)	(49)
MIXED TUMOR, MALIGNANT		1 (2%)	
#LEFT KIDNEY	(50)	(49)	(49)
MIXED TUMOR, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(43)
CHROMOPHOBE ADENOMA	28 (58%)	25 (53%)	19 (44%)
#ADRENAL	(50)	(49)	(49)
PHEOCHROMOCYTOMA		1 (2%)	3 (6%)
MIXED TUMOR, METASTATIC		1 (2%)	
#THYROID	(48)	(47)	(46)
FOLLICULAR-CELL ADENOMA	2 (4%)	2 (4%)	2 (4%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
C-CELL CARCINOMA	1 (2%)	2 (4%)	2 (4%)
#PANCREATIC ISLETS	(50)	(47)	(49)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(49)
ADENOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS	1 (2%)	3 (6%)	
CYSTADENOMA, NOS	1 (2%)		
FIBROADENOMA	20 (40%)	10 (20%)	7 (14%)
*PREPUTIAL GLAND	(50)	(49)	(49)
CARCINOMA,NOS	2 (4%)	2 (4%)	1 (2%)
#UTERUS	(50)	(49)	(48)
ADENOCARCINOMA, NOS		1 (2%)	
ENDOMETRIAL STROMAL POLYP	6 (12%)	6 (12%)	8 (17%)
#UTERUS/ENDOMETRIUM	(50)	(49)	(48)
SARCOMA, NOS	1 (2%)		2 (4%)
ENDOMETRIAL STROMAL POLYP	1 (2%)		
#OVARY	(49)	(49)	(48)
FIBROMA	1 (2%)		
SEMINOMA/DYSGERMINOMA	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(48)	(49)	(49)
GLIOMA, NOS	1 (2%)		
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
*ZYMBAL'S GLAND	(50)	(49)	(49)
CARCINOMA,NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*FEMUR	(50)	(49)	(49)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	11	14	8
MORIBUND SACRIFICE	3	1	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	35	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	41	36	32
TOTAL PRIMARY TUMORS	83	67	53
TOTAL ANIMALS WITH BENIGN TUMORS	38	32	29
TOTAL BENIGN TUMORS	62	46	40
TOTAL ANIMALS WITH MALIGNANT TUMORS	19	15	10
TOTAL MALIGNANT TUMORS	20	20	12
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	
TOTAL SECONDARY TUMORS	1	13	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	1
TOTAL UNCERTAIN TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED dl-MENTHOL IN THE DIET

TABLE B1.
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	47	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	47	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(47)	(49)	(48)
FIBROSARCOMA, METASTATIC			1 (2%)
*SUBCUT TISSUE	(47)	(49)	(48)
FIBROMA	4 (9%)	1 (2%)	6 (13%)
FIBROSARCOMA	8 (17%)	6 (12%)	4 (8%)
HEMANGIOSARCOMA	1 (2%)	2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(46)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (11%)	6 (12%)	6 (13%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	2 (4%)	
FIBROSARCOMA, METASTATIC		1 (2%)	1 (2%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(47)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (9%)	1 (2%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	2 (4%)
GRANULOCYTIC LEUKEMIA		2 (4%)	
#SPLEEN	(47)	(49)	(48)
HEMANGIOSARCOMA			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#MESENTERIC L. NODE	(47)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#SMALL INTESTINE	(47)	(49)	(47)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#PEYERS PATCH	(47)	(49)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(46)	(49)	(48)
HEMANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(47)	(49)	(48)
HEPATOCELLULAR CARCINOMA	8 (17%)	8 (16%)	14 (29%)
HEMANGIOSARCOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(43)	(46)	(44)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#TESTIS	(47)	(49)	(48)
INTERSTITIAL-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(47)	(49)	(48)
ADENOMA, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*HARDERIAN GLAND	(47)	(49)	(48)
ADENOMA, NOS		1 (2%)	3 (6%)
MUSCULOSKELETAL SYSTEM			
*ABDOMINAL MUSCLE	(47)	(49)	(48)
FIBROSARCOMA, METASTATIC			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(47)	(49)	(48)
MESOTHELIOMA, NOS	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	17	18	15
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1		
TERMINAL SACRIFICE	32	32	35
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	29	28	33
TOTAL PRIMARY TUMORS	36	35	48
TOTAL ANIMALS WITH BENIGN TUMORS	10	9	15
TOTAL BENIGN TUMORS	10	9	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	23	26
TOTAL MALIGNANT TUMORS	25	25	32
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	2
TOTAL SECONDARY TUMORS		2	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		
TOTAL UNCERTAIN TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	47	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(49)	(47)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
LEIOMYOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(47)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	6 (12%)	5 (11%)	7 (15%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	12 (24%)	8 (17%)	3 (6%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		
#SPLEEN	(49)	(47)	(48)
HEMANGIOSARCOMA			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#CERVICAL LYMPH NODE	(48)	(47)	(48)
HEMANGIOSARCOMA	1 (2%)		
#MESENTERIC L. NODE	(48)	(47)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#THYMUS	(23)	(26)	(29)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(49)	(47)	(48)
HEPATOCELLULAR CARCINOMA	1 (2%)	3 (6%)	3 (6%)
#STOMACH	(48)	(47)	(48)
LEIOMYOSARCOMA	1 (2%)		
#LARGE INTESTINE	(48)	(46)	(48)
LEIOMYOSARCOMA, METASTATIC	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(47)	(48)
LEIOMYOSARCOMA, METASTATIC	1 (2%)		
#URINARY BLADDER	(47)	(45)	(47)
LEIOMYOSARCOMA, METASTATIC	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(35)	(39)
CHROMOPHOBE ADENOMA	3 (9%)		1 (3%)
#THYROID	(43)	(46)	(46)
FOLLICULAR-CELL ADENOMA	3 (7%)	3 (7%)	
FOLLICULAR-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(47)	(48)
ADENOCARCINOMA, NOS	1 (2%)	3 (6%)	2 (4%)
#UTERUS	(48)	(46)	(48)
LEIOMYOSARCOMA, METASTATIC	1 (2%)		
ENDOMETRIAL STROMAL POLYP		1 (2%)	
HEMANGIOSARCOMA	1 (2%)		
#OVARY	(47)	(47)	(48)
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
TERATOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(47)	(48)
HEMANGIOSARCOMA	1 (2%)		
*MESENTERY	(49)	(47)	(48)
LEIOMYOSARCOMA, METASTATIC	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	4	10	12
MORIBUND SACRIFICE			2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	45	40	36
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	30	20	24
TOTAL PRIMARY TUMORS	34	27	26
TOTAL ANIMALS WITH BENIGN TUMORS	6	7	5
TOTAL BENIGN TUMORS	7	7	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	26	17	19
TOTAL MALIGNANT TUMORS	27	20	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		1
TOTAL SECONDARY TUMORS	6		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS ADMINISTERED dl-MENTHOL IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)	1 (2%)	1 (2%)
METAPLASIA, SQUAMOUS	1 (2%)		
*SUBCUT TISSUE	(49)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	1 (2%)
ABSCESS, NOS		1 (2%)	
GRANULOMA, NOS		1 (2%)	
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	5 (10%)	1 (2%)	7 (14%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#HEART	(49)	(50)	(50)
THROMBOSIS, NOS	1 (2%)		
THROMBUS, ORGANIZED		1 (2%)	
FIBROSIS	1 (2%)		
DEGENERATION, NOS	1 (2%)		
#AURICULAR APPENDAGE	(49)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(49)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS		6 (12%)	
DEGENERATION, NOS	1 (2%)	6 (12%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
PELIOSIS HEPATIS	1 (2%)		
METAMORPHOSIS FATTY	1 (2%)		
FOCAL CELLULAR CHANGE			1 (2%)
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
NECROSIS, NOS	1 (2%)	1 (2%)	
*BILE DUCT	(49)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#PANCREAS	(49)	(50)	(50)
PERIARTERITIS	2 (4%)	3 (6%)	1 (2%)
ATROPHY, NOS	1 (2%)		
#STOMACH	(49)	(48)	(48)
ULCER, FOCAL		2 (4%)	1 (2%)
CALCIUM DEPOSIT			1 (2%)
HYPERKERATOSIS		2 (4%)	
ACANTHOSIS		2 (4%)	
#COLON	(49)	(50)	(50)
PARASITISM	3 (6%)		
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
MINERALIZATION			4 (8%)
HYDRONEPHROSIS		1 (2%)	
PYELONEPHRITIS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	29 (59%)	41 (82%)	41 (82%)
AMYLOIDOSIS	1 (2%)		
PIGMENTATION, NOS		1 (2%)	1 (2%)
ATROPHY, NOS		1 (2%)	
#KIDNEY/PELVIS	(49)	(50)	(50)
MINERALIZATION		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL	(49)	(50)	(50)
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(49)	(50)	(50)
DEGENERATION, NOS	1 (2%)		
HYPERPLASIA, NOS			1 (2%)
#THYROID	(49)	(48)	(48)
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL			2 (4%)
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)	1 (2%)	1 (2%)
#PARATHYROID	(31)	(39)	(35)
HYPERPLASIA, NOS			1 (3%)
#PANCREATIC ISLETS	(49)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
GALACTOCELE		1 (2%)	
LACTATION		1 (2%)	
#PROSTATE	(47)	(48)	(50)
INFLAMMATION, NOS	1 (2%)	1 (2%)	3 (6%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#TESTIS	(49)	(50)	(50)
ATROPHY, NOS	3 (6%)	1 (2%)	
*EPIDIDYMIS	(49)	(50)	(50)
GRANULOMA, SPERMATIC		1 (2%)	
NECROSIS, FAT			1 (2%)
*SCROTUM	(49)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(50)
HYDROCEPHALUS, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(49)	(50)	(50)
SYNECHIA, ANTERIOR			1 (2%)
SYNECHIA, POSTERIOR		1 (2%)	1 (2%)
CATARACT		1 (2%)	2 (4%)
LENTICULAR OPACITIES		1 (2%)	
*EYE/CORNEA	(49)	(50)	(50)
PANNUS			1 (2%)
CATARACT			1 (2%)
VASCULARIZATION			1 (2%)
*EYE/CRYSTALLINE LENS	(49)	(50)	(50)
RUPTURE			1 (2%)
*HARDERIAN GLAND	(49)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
NECROSIS, FAT		3 (6%)	4 (8%)
*PERICARDIUM	(49)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
*MESENTERY	(49)	(50)	(50)
PERIARTERITIS	1 (2%)	2 (4%)	2 (4%)
NECROSIS, FAT	2 (4%)	3 (6%)	3 (6%)
ALL OTHER SYSTEMS			
DIAPHRAGM			
HERNIA, NOS		2	4
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTOLYSIS/NO NECROPSY	1		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(49)
NECROSIS, FAT	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(48)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE	1 (2%)		
PNEUMONIA, CHRONIC MURINE	3 (6%)	4 (8%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	
#SPLEEN	(50)	(49)	(49)
HEMORRHAGE			1 (2%)
PIGMENTATION, NOS		2 (4%)	
HEMATOPOIESIS		1 (2%)	1 (2%)
#CERVICAL LYMPH NODE	(50)	(47)	(47)
INFLAMMATION, NOS	3 (6%)		
CIRCULATORY SYSTEM			
#HEART	(50)	(49)	(48)
PERIARTERITIS			1 (2%)
CALCIUM DEPOSIT		1 (2%)	
#MYOCARDIUM	(50)	(49)	(48)
INFLAMMATION, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS	1 (2%)	4 (8%)	4 (8%)
DEGENERATION, NOS	1 (2%)	4 (8%)	4 (8%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
INFLAMMATION, NOS			1 (2%)
PELIOSIS HEPATIS			1 (2%)
METAMORPHOSIS FATTY	2 (4%)		
FOCAL CELLULAR CHANGE	3 (6%)	1 (2%)	2 (4%)
ANGIECTASIS	1 (2%)		
#STOMACH	(50)	(48)	(48)
ULCER, NOS	1 (2%)		
ULCER, FOCAL	1 (2%)		
#COLON	(50)	(49)	(49)
PARASITISM		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
PYELONEPHRITIS, NOS	1 (2%)		2 (4%)
INFLAMMATION, CHRONIC	19 (38%)	5 (10%)	2 (4%)
#KIDNEY/PELVIS	(50)	(49)	(49)
INFLAMMATION, NOS	1 (2%)		
#URINARY BLADDER	(47)	(44)	(39)
CALCULUS, NOS			1 (3%)
INFLAMMATION, NOS		1 (2%)	2 (5%)
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(43)
CYST, NOS	2 (4%)	1 (2%)	
ANGIECTASIS			2 (5%)
#ADRENAL	(50)	(49)	(49)
CYST, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANGIECTASIS	1 (2%)	1 (2%)	
#ADRENAL MEDULLA	(50)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
#THYROID	(48)	(47)	(46)
CYSTIC FOLLICLES			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(49)
GALACTOCELE	2 (4%)	9 (18%)	8 (16%)
LACTATION	1 (2%)	8 (16%)	5 (10%)
*VAGINA	(50)	(49)	(49)
PROLAPSE			1 (2%)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, CHRONIC			1 (2%)
#UTERUS	(50)	(49)	(48)
HYDROMETRA	7 (14%)	1 (2%)	5 (10%)
CYST, NOS	2 (4%)		
THROMBUS, ORGANIZED	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(49)	(48)
INFLAMMATION, NOS			2 (4%)
HYPERPLASIA, CYSTIC		1 (2%)	
#OVARY/PAROVARIAN	(50)	(49)	(48)
NECROSIS, FAT			1 (2%)
#OVARY	(49)	(49)	(48)
CYST, NOS	1 (2%)	3 (6%)	
FOLLICULAR CYST, NOS		1 (2%)	
PAROVARIAN CYST			2 (4%)
FIBROSIS		1 (2%)	
CALCIUM DEPOSIT		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(48)	(49)	(49)
THROMBOSIS, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(50)	(49)	(49)
CONGENITAL MALFORMATION, NOS		1 (2%)	
INFLAMMATION, NOS			1 (2%)
SYNECHIA, ANTERIOR		3 (6%)	
CATARACT	1 (2%)	3 (6%)	1 (2%)
*EYE/CORNEA	(50)	(49)	(49)
VASCULARIZATION		1 (2%)	
*HARDERIAN GLAND	(50)	(49)	(49)
HYPERPLASIA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(49)	(49)
NECROSIS, FAT	3 (6%)	2 (4%)	1 (2%)
*ABDOMINAL WALL	(50)	(49)	(49)
NECROSIS, FAT		1 (2%)	
*PERITONEAL CAVITY	(50)	(49)	(49)
NECROSIS, FAT	1 (2%)		
*MESENTERY	(50)	(49)	(49)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
DIAPHRAGM			
HERNIA, NOS		3	3
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	3	9

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTOLYSIS/NO NECROPSY		1	1

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED dl-MENTHOL IN THE DIET

TABLE D1.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	47	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	47	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(47)	(49)	(48)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, NOS	3 (6%)	3 (6%)	1 (2%)
FIBROSIS			1 (2%)
ACARIASIS			1 (2%)
HYPERKERATOSIS	2 (4%)		
ACANTHOSIS	2 (4%)	1 (2%)	
METAPLASIA, OSSEOUS			1 (2%)
*SUBCUT TISSUE	(47)	(49)	(48)
GRANULOMA, NOS	1 (2%)		
FIBROSIS		1 (2%)	
NECROSIS, FAT	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(46)	(49)	(48)
INFLAMMATION, CHRONIC		1 (2%)	
#LUNG	(46)	(49)	(48)
PNEUMONIA, CHRONIC MURINE	1 (2%)	1 (2%)	5 (10%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(47)	(49)	(48)
AMYLOIDOSIS			1 (2%)
HEMATOPOIESIS	3 (6%)	3 (6%)	2 (4%)
#LUMBAR LYMPH NODE	(47)	(49)	(48)
INFLAMMATION, NOS			1 (2%)
#MESENTERIC L. NODE	(47)	(49)	(48)
LYMPHANGIECTASIS	1 (2%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGE	1 (2%)		
INFLAMMATION, NOS		4 (8%)	3 (6%)
ANGIECTASIS		11 (22%)	4 (8%)
CIRCULATORY SYSTEM			
#AURICULAR APPENDAGE	(46)	(49)	(48)
THROMBOSIS, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(47)	(49)	(48)
THROMBUS, ORGANIZED		1 (2%)	1 (2%)
INFARCT, FOCAL		1 (2%)	
#LIVER/CENTRILOBULAR	(47)	(49)	(48)
NECROSIS, NOS		2 (4%)	
*GALLBLADDER	(47)	(49)	(48)
THROMBUS, ORGANIZED		1 (2%)	
#PANCREAS	(47)	(48)	(48)
DILATATION/DUCTS			1 (2%)
CYST, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
#STOMACH	(47)	(49)	(48)
ULCER, FOCAL			1 (2%)
HYPERKERATOSIS	2 (4%)	1 (2%)	
ACANTHOSIS	2 (4%)	1 (2%)	
#LARGE INTESTINE	(45)	(49)	(46)
NEMATODIASIS	1 (2%)		
PARASITISM			1 (2%)
NECROSIS, FAT			1 (2%)
*ANUS	(47)	(49)	(48)
PROLAPSE		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(47)	(49)	(48)
HYDRONEPHROSIS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
PYELONEPHRITIS, NOS		3 (6%)	
PYELONEPHRITIS SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC		2 (4%)	
#URINARY BLADDER	(46)	(47)	(48)
CALCULUS, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC		5 (11%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(43)	(46)	(44)
INFLAMMATION, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*PENIS	(47)	(49)	(48)
INFLAMMATION, CHRONIC		2 (4%)	
*PREPUCE	(47)	(49)	(48)
INFLAMMATION, CHRONIC		2 (4%)	
*PREPUTIAL GLAND	(47)	(49)	(48)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		
#PROSTATE	(46)	(47)	(47)
INFLAMMATION, SUPPURATIVE		3 (6%)	1 (2%)
*EPIDIDYMIS	(47)	(49)	(48)
NECROSIS, FAT	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(47)	(49)	(48)
INFLAMMATION, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
VASCULARIZATION	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*KNEE JOINT	(47)	(49)	(48)
OSTEOARTHRITIS			4 (8%)
OSTEOSCLEROSIS		3 (6%)	
BODY CAVITIES			
*ABDOMINAL CAVITY	(47)	(49)	(48)
NECROSIS, FAT	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	8	7	7
AUTO/NECROPSY/HISTO PERF	1		1
AUTOLYSIS/NO NECROPSY	3	1	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED dl-MENTHOL IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	47	48
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(47)	(48)
INFLAMMATION, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(47)	(48)
PNEUMONIA, CHRONIC MURINE	3 (6%)	4 (9%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(47)	(48)
HEMATOPOIESIS			1 (2%)
#THYMUS	(23)	(26)	(29)
FOLLICULAR CYST, NOS			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(49)	(47)	(48)
FIBROSIS		1 (2%)	
DEGENERATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(47)	(48)
INFLAMMATION, NOS			1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	
ANGIECTASIS			1 (2%)
#PANCREAS	(49)	(47)	(47)
CYSTIC DUCTS	2 (4%)		2 (4%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS		1 (2%)	
PERIARTERITIS		1 (2%)	
ATROPHY, NOS	1 (2%)		
ANGIECTASIS			1 (2%)
#STOMACH	(48)	(47)	(48)
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		
#SMALL INTESTINE	(49)	(47)	(48)
DIVERTICULUM		1 (2%)	
#COLON	(48)	(46)	(48)
PARASITISM			1 (2%)
URINARY SYSTEM			
#KIDNEY	(49)	(47)	(48)
INFLAMMATION, CHRONIC	1 (2%)		
#URINARY BLADDER	(47)	(45)	(47)
INFLAMMATION, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(35)	(39)
ANGIECTASIS			1 (3%)
#ADRENAL	(48)	(46)	(48)
ANGIECTASIS		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(48)	(46)	(48)
HYDROMETRA	6 (13%)	5 (11%)	2 (4%)
INFLAMMATION, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(48)	(46)	(48)
HYPERPLASIA, CYSTIC	17 (35%)	27 (59%)	23 (48%)
#OVARY	(47)	(47)	(48)
CYST, NOS	10 (21%)	12 (26%)	10 (21%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FOLLICULAR CYST, NOS	1 (2%)	3 (6%)	
PAROVARIAN CYST	1 (2%)		
INFLAMMATION, NOS	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(49)	(47)	(47)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(49)	(47)	(48)
SYNECHIA, POSTERIOR			1 (2%)
CATARACT			1 (2%)
PHTHISIS BULBI		1 (2%)	
*EYE/LACRIMAL GLAND	(49)	(47)	(48)
INFLAMMATION, NOS		1 (2%)	
*HARDERIAN GLAND	(49)	(47)	(48)
HYPERPLASIA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*KNEE JOINT	(49)	(47)	(48)
OSTEOARTHRITIS		1 (2%)	
BODY CAVITIES			
*PERITONEUM	(49)	(47)	(48)
INFLAMMATION, NOS		1 (2%)	
*MESENTERY	(49)	(47)	(48)
NECROSIS, FAT			2 (4%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	1	8

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY		3	2

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED dl-MENTHOL IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Sarcoma[b]	3/49 (6)	3/50 (6)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.980	1.307
Lower Limit		0.137	0.233
Upper Limit		6.989	8.508
Weeks to First Observed Tumor	105	91	98
Hematopoietic System:			
Monocytic Leukemia[b]	14/49 (29)	14/50 (28)	11/50 (22)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.980	0.770
Lower Limit		0.487	0.353
Upper Limit		1.976	1.639
Weeks to First Observed Tumor	81	76	80

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: All Leukemias or Lymphomas[b]	14/49 (29)	14/50 (28)	12/50 (24)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.980	0.840
Lower Limit		0.487	0.397
Upper Limit		1.976	1.752
Weeks to First Observed Tumor	81	76	70
Liver: Hepatocellular Carcinoma[b]	0/49 (0)	3/50 (6)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.590	0.290
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	97	105

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	5/48 (10)	7/49 (14)	2/46 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.371	0.417
Lower Limit		0.403	0.041
Upper Limit		5.119	2.404
Weeks to First Observed Tumor	103	105	105
Adrenal: Pheochromocytoma[b]	7/49 (14)	7/50 (14)	7/50 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.980	0.980
Lower Limit		0.317	0.317
Upper Limit		3.032	3.032
Weeks to First Observed Tumor	67	104	97

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	1/49 (2)	1/48 (2)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.021	3.063
Lower Limit		0.013	0.257
Upper Limit		78.494	157.336
Weeks to First Observed Tumor	105	105	91
Thyroid: C-cell Carcinoma[b]	4/49 (8)	0/48 (0)	1/48 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.255
Lower Limit		0.000	0.005
Upper Limit		1.100	2.457
Weeks to First Observed Tumor	81	--	105

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	4/49 (8)	1/48 (2)	1/48 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.255	0.255
Lower Limit		0.005	0.005
Upper Limit		2.457	2.457
Weeks to First Observed Tumor	81	105	105
Mammary Gland: Fibroadenoma[b]	1/49 (2)	4/50 (8)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		3.920	1.960
Lower Limit		0.407	0.106
Upper Limit		188.989	113.312
Weeks to First Observed Tumor	105	97	105

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Preputial Gland: Carcinoma, NOS[b]	2/49 (4)	2/50 (4)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.980	1.960
Lower Limit		0.074	0.296
Upper Limit		13.058	20.886
Weeks to First Observed Tumor	105	105	105
Testis: Interstitial-Cell Tumor[b]	45/49 (92)	47/50 (94)	50/50 (100)
P Values[c,d]	P = 0.046	N.S.	N.S.
Relative Risk[f]		1.024	1.089
Lower Limit		0.912	0.985
Upper Limit		1.130	Infinite
Weeks to First Observed Tumor	78	72	70

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Mesothelioma, NOS, or Malignant Mesothelioma[b]	2/49 (4)	5/50 (10)	1/50 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.450	0.490
Lower Limit		0.424	0.008
Upper Limit		24.778	9.103
Weeks to First Observed Tumor	94	76	105

[a]Dosed groups received 3,750 or 7,500 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered dl-Menthol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	3/50 (6)	0/49 (0)	0/48 (0)
P Values[c,d]	P = 0.040(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.696	1.730
Weeks to First Observed Tumor	105	--	--
Hematopoietic System: Monocytic Leukemia[b]	10/50 (20)	5/49 (10)	6/49 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.510	0.612
Lower Limit		0.147	0.198
Upper Limit		1.510	1.708
Weeks to First Observed Tumor	94	89	95

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: All Leukemia[b]	10/50 (20)	6/49 (12)	6/49 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.612	0.612
Lower Limit		0.198	0.198
Upper Limit		1.708	1.708
Weeks to First Observed Tumor	94	89	95
Pituitary: Chromophobe Adenoma[b]	28/48 (58)	25/47 (53)	19/43 (44)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.912	0.757
Lower Limit		0.616	0.481
Upper Limit		1.348	1.178
Weeks to First Observed Tumor	85	69	81

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	0/50 (0)	1/49 (2)	3/49 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.055	0.614
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	98	105
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	2/48 (4)	3/47 (6)	2/46 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.532	1.043
Lower Limit		0.184	0.078
Upper Limit		17.658	13.866
Weeks to First Observed Tumor	105	105	86

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma, NOS[b]	1/50 (2)	3/49 (6)	0/49 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		3.061	0.000
Lower Limit		0.256	0.000
Upper Limit		157.341	19.032
Weeks to First Observed Tumor	94	105	--
Mammary Gland: Fibroadenoma[b]	20/50 (40)	10/49 (20)	7/49 (14)
P Values[c,d]	P = 0.003(N)	P = 0.028(N)	P = 0.004(N)
Relative Risk[f]		0.510	0.357
Lower Limit		0.240	0.142
Upper Limit		1.015	0.789
Weeks to First Observed Tumor	98	95	95

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus/Endometrium: Endometrial Stromal Polyp[b]	7/50 (14)	6/49 (12)	8/48 (17)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.875	1.190
Lower Limit		0.261	0.409
Upper Limit		2.820	3.557
Weeks to First Observed Tumor	92	98	62

[a]Dosed groups received 3,750 or 7,500 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED dl-MENTHOL IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered dl-Menthol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibroma of the Subcutaneous Tissue[b]	4/47 (9)	1/49 (2)	6/48 (13)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.240	1.469
Lower Limit		0.005	0.373
Upper Limit		2.309	6.658
Weeks to First Observed Tumor	98	104	90
Integumentary System: Fibrosarcoma of the Subcutaneous Tissue[b]	8/47 (17)	6/49 (12)	4/48 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.719	0.490
Lower Limit		0.222	0.115
Upper Limit		2.182	1.695
Weeks to First Observed Tumor	89	94	89

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	6/46 (13)	7/49 (14)	6/48 (13)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.095	0.958
Lower Limit		0.341	0.276
Upper Limit		3.661	3.330
Weeks to First Observed Tumor	104	94	89
Hematopoietic System: Lymphoma[b]	6/47 (13)	6/49 (12)	10/48 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.959	1.632
Lower Limit		0.276	0.587
Upper Limit		3.338	5.035
Weeks to First Observed Tumor	74	84	95

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia[b]	6/47 (13)	8/49 (16)	10/48 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.279	1.632
Lower Limit		0.422	0.587
Upper Limit		4.143	5.035
Weeks to First Observed Tumor	74	84	95
All Sites: Hemangiosarcoma[b]	2/47 (4)	2/49 (4)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.959	1.469
Lower Limit		0.072	0.176
Upper Limit		12.769	16.939
Weeks to First Observed Tumor	84	76	92

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	8/47 (17)	8/49 (16)	14/48 (29)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.959	1.714
Lower Limit		0.342	0.745
Upper Limit		2.692	4.262
Weeks to First Observed Tumor	89	71	91
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	0/43 (0)	0/46 (0)	2/44 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.290
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	80

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Harderian Gland: Adenoma, NOS[b]	0/47 (0)	1/49 (2)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.051	0.590
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	104

[a]Dosed groups received 2,000 or 4,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered dl-Menthol in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/49 (2)	3/47 (6)	5/48 (10)
P Values[c,d]	N.S.	N.S.	N.s.
Relative Risk[f]		3.128	5.104
Lower Limit		0.262	0.602
Upper Limit		160.605	236.011
Weeks to First Observed Tumor	104	97	104
Hematopoietic System: All Lymphomas[b]	20/49 (41)	14/47 (30)	12/48 (25)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.730	0.613
Lower Limit		0.391	0.311
Upper Limit		1.329	1.159
Weeks to First Observed Tumor	81	61	88

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma[b]	3/49 (6)	0/47 (0)	1/48 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.340
Lower Limit		0.000	0.007
Upper Limit		1.730	4.060
Weeks to First Observed Tumor	102	--	69
Liver: Hepatocellular Carcinoma[b]	1/49 (2)	3/47 (6)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		3.128	3.063
Lower Limit		0.262	0.257
Upper Limit		160.605	157.336
Weeks to First Observed Tumor	104	96	104

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered dl-Menthol in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	3/33 (9)	0/35 (0)	1/39 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.282
Lower Limit		0.000	0.006
Upper Limit		1.546	3.322
Weeks to First Observed Tumor	104	--	104
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	3/43 (7)	3/46 (7)	1/46 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.935	0.312
Lower Limit		0.132	0.006
Upper Limit		6.634	3.707
Weeks to First Observed Tumor	104	104	76

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered dl-Menthol in te Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinma, NOS[b]	1/49 (2)	3/47 (6)	2/48 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		3.128	2.042
Lower Limit		0.262	0.110
Upper Limit		160.605	117.915
Weeks to First Observed Tumor	104	97	92

[a]Dosed groups received 2,000 or 4,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR dl-MENTHOL CONCENTRATION

APPENDIX G

Analysis of Formulated Diets for dl-Menthol Concentration

Duplicate 10-g dosed feed samples were extracted with 20 ml of carbon disulfide, and aliquots of the extract analyzed by gas chromatography (thermal conductivity detector). Spiked samples containing 0.2, 0.4, and 0.8% dl-menthol were worked up simultaneously with each set of dosed feed samples. The average recoveries from these spiked samples were greater than 90%.

Theoretical Concentrations (% in diet)	No. of Samples	Sample Analytical Mean (% in diet)	Coefficient of Variation (%)	Range (% in diet)
0.375	9	0.34	15.23	0.23-0.40*
0.75	9	0.70	14.69	0.53-0.85*
0.20	6	0.19	13.81	0.16-0.23*
0.40	6	0.36	13.71	0.28-0.41*

*See Section II, B, Dietary Preparation for discussion of these data.

Review of the Bioassay of dl-Menthol* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of dl-Menthol for carcinogenicity.

The primary reviewer agreed with the conclusion in the report that dl-Menthol was not carcinogenic in rats or mice, under the conditions of test. After a brief description of the experimental design, the primary reviewer said that the study was adequate to serve as the basis for the negative findings. Based on the results of the study, he said that dl-Menthol would not appear to pose a carcinogenic risk to humans. However, he indicated that the negative findings could not be extrapolated to evaluate the potential human hazard of menthol in tobacco products.

The secondary reviewer agreed with the conclusion that dl-Menthol was not carcinogenic, under the conditions of test. He indicated that the study was well conducted and that the data suggested the compound did not pose a risk to man.

There was no objection to a recommendation that the report on the bioassay of dl-Menthol be accepted as written.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund

(Verald K. Rowe, Dow Chemical USA, submitted a written review)
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-281-217/3267

DHEW Publication No. (NIH) 79-1348