NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 381

TOXICOLOGY AND CARCINOGENESIS STUDIES OF *d*-CARVONE

(CAS NO. 2244-16-8)

IN B6C3F$_1$ MICE

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a comprehensive audit before being presented for public review. This Technical Report has been reviewed and approved by the NTP Board of Scientific Counselors' Peer Review Panel in public session; the interpretations described herein represent the official scientific position of the NTP.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF *d*-CARVONE

(CAS NO. 2244-16-8)

IN B6C3F$_1$ MICE

(GAVAGE STUDIES)

Po C. Chan, Ph.D., Study Scientist

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

February 1990

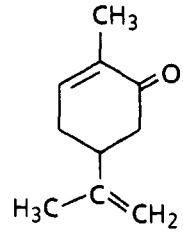
NTP TR 381

NIH Publication No. 90-2836

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTENTS

APPENDIXES



d-CARVONE

CAS No. 2244-16-8

$C_{10}H_{14}O$ Molecular weight 150.2

Synonyms for *d*-carvone: (+)-carvone; *d*(+)-carvone; (*S*)-carvone; (*S*)-(+)-carvone; (*S*)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one; (*S*)-*d*-*p*-mentha-6-8,(9)-dien-2-one; (*S*)-(+)-*p*-mentha-6,8-dien-2-one; *d*-1-methyl-4-isopropenyl-6-cyclohexen-2-one.
Carvol is a synonym for carvone (*d*, *l* not specified)

ABSTRACT

d-Carvone occurs naturally in caraway and dill seeds and in many essential oils; it has been used as a carminative and in perfumes and soaps. Toxicity and carcinogenesis studies were conducted by administering d-carvone (approximately 96% pure) in corn oil by gavage to groups of male and female B6C3F$_1$ mice for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Sixteen-Day Studies: All mice that received 1,600 or 3,500 mg/kg died within 7 days. Relative liver weights were increased for dosed male mice, and relative thymus weights were decreased for dosed female mice. No compound-related lesions were observed.

Thirteen-Week Studies: All male mice and 9/10 female mice that received the top dose of 1,500 mg/kg died before the end of the studies. No compound-related histopathologic changes were observed.

Based on survival at the higher doses in the 13-week studies, 2-year toxicology and carcinogenesis studies were conducted by administering *d*-carvone in corn oil by gavage to groups of 50 male and 50 female mice at doses of 375 or 750 mg/kg, 5 days per week for 103 weeks.

Two-Year Studies: Mean body weights of dosed and vehicle control mice were similar throughout the studies. Survival of dosed male mice was similar to that of vehicle controls (vehicle control, 37/50; low dose, 42/50; high dose, 36/50); survival of dosed female mice was greater than that of vehicle control female mice (14/50; 29/50; 38/50). Apparently, abscesses in the urogenital system caused the early deaths of many vehicle control female mice.

No neoplastic lesions attributed to *d*-carvone dosing were observed in mice.

Genetic Toxicology: *d*-Carvone was not mutagenic in *S. typhimurium* but induced sister chromatid exchanges and chromosomal aberrations in CHO cells.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of *d*-carvone for male or female B6C3F$_1$ mice administered 375 or 750 mg/kg, 5 days per week for 2 years.

SUMMARY OF THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE

Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses 0, 375, or 750 mg/kg *d*-carvone in corn oil, 5 d/wk	0, 375, or 750 mg/kg *d*-carvone in corn oil, 5 d/wk
Body weights in the 2-year study Dosed and vehicle controls similar	Dosed and vehicle controls similar
Survival rates in the 2-year study 37/50; 42/50; 36/50	14/50; 29/50; 38/50
Nonneoplastic effects None	None
Neoplastic effects None	None
Level of evidence of carcinogenic activity No evidence	No evidence

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 5.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 8-9.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.

- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of *d*-Carvone is based on 13-week studies that began in August 1981 and ended in November 1981 and on 2-year studies that began in June 1982 and ended in June 1984 at the International Research and Development Corporation (Mattawan, MI).

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

Po C. Chan, Ph.D., Study Scientist

John R. Bucher, Ph.D.
Scot L. Eustis, D.V.M., Ph.D.
Joseph K. Haseman, Ph.D.
James Huff, Ph.D.
Joseph H. Roycroft, Ph.D.

(Discipline Leaders and Principal Contributors)

Jack Bishop, Ph.D.
Douglas W. Bristol, Ph.D.
R. Chhabra, Ph.D.
R. Griesemer, D.V.M., Ph.D.
C.W. Jameson, Ph.D.
G.N. Rao, D.V.M., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
Douglas Walters, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report on 5/24/88)

Frank Voelker, D.V.M., Ph.D., D.A.C.V.P. (Chair)
Pathology Associates, Inc.
John Cullen, V.M.D., Ph.D. (North Carolina State University)
Michael Elwell, D.V.M., Ph.D. (NTP)
Bradley F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
Micheal Jokinen, D.V.M. (NTP)
Steven Stefanski, D.V.M. (NTP)

Principal Contributors at International Research and Development Corporation
(Conducted Studies and Evaluated Tissues)

Barrie M. Phillips, Ph.D.
D. Clifford Jessup, Ph.D.
Joseph H. Thorstenson, Ph.D.

Principal Contributor at Experimental Pathology Laboratories, Inc.
(Provided Pathology Quality Assurance)

Bradley F. Hamilton, D.V.M., Ph.D.

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D. Theriault, Ph.D.
Abigail C. Jacobs, Ph.D.
John Warner, M.S.
Naomi Levy, B.A.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on *d*-carvone on June 27, 1989, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D. (Chair)
Senior Scientific Advisor, Medicine and Environmental Health Department
Research and Environmental Health Division, Exxon Corporation
East Millstone, NJ

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Imperial Chemical Industries, PLC
Central Toxicology Laboratory
Alderley Park, England

Robert H. Garman, D.V.M.
Bushy Run Laboratories
Export, PA
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D.
University of California
Lawrence Berkeley Laboratory
Berkeley, CA

Curtis D. Klaassen, Ph.D. (Principal Reviewer)
Professor, Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

William Lijinsky, Ph.D. (Principal Reviewer)
Director, Chemical Carcinogenesis
Frederick Cancer Research Facility
Frederick, MD

Barbara McKnight, Ph.D.
Assistant Professor, Department of Biostatistics, University of Washington
Seattle, WA

Franklin E. Mirer, Ph.D.
Director, Health and Safety Department
International Union, United Auto Workers, Detroit, MI

Paul M. Newberne, D.V.M., Ph.D.*
Professor, Mallory Institute of Pathology
Boston, MA

James A. Popp, D.V.M., Ph.D.
(Principal Reviewer)
Head, Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

*Unable to attend

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF *d*-CARVONE

On June 27, 1989, the draft Technical Report on the toxicology and carcinogenesis studies of *d*-carvone received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. Po C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of *d*-carvone by reviewing the experimental design, results, and proposed conclusions (no evidence of carcinogenic activity of *d*-carvone for male or female mice administered 375 or 750 mg/kg, 5 days per week).

Dr. Chan reported that planned 2-year studies of *d*-carvone in F344/N rats were terminated after 13 months and the tissues were not evaluated. The decision to terminate the studies in rats was made because of several performance problems in the laboratory, which were unrelated to the studies in mice.

Dr. Lijinsky, a principal reviewer, agreed with the conclusions. He wondered if the higher mortality in vehicle control female mice than in dosed groups might suggest that the animals were not randomized. Dr. Chan responded that the animals were properly randomized. Dr. S. Eustis, NIEHS, explained that the mortality was due to utero-ovarian infections and it appeared that the chemical may have prevented the infections. Dr. Lijinsky asked about the volume of dose solution administered and whether it might not have been excessive in some cases. Dr. Chan pointed out that the volume of dose solution was constant among groups, 10 ml/kg. In view of the early termination of the rat studies, Dr. Lijinsky thought that speculation in the Discussion about possible renal effects of *d*-carvone in rats should be deleted.

Because Dr. Newberne, the second principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read his review into the record. Dr. Newberne agreed with the conclusions. He asked that more information be added on reasons for the cancellation of the studies in rats. He said that speculative discussion comparing renal effects of *d*-limonene in male rats with unknown renal effects of *d*-carvone should be deleted.

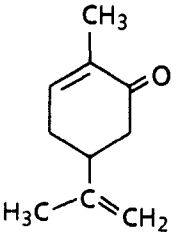
Dr. Klaassen, the third principal reviewer, agreed with the conclusions. He said that reasons for cancelling and not reporting the studies in rats should be given near the beginning of the Report. Dr. McKnight asked for clarification as to whether the problems with the studies in rats might have impinged on the studies in mice. Dr. J. Huff, NIEHS, said the inlife portion of the studies in mice was completed and proceeding through the histopathology phase by the time the 2-year studies in rats were begun. About halfway through the studies in rats, the National Toxicology Program (NTP) made the decision to terminate them, based on the judgment that collective performance of the contract laboratory was inadequate at that time. There was no evidence of fraud or deceit. Information on the short-term studies in rats was included in the draft Report because they were done concomitantly with the 2-year studies in mice and are considered valid. Further, he said that, based on the audit and knowledge of laboratory performance at the time the studies in mice were done, the NTP believed that the studies in mice are adequate for evaluation. Dr. Popp commented that the Audit Summary supported this belief. Dr. Ashby and Dr. Lijinsky proposed that all references to the studies in rats be deleted from the Report. Dr. Chan agreed. Dr. Scala said the discussion was helpful in providing assurances to the Panel concerning the reliability of the data from the studies in mice.

Dr. Lijinsky moved that the Technical Report on *d*-carvone be accepted with the revisions discussed, including deletion of all references to the studies in rats, as well as the comparisons to *d*-limonene, and with the conclusions as written for male and female mice, no evidence of carcinogenic activity. Dr. Klaassen seconded the motion. Dr. Gold offered an amendment to the motion, to add a phrase to the conclusions stating that the mice may have been able to tolerate a higher dose. The vote on this amendment resulted in a tie, with four negative votes (Drs. Ashby, Klaassen, Lijinsky, and Popp) and four affirmative votes (Drs. Garman, Gold, McKnight, and Mirer). To resolve the tie, Dr. Scala, the Chair, voted no, resulting in defeat of the amendment. The original motion by Dr. Lijinsky then was accepted unanimously by the Panel.

I. INTRODUCTION

Physical Properties
Use and Production
Environmental Occurrence
Regulatory Status
Absorption, Metabolism, and Biologic Effects
Toxicity
Genetic Toxicology
Carcinogenicity
Study Rationale

I. INTRODUCTION



***d*-CARVONE**

CAS No. 2244-16-8

$C_{10}H_{14}O$ Molecular weight 150.2

Synonyms for *d*-carvone: (+)-carvone; *d*(+)-carvone; (*S*)-carvone; (*S*)-(+)-carvone; (*S*)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one; (*S*)-*d*-*p*-mentha-6-8,(9)-dien-2-one; (*S*)-(+)-*p*-mentha-6,8-dien-2-one; *d*-1-methyl-4-isopropenyl-6-cyclohexen-2-one.
Carvol is a synonym for carvone (*d*, *l* not specified)

Physical Properties

d-Carvone is a colorless to pale yellow liquid with a characteristic caraway or dill odor and taste. *d*-Carvone is insoluble in water and glycerin, miscible with ethanol, and soluble in propylene glycol, fixed oils, and mineral oil (Condensed Chemical Dictionary, 1981). The boiling point of *d*-carvone is 230° C at 755 mm, and its specific gravity is 0.965 at 20° C (Merck, 1983).

Use and Production

d-Carvone is used as a flavoring agent in beverages (at a reported average concentration of 850 ppm), liquors and liqueurs (130 ppm), ice cream and other frozen desserts (120 ppm), baked goods (110 ppm), and candy (180 ppm). It is also used as a fragrance in perfumes (400-2,000 ppm), soaps (100-1,000 ppm), creams and lotions (50-300 ppm), and detergents (10-100 ppm) (Fenaroli, 1975; Opdyke, 1978). Approximately 1,500 kg of *d*-carvone per year has been used in fragrances in the United States (Opdyke, 1978).

d-Carvone is usually prepared by fractional distillation of caraway oil (Fenaroli, 1975). Production of *d*-carvone in the United States in 1976 was estimated to exceed 0.45×10^6 kg (SRI, 1977). Worldwide production of carvone was 0.3×10^6 kg in 1981 (Kirk-Othmer, 1981). In 1983, U.S. production of carvone (both optical isomers and the racemate) reportedly was 0.7×10^6 to 7×10^6 kg. One major manufacturer reported production of approximately 0.7×10^6 kg.

Environmental Occurrence

Carvone occurs naturally in both the dextrorotatory and levorotatory forms. *d*-Carvone is present in the aerial parts and seeds ("greens") of dill plants, *Anethum graveolus* L. (Lichtenstein et al., 1974). It is also found in spearmint oil isolated from the flowering tops of *Mentha spicata* L. (*M. virdis* L.), oil distilled from the dried ripe fruit of *Carum carvi* L., Umbelliferae, oil from the leaves and flowers of *Chrysanthemum indicum* L., Compositae, caraway seed (*Carum carvi*) oil, mandarin peel oil (*Citrus reticulata blanco*, Rutaceae), ginger-grass oil, kuromoji oil, lavender oil, and many other essential oils (Fenaroli, 1975; Andersen, 1978).

Shackelford and Keith (1976) reported that *d*-carvone has been detected in the effluent discharge from a sewage treatment plant.

Regulatory Status

d-Carvone was given generally-recognized-as-safe status by the Flavoring Extract Manufacturers' Association in 1965 (FEMA, 1965) and is approved by the Food and Drug Administration

for use in food (Fed. Regist., 1961). An acceptable daily intake of 1.25 mg/kg was established for *d*-carvone by the Council of Europe in 1974 (Council of Europe, 1974) and by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives in 1967 (FAO/WHO, 1967).

Absorption, Metabolism, and Biologic Effects

Little is known about the absorption and metabolism of carvone, except that it was reportedly metabolized by rabbits to carbinol and 1,5-dimethyl-1,5-hexadien-1,6-dicarboxylic acid (Fischer and Bielig, 1940). It was suggested that these metabolites may be excreted as glucuronide conjugates, on the basis of a study in which a glucuronide was detected in rabbit urine after exposure to carvone (Hildebrandt, 1902). Dihydrocarveol has been reported to be excreted by rabbits as a glucuronide conjugate (Hamalainen, 1912).

d-Carvone was identified in profiles of volatile metabolites in urine samples from normal humans (Zlatkis et al., 1973). The dietary history of the subjects was not recorded.

d-Carvone fed to male Sprague Dawley rats (200-225 g) stimulated liver degradation of parathion and paraoxon (Fuhremann et al., 1978). When *d*-carvone was injected simultaneously with pentobarbital, it prolonged the sleeping time of mice (Marcus and Lichtenstein, 1982). These data suggest that metabolism of *d*-carvone involves the cytochrome P450 oxidase enzyme system.

d-Carvone has been reported to increase the synthesis and excretion of ascorbate in rats (Longenecker et al., 1939). Ritz et al. (1940) observed that *d*-carvone primarily stimulated ascorbate production and concentration in liver. It was postulated that ascorbate is connected with detoxification of *d*-carvone (Longenecker et al., 1939).

d-Carvone is considered to have insecticidal properties (Lichtenstein et al., 1974). When used at sublethal dosages, *d*-carvone increased the toxicity of carbaryl, carbofuran, and parathion to insects and is therefore considered synergistic for carbamate and organophosphorus insecticides (Fuhremann and Lichtenstein, 1979).

Toxicity

Reported LD_{50} values for *d*-carvone are 1,500 mg/kg for mice (after intravenous injection) (Lichtenstein et al., 1974), 1,640 mg/kg for rats (after oral administration), and 766 mg/kg for guinea pigs (after oral administration) (Jenner et al., 1964). Stoner et al. (1973) reported that no adverse effects were observed in mice given six intraperitoneal injections of 0.25 g/kg *d*-carvone over a 2-week period.

d-Carvone (100%) applied to intact or abraded rabbit skin for 24 hours produced erythema lasting for 24 hours (Levenstein, 1976). In patch tests with humans, *d*-carvone produced no irritation after 48 hours at 2% in petrolatum (Kligman, 1976) and showed a low sensitizing potential at 5% in petrolatum (Andersen, 1978).

Genetic Toxicology

d-Carvone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without S9 activation. Carvone, a mixture of the dextrorotatory and levorotatory isomers, and carveol, a structurally related alcohol, were also negative for gene mutation induction in Salmonella (Florin et al., 1980; Mortelmans et al., 1986).

Rockwell and Raw (1979) reported that no mutagenic activity was detected in *S. typhimurium* strains TA98 or TA100 exposed to extracts of urine of rats fed carvone.

In mammalian cell tests, *d*-carvone induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells with and without S9 (Appendix F).

Carcinogenicity

No epidemiologic studies on the relationship between exposure to carvone or dihydrocarveol and cancer incidences have been reported in the literature. Stoner et al. (1973) reported that carvone was inactive in the short-term pulmonary lung adenoma test in strain A mice given

intraperitoneal injections of *d*-carvone and *l*-carvone three times per week for 8 weeks (total dose, 1.2 or 6 g/kg).

Hagan et al. (1967) reported that rats fed diets containing 10,000 ppm carvone for 16 weeks had growth retardation and testicular atrophy, whereas no effects were observed in rats given 1,000 ppm for 28 weeks or 2,500 ppm for 1 year.

Study Rationale

d-Carvone was selected for toxicity and carcinogenesis studies because human exposure to *d*-carvone in food has been estimated to be 3.1×10^8 g per year (NCI, 1979). The gavage route of administration was selected because the volatility of the chemical precluded its administration in feed.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *d*-CARVONE

CHARACTERIZATION OF DOSE MIXTURES

SIXTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Study Design

Source and Specifications of Animals

Animal Maintenance

Clinical Examinations and Pathology

Statistical Methods

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *d*-CARVONE

d-Carvone was obtained as a clear, pale yellow liquid in a single lot (lot no. K-332) from Fritzsche, Dodge, and Olcott, Inc. (New York, NY), with the purity indicated on the label as food grade (Food Chemical Codex purity of not less than 95%). Purity and identity analyses were conducted at Midwest Research Institute (Kansas City, MO) (Appendix E).

d-Carvone was identified by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy and by optical rotation; all spectra were consistent with those in the literature (Sadtler Standard Spectra). The specific rotation, +60.3°, was consistent with a literature value (Merck, 1968) and confirmed that the sample was the *d*-isomer.

The purity of *d*-carvone was found to be approximately 96%, as determined by elemental analysis, Karl Fischer water analysis, titration of the carbonyl group after reaction with excess hydroxylamine hydrochloride dissolved in alcoholic potassium hydroxide and back-titration of excess potassium hydroxide with hydrochloric acid (FCC, 1966), thin-layer chromatography, and gas chromatography.

Lot no. K-332 had a water content of 0.88%. Titration of the carbonyl group indicated a purity of 98.0%. Gas chromatography by one system detected a major peak preceded by 16 impurities and followed by 6 impurities, with a total relative area of 4.6%. Eight impurities, seven preceding and one following the major peak, had individual relative areas greater than 0.1% with a total relative area of 4.1%. Gas chromatography by a second system detected a major peak preceded by 11 impurities and followed by 8 impurities, with a total relative area of 4.2%. Eight impurities, five preceding and three following the major peak, had relative areas greater than 0.1% and totaled 4.0%.

The complete battery of Food Chemicals Codex (FCC) tests (FCC, 1981) for *d*-carvone was performed and indicated that lot no. K-332, a synthetic *d*-carvone, met the FCC specifications that were in effect at the time of purchase (FCC, 1972). Under present FCC requirements (FCC, 1981), results of the titrimetric assay (96.5%) met the specification for natural *d*-carvone (95.0%) but were slightly lower than that required for the synthetic material (97.0%).

The identity of the chemical at the study laboratory was confirmed by infrared spectroscopy. The stability of the bulk chemical was monitored during the 2-year studies by gas chromatography and titration of the carbonyl group. No deterioration of *d*-carvone was seen throughout the studies.

CHARACTERIZATION OF DOSE MIXTURES

The 2-week stability of *d*-carvone mixed with NIH 07 Rat and Mouse Ration at 10,000 ppm and stored at temperatures varying from −20° C to room temperature was determined. The feed mixtures were extracted with acetonitrile and analyzed by gas chromatography with a 10% Carbowax 2M column and flame ionization detection. The study found that more than 4% of the *d*-carvone was lost during the feed blending process. Formulated diets stored open to air and light under simulated animal exposure conditions lost 5.5%, 11.2%, and 22.6% of the study chemical after 1, 3, and 7 days, respectively. The same feed stored in the dark for 2 weeks in sealed containers lost 0%, 2%, and 3% of the study chemical at −20° C, 5° C, and room temperature.

Because the feed blends of *d*-carvone were found to be unstable under the feed blending and simulated animal exposure conditions and because *d*-carvone is insoluble in water, corn oil gavage was selected as the route of administration for these studies. The 21-day stability of *d*-carvone in corn oil at 0.5% (5 mg/g) stored at room temperature or at 5° C was determined. The corn oil solutions were extracted with methanol and analyzed by high-performance liquid chromatography with a Brownlee RP-18 column and ultraviolet detection at 229 nm. The *d*-carvone/corn oil solutions were found to be stable for at least 21 days when stored in the dark at room temperature or at 5° C. The corn oil solutions were also stable under simulated dosing conditions for at least 3 hours.

The chemical in corn oil was found by gas chromatography to be stable for at least 1 week in the dark at room temperature. During the 2-year studies, the dose mixtures were analyzed at approximately 8-week intervals by extraction with methanol, followed by analysis with ultraviolet spectroscopy at 236 nm. For the *d*-carvone studies, it was estimated that the mixtures were formulated within ±10% of the target concentrations 100% (46/46) of the time throughout the studies (Table E3). Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results from the study laboratory (Table E4).

SIXTEEN-DAY STUDIES

Male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and were held for 14 days before the studies began. The mice were 7-8 weeks old when placed on study.

Groups of five mice of each sex were administered 0, 150, 328, 723, 1,590, or 3,500 mg/kg *d*-carvone in corn oil by gavage, 5 days per week, for 12 doses over 16 days.

Animals were housed five per cage. Water and feed were available ad libitum. The mice were observed twice per day and were weighed on days 1, 7, and 14 and at the end of the studies. A necropsy was performed on all animals. The brain, heart, liver, right kidney, lungs and bronchi, and thymus were weighed. Histopathologic examinations of animals that received 3,500 mg/kg were performed. The lungs and liver of all dosed mice were examined. Further details are presented in Table 1.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of *d*-carvone and to determine the doses to be used in the 2-year studies.

Five- to 7-week-old male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories, observed for 16 days, and assigned to dose groups according to a table of random numbers. Mean body weights were then checked to assure homogeneity of the groups.

Groups of 10 female mice and groups of 30 male mice, including groups of 20 animals originally designated for other studies to assess effects on reproductive functions (sperm morphology), were administered 0, 93, 375, or 1,500 mg/kg *d*-carvone in corn oil by gavage, 5 days per week for 13 weeks. Groups of 10 mice of each sex were administered 187 or 750 mg/kg on the same schedule.

Mice were housed five per cage. Feed and water were available ad libitum. Further experimental details are summarized in Table 1.

Animals were observed two times per day; moribund animals were humanely killed. Individual animal weights were recorded at the beginning of the studies, once per week thereafter, and at the end of the studies.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed. Histopathologic examinations were conducted on vehicle controls; animals killed at 2, 4, 6, and 8 weeks; animals that received 750 mg/kg; and animals that died before the end of the studies. Tissues and groups examined are listed in Table 1.

TWO-YEAR STUDIES

Study Design

Groups of 50 male and 50 female mice were administered 0, 375, or 750 mg/kg *d*-carvone in corn oil by gavage, 5 days per week for 103 weeks.

Source and Specifications of Animals

The male and female B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Frederick Cancer Research Facility. Breeding stock for the foundation colony at the production facility originated at the National Institutes of Health Repository. Animals

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF *d*-CARVONE

Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 male and 5 female mice	10 (187 and 750 mg/kg groups) or 30 (0, 93, 375, and 1,500 mg/kg groups) male and 10 female mice	50 male and 50 female mice
Doses		
0, 150, 328, 723, 1,590, or 3,500 mg/kg *d*-carvone in corn oil by gavage; dose vol--10 ml/kg	0, 93, 187, 375, 750, or 1,500 mg/kg *d*-carvone in corn oil by gavage; dose vol--10 ml/kg	0, 375, or 750 mg/kg *d*-carvone in corn oil gavage; dose vol--10 ml/kg
Date of First Dose		
5/18/81	8/6/81-8/7/81	6/29/82
Date of Last Dose		
6/2/81	11/5/81	6/20/84
Duration of Dosing		
12 doses over 16 d	5 d/wk for 13 wk	5 d/wk for 103 wk (some mice received 1 or 2 doses during wk 104)
Type and Frequency of Observation		
Observed 2 × d; weighed initially and then 1 × wk	Same as 16-d studies	Observed 2 × d; weighed initially, 1 × wk for 13 wk, and then at least 1 × mo
Necropsy and Histologic Examinations		
Necropsy performed on all animals; the following tissues were examined histologically for vehicle control and highest dose groups: adrenal glands, aorta, brain, colon, epididymis/tunica vaginalis/prostate/testes/scrotal sac or ovaries/uterus, esophagus, gallbladder, gross lesions, heart, jejunum, kidneys, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, salivary glands, spinal cord, spleen, sternebrae including marrow, stomach, thymus, thyroid gland, tissue masses, trachea, and urinary bladder. Liver and lungs examined for all other groups of mice. Organ weights obtained at necropsy	Necropsy performed on all animals; the following tissues were examined histologically for vehicle controls, mice receiving 750 mg/kg, and all animals dying or killed before the end of the studies: adrenal glands, aorta, brain, cecum, colon, duodenum, epididymis/prostate/seminal vesicles/testes or ovaries/uterus, esophagus, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, mesenteric lymph nodes, nasal cavity, pancreas, parathyroid glands, pituitary gland, preputial gland, rectum, salivary glands, spinal cord, spleen, sternebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Organ weights obtained at necropsy	Necropsy and histologic exams performed on all animals; the following tissues were examined: adrenal glands, aorta, brain, cecum, colon, duodenum, epididymis/prostate/seminal vesicles/testes or ovaries/uterus, esophagus, femur, gallbladder, gross lesions, Harderian gland, heart, ileum, jejunum, kidneys, larnyx, liver, lungs and bronchi, mammary gland, mesenteric lymph nodes, nasal cavity and turbinates, pancreas, pancreatic islets, parathyroid and pituitary glands, preputialgland, rectum, salivary glands, skin, skeletal muscle, spinal cord, spleen, sternebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
$B6C3F_1$ mice	$B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source		
Charles River Breeding Laboratories	Charles River Breeding Laboratories (Portage, MI)	Frederick Cancer Research Facility (Frederick, MD)
Study Laboratory		
International Research and Development Corporation	International Research and Development Corporation	International Research and Development Corporation

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF *d*-CARVONE (Continued)

Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Method of Animal Identification Toe clip; rats--ear tag	Toe clip	Toe clip
Time Held Before Study 14 d	16 d	13 d
Age When Placed on Study 7-8 wk	7-9 wk	7 wk
Age When Killed 9-10 wk	20-22 wk	111 wk
Necropsy Dates 6/3/81	11/6/81	6/26/84-6/28/84
Method of Animal Distribution Animals distributed to weight classes and then assigned to cages according to one table of random numbers and to groups according to another table of random numbers	Animals assigned to groups according to a table of random numbers	Same as 16-d studies
Diet NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 16-d studies	Same as 16-d studies
Bedding Beta Chips (Northeastern Products, Inc., Warrensburg, NY)	Same as 16-d studies	Same as 16-d studies
Water Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 16-d studies	Same as 16-d studies
Cages Polycarbonate (Hazleton Systems, Inc., Aberdeen, MD)	Same as 16-d studies	Same as 16-d studies
Cage Filters Reemay® spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 16-d studies	Same as 16-d studies
Animals per Cage 5	5	5
Other Chemicals on Study in the Same Room None	None	None
Animal Room Environment Temp--74° ± 2° F; hum--50% ± 10%; fluorescent light 12 h/d; 6-12 room air changes/h	Temp--average, 74.1° F; hum--average, 53.6%; fluorescent light 12 h/d; 6-12 room air changes/h	Temp--60°-83° F; hum--23%-90%; fluorescent light 12 h/d; 6-12 room air changes/h

shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Mice were shipped to the study laboratory at 5 weeks of age. The animals were quarantined at the study laboratory for 2 weeks. Thereafter, a complete necropsy was performed on five animals of each sex to assess their health status. The mice were placed on study at 7 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C).

Animal Maintenance

Animals were housed five per cage. Feed (Appendix D) and water were available ad libitum. Cages were rotated on the racks throughout the studies. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All animals were observed two times per day. Body weights were recorded once per week for the first 13 weeks of the study and at least once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, except for tissues that were excessively autolyzed or missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined are listed in Table 1.

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Toxicology Data Management System, the slides, paraffin blocks, and residual formalin-fixed tissues were sent to the NTP Archives. The slides, blocks, and residual wet tissues were audited for accuracy of labeling and animal identification and for thoroughness of tissue trimming. The slides, individual animal necropsy records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tissues with a tumor diagnosis and all tissues from a randomly selected 10% of the animals were re-evaluated microscopically by a quality assessment pathologist. Nonneoplastic lesions were evaluated for accuracy and consistency of diagnosis only in the potential target organs, in the randomly selected 10% of animals, and in tissues with unusual incidence patterns or trends. Tissues are generally not evaluated in a "blinded" fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle.

The quality assessment report and slides were submitted to a Pathology Working Group (PWG) Chairperson, who reviewed microscopically all potential target tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative examples of potential chemical-related nonneoplastic lesions of the nose and examples of disagreements in diagnosis between the laboratory and quality assessment pathologists were shown to the PWG. The PWG included the quality assessment pathologist and other pathologists experienced in rodent toxicology, who examined the tissues without knowledge of dose group or previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the opinion of the PWG. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final pathology data represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Statistical Methods

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead from other than natural causes; animals dying from natural causes were not 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) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: The majority of tumors in this study were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and vehicle control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart et al., 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance include pairwise comparisons of each dosed group with vehicle controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. (For further discussion of these statistical methods, see Haseman, 1984.)

III. RESULTS

MICE

SIXTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

GENETIC TOXICOLOGY

SIXTEEN-DAY STUDIES

All mice that received 1,590 or 3,500 mg/kg died on day 2 or 3 (Table 2). Mean necropsy body weights of dosed and vehicle control mice were similar. Ataxia, impaired grasping reflex, ocular discharge, corneal opacity, body tremors, prostration, gasping, clonic or tonic convulsions, or impaired righting reflex were observed at 1,590 and 3,500 mg/kg; lacrimation, piloerection, hypoactivity, bradypnea, or ptosis were observed at 723, 1,590, and 3,500 mg/kg. Relative liver weights were increased for dosed male mice; relative thymus weights were decreased for dosed female mice (Table 3). No compound-related histologic changes were seen.

TABLE 2. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SIXTEEN-DAY GAVAGE STUDIES OF *d*-CARVONE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	22.6 ± 0.9	23.6 ± 0.6	+1.0 ± 0.5	
150	5/5	21.2 ± 1.1	24.8 ± 1.0	+3.6 ± 1.2	105.1
328	5/5	22.8 ± 0.9	24.8 ± 1.1	+2.0 ± 0.4	105.1
723	5/5	23.4 ± 1.0	25.2 ± 0.9	+1.8 ± 0.2	106.8
1,590	(d) 0/5	22.8 ± 0.9	(e)	(e)	(e)
3,500	(f) 0/5	22.8 ± 0.6	(e)	(e)	(e)
FEMALE					
0	5/5	18.2 ± 0.7	19.2 ± 0.9	+1.0 ± 0.3	
150	5/5	18.6 ± 0.2	20.0 ± 0.3	+1.4 ± 0.2	104.2
328	5/5	18.4 ± 0.7	20.0 ± 0.7	+1.6 ± 0.2	104.2
723	5/5	19.0 ± 0.5	20.8 ± 0.4	+1.8 ± 0.2	108.3
1,590	(g) 0/5	18.6 ± 0.5	(e)	(e)	(e)
3,500	(h) 0/5	18.8 ± 0.4	(e)	(e)	(e)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean.
(c) Mean body weight change of the group ± standard error of the mean
(d) Day of death: all 3
(e) No data are reported due to 100% mortality in this group.
(f) Day of death: all 2
(g) Day of death: 2,2,2,3,3
(h) Day of death: 2,2,2,2,3

TABLE 3. ORGAN WEIGHT TO BODY WEIGHT RATIOS FOR MICE IN THE SIXTEEN-DAY GAVAGE STUDIES OF *d*-CARVONE (a)

Organ	Vehicle Control	150 mg/kg	328 mg/kg	723 mg/kg
MALE				
Body weight (grams)	23.6 ± 0.60	24.8 ± 0.97	24.8 ± 1.07	25.2 ± 0.86
Liver	54.5 ± 1.08	**62.7 ± 1.52	58.0 ± 2.02	**63.3 ± 1.81
Right kidney	(b) 9.3 ± 0.41	10.3 ± 0.39	9.9 ± 0.26	10.2 ± 0.25
Heart	5.4 ± 0.24	6.1 ± 0.11	5.8 ± 0.33	5.4 ± 0.22
Lungs/bronchi	7.1 ± 0.43	7.4 ± 0.19	7.6 ± 0.33	7.3 ± 0.42
Thymus	2.1 ± 0.19	(c) 2.0 ± 0.25	1.8 ± 0.15	1.8 ± 0.13
Brain	18.1 ± 0.18	17.4 ± 0.69	18.0 ± 0.60	17.3 ± 0.32
FEMALE				
Body weight (grams)	19.2 ± 0.86	20.0 ± 0.32	20.0 ± 0.71	20.8 ± 0.37
Liver	59.8 ± 0.55	61.6 ± 0.89	61.4 ± 0.62	61.1 ± 2.17
Right kidney	9.2 ± 0.39	9.2 ± 0.21	8.8 ± 0.40	9.2 ± 0.22
Heart	6.2 ± 0.35	6.1 ± 0.29	5.8 ± 0.38	6.4 ± 0.22
Lungs/bronchi	8.4 ± 0.16	9.5 ± 0.56	9.8 ± 0.55	8.9 ± 2.14
Thymus	3.0 ± 0.19	*2.5 ± 0.05	2.6 ± 0.17	**2.2 ± 0.08
Brain	22.6 ± 0.94	22.7 ± 0.21	21.8 ± 0.90	22.0 ± 0.38

(a) Mean ± standard error in milligrams per gram for groups of five animals, unless otherwise specified; P values vs. the vehicle controls by Dunnett's test (Dunnett, 1955).
(b) Four kidneys were weighed.
(c) Four thymuses were weighed.
*P<0.05
**P<0.01

THIRTEEN-WEEK STUDIES

All 30 male and 9/10 female mice that received 1,500 mg/kg and 1/10 males that received 93 mg/kg died before the end of the studies (Table 4). Final mean body weights of dosed and vehicle control male mice were similar. The final mean body weight of the female survivor at 1,500 mg/kg was 12% lower than that of vehicle controls. Hypoactivity, ataxia, and hypersensitivity to touch were seen at 1,500 mg/kg. The female that received 1,500 mg/kg and survived to the end of the study had body tremors, hypersensitivity to touch, and impaired hind limb function during the study. The relative liver weights for mice that received 750 mg/kg were significantly greater than those for vehicle controls (Table 5). No compound-related lesions were observed.

Dose Selection Rationale: Because 1,500 mg/kg was lethal to both male and female mice in the 13-week studies, doses of *d*-carvone selected for mice for the 2-year studies were 375 and 750 mg/kg, administered in corn oil by gavage, 5 days per week.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF *d*-CARVONE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	(d) 10/10	21.6 ± 0.5	27.4 ± 0.7	+5.8 ± 0.6	
93	(e) 9/10	22.5 ± 0.6	30.0 ± 0.7	+7.3 ± 0.5	109.5
187	10/10	20.3 ± 0.6	28.7 ± 0.6	+8.4 ± 0.8	104.7
375	(d) 10/10	23.6 ± 0.5	30.0 ± 0.8	+6.4 ± 0.6	109.5
750	10/10	21.4 ± 0.5	27.7 ± 0.7	+6.3 ± 0.7	101.1
1,500	(f) 0/30	22.6 ± 0.2	(g)	(g)	(g)
FEMALE					
0	10/10	18.5 ± 0.3	23.9 ± 0.6	+5.4 ± 0.5	
93	10/10	18.8 ± 0.6	25.0 ± 0.9	+6.2 ± 0.5	104.6
187	10/10	18.9 ± 0.4	24.5 ± 0.6	+5.6 ± 0.5	102.5
375	10/10	18.9 ± 0.4	24.0 ± 0.6	+5.1 ± 0.4	100.4
750	10/10	18.2 ± 0.4	23.6 ± 0.5	+5.4 ± 0.6	98.7
1,500	(h) 1/10	18.9 ± 0.4	21.0	+2.0	87.9

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) An additional 20 animals were originally included in this group to be used for other studies.
(e) An additional 20 animals were originally included in this group to be used for other studies; week of death: 1.
(f) Includes 20 animals originally designated for other studies; all deaths occurred during week 1.
(g) No data are reported due to 100% mortality in this group.
(h) Week of death: 1,1,1,1,1,1,1,1,12

TABLE 5. ORGAN WEIGHT TO BODY WEIGHT RATIOS FOR MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF *d*-CARVONE (a)

Organ	Vehicle Control	93 mg/kg	187 mg/kg	375 mg/kg	750 mg/kg
MALE					
Number weighed (b)	10	9	10	10	10
Body weight (grams)	27.5 ± 0.62	*30.1 ± 0.79	28.8 ± 0.55	*30.0 ± 0.77	26.5 ± 0.43
Liver	43.6 ± 0.46	47.2 ± 1.23	43.9 ± 1.11	45.8 ± 0.80	**50.0 ± 1.52
Right kidney	7.5 ± 0.26	8.0 ± 0.24	7.2 ± 0.19	8.2 ± 0.20	8.0 ± 0.20
Right testis	4.1 ± 0.14	3.7 ± 0.18	3.9 ± 0.15	3.9 ± 0.12	4.3 ± 0.10
Heart	5.3 ± 0.21	5.1 ± 0.10	5.0 ± 0.12	5.3 ± 0.16	5.0 ± 0.11
Lungs/bronchi	7.2 ± 0.27	6.9 ± 0.17	*6.4 ± 0.19	7.2 ± 0.13	7.5 ± 0.19
Thymus	1.2 ± 0.10	1.0 ± 0.07	1.1 ± 0.11	(c) 1.2 ± 0.06	1.3 ± 0.09
Brain	15.2 ± 0.36	*13.8 ± 0.20	14.5 ± 0.19	14.0 ± 0.50	15.1 ± 0.34
FEMALE					
Number weighed	10	10	10	10	10
Body weight (grams)	23.5 ± 0.43	24.4 ± 0.62	23.6 ± 0.43	24.1 ± 0.67	23.1 ± 0.43
Liver	44.5 ± 0.87	43.5 ± 0.90	43.6 ± 0.71	44.3 ± 0.79	*49.1 ± 1.80
Right kidney	6.7 ± 0.08	6.6 ± 0.19	6.5 ± 0.16	6.9 ± 0.18	6.7 ± 0.17
Heart	5.0 ± 0.08	5.0 ± 0.14	5.0 ± 0.13	5.0 ± 0.20	5.1 ± 0.24
Lungs/bronchi	7.8 ± 0.18	8.1 ± 0.12	8.4 ± 0.37	8.1 ± 0.29	8.1 ± 0.35
Thymus	1.6 ± 0.05	1.7 ± 0.10	1.6 ± 0.08	1.6 ± 0.13	1.6 ± 0.07
Brain	18.3 ± 0.34	18.4 ± 0.47	18.3 ± 0.41	18.2 ± 0.41	17.9 ± 0.44

(a) Mean ± standard error in milligrams per gram unless otherwise specified; P values vs. the vehicle controls by Dunnett's test (Dunnett, 1955).
(b) Unless otherwise specified
(c) Nine thymuses were weighed.
*P<0.05
**P<0.01

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed and vehicle control male mice were similar throughout most of the studies; mean body weights of dosed female mice were within 7% of those of vehicle controls throughout most of the studies (Table 6 and Figure 1). No compound-related clinical signs were observed.

TABLE 6. MEAN BODY WEIGHTS OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE

Week on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control Number Weighed	375 mg/kg Av. Wt. (grams)	375 mg/kg Wt. (percent of vehicle controls)	375 mg/kg Number Weighed	750 mg/kg Av. Wt. (grams)	750 mg/kg Wt. (percent of vehicle controls)	750 mg/kg Number Weighed
MALE								
1	20.2	50	20.4	101.0	50	20.1	99.5	50
2	22.8	50	22.9	100.4	50	22.7	99.6	47
3	22.9	50	23.0	100.4	50	23.0	100.4	47
4	24.8	50	24.7	99.6	50	24.6	99.2	47
5	25.9	50	25.3	97.7	50	25.2	97.3	47
6	26.0	50	25.4	97.7	50	25.7	98.8	47
7	27.3	50	26.3	96.3	50	26.0	95.2	47
8	27.8	50	27.0	97.1	50	26.5	95.3	47
9	26.4	50	25.7	97.3	50	25.4	96.2	47
10	28.2	50	27.7	98.2	50	26.6	94.3	47
11	28.6	50	27.9	97.6	50	27.5	96.2	47
12	29.1	50	28.8	99.0	50	27.9	95.9	47
13	30.1	50	29.2	97.0	50	27.9	92.7	47
17	32.0	50	30.5	95.3	50	29.6	92.5	47
21	32.8	50	31.7	96.6	50	31.0	94.5	46
25	33.7	50	32.7	97.0	50	32.3	95.8	45
29	33.1	49	33.1	100.0	50	32.2	97.3	45
33	35.0	49	34.0	97.1	49	33.9	96.9	45
37	34.7	49	34.8	100.3	49	34.1	98.3	45
41	35.3	49	35.7	101.1	49	34.3	97.2	45
45	35.3	48	35.7	101.1	49	34.5	97.7	45
49	34.8	47	35.0	100.6	48	35.0	100.6	44
54	35.2	47	35.3	100.3	48	33.7	95.7	44
57	34.4	47	35.0	101.7	48	34.1	99.1	44
61	35.5	47	35.8	100.8	47	35.0	98.6	43
65	35.2	47	36.1	102.6	47	34.8	98.9	43
69	35.2	46	35.8	101.7	47	35.2	100.0	42
73	35.2	45	35.1	99.7	47	35.0	99.4	41
77	34.8	45	36.0	103.4	46	35.4	101.7	41
81	35.5	45	36.4	102.5	45	35.6	100.3	41
85	35.0	43	36.9	105.4	45	35.6	101.7	40
89	34.6	42	37.2	107.5	45	35.5	102.6	39
91	33.2	41	36.2	109.0	45	34.6	104.2	39
93	35.1	41	36.9	105.1	45	35.5	101.1	39
95	35.0	41	37.3	106.6	44	35.6	101.7	39
97	34.1	40	37.0	108.5	43	35.7	104.7	39
99	34.8	38	37.4	107.5	43	36.4	104.6	39
101	34.5	37	36.1	104.6	43	35.1	101.7	38
103	34.2	37	36.1	105.6	42	34.4	100.6	36
Mean for weeks								
1-13	26.2		25.7	98		25.3	97	
17-49	34.1		33.7	99		33.0	97	
54-103	34.8		36.3	104		35.1	101	
FEMALE								
1	16.3	50	16.3	100.0	50	16.3	100.0	50
2	17.6	50	18.2	103.4	49	18.3	104.0	49
3	17.8	50	18.4	103.4	49	19.0	106.7	49
4	20.0	50	19.9	99.5	49	19.9	99.5	49
5	20.0	50	20.4	102.0	49	20.6	103.0	49
6	20.0	50	19.7	98.5	49	20.9	104.5	49
7	20.8	50	20.9	100.5	49	20.7	99.5	49
8	21.3	50	21.4	100.5	49	21.3	100.0	49
9	19.3	50	19.8	102.6	49	20.6	106.7	49
10	21.5	50	21.7	100.9	49	21.5	100.0	49
11	21.7	50	21.9	100.9	49	21.7	100.0	49
12	21.9	50	22.3	101.8	49	22.1	100.9	49
13	22.6	50	22.5	99.6	49	22.1	97.8	49
17	23.0	50	23.3	101.3	49	23.2	100.9	49
21	24.1	50	24.3	100.8	49	24.1	100.0	49
25	25.0	50	25.1	100.4	49	24.8	99.2	49
29	24.7	50	25.6	103.6	49	25.1	101.6	49
33	26.7	50	26.3	98.5	49	26.7	100.0	49
37	27.7	50	27.1	97.8	49	27.3	98.6	49
41	28.6	50	28.1	98.3	49	27.4	95.8	49
45	28.6	50	27.4	95.8	48	28.1	98.3	49
49	28.5	50	28.3	99.3	48	27.9	97.9	49
54	28.9	50	27.9	96.5	48	27.3	94.5	49
57	29.4	50	28.1	95.6	47	27.9	94.9	47
61	29.7	47	28.7	96.6	47	28.1	94.6	47
65	29.8	46	28.5	95.6	46	28.6	96.0	47
69	29.8	45	28.4	95.3	46	28.5	95.6	47
73	29.6	43	27.7	93.6	44	28.3	95.6	47
77	29.0	40	28.0	96.6	44	29.1	100.3	46
81	29.1	39	28.6	98.3	42	28.9	99.3	43
85	29.3	36	28.9	98.6	40	28.7	98.0	42
89	28.9	33	29.2	101.0	37	29.0	100.3	40
91	29.3	32	28.9	98.6	35	29.1	99.3	40
93	29.6	29	29.0	98.0	33	29.2	98.6	40
95	29.7	27	28.7	96.6	32	29.3	98.7	40
97	29.6	25	28.7	97.0	31	29.8	100.7	40
99	30.4	22	29.1	95.7	31	30.1	99.0	38
101	30.5	18	28.9	94.8	29	29.1	95.4	38
103	30.7	14	28.5	92.8	29	29.1	94.8	38
Mean for weeks								
1-13	20.1		20.3	101		20.4	101	
17-49	26.3		26.2	100		26.1	100	
54-103	29.6		28.6	97		28.8	97	

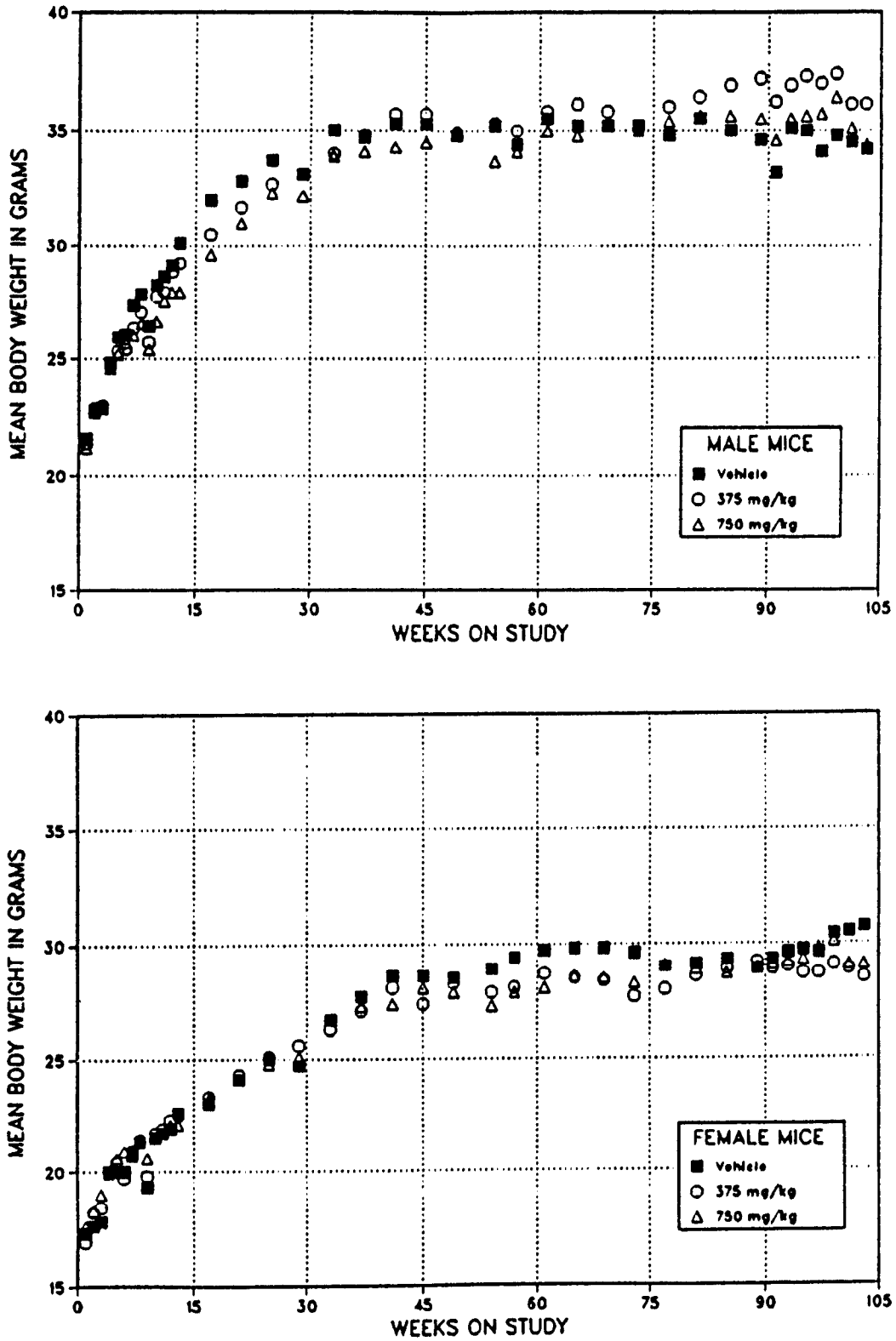


FIGURE 1. GROWTH CURVES FOR MICE ADMINISTERED *d*-CARVONE IN CORN OIL BY GAVAGE FOR TWO YEARS

III. RESULTS: MICE

Survival

Estimates of the probabilities of survival for male and female mice administered *d*-carvone at the doses used in these studies and for vehicle controls are shown in Table 7 and in the Kaplan and Meier curves in Figure 2. The survival of both the low dose (after week 101) and high dose (after week 92) groups of female mice was significantly greater than that of the vehicle controls. No significant differences were observed between any groups of male mice.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of mice with nonneoplastic lesions of the nasal cavity, subcutaneous tissue, circulatory system, and urogenital system. No increase in neoplastic lesions was observed in dosed mice.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group are presented in Appendixes A and B for male and female mice, respectively.

TABLE 7. SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	6	6	7
Moribund kills	7	2	3
Killed accidentally	0	0	4
Animals surviving until study termination	37	42	36
Mean survival (days)	679	694	631
Survival P values (b)	0.675	0.329	0.784
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	13	10	7
Moribund kills	23	10	4
Killed accidentally	0	2	1
Animals surviving until study termination	14	(c) 29	38
Mean survival (days)	639	652	676
Survival P values (b)	<0.001	0.006	<0.001

(a) First day of termination period: 729
(b) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.
(c) One animal died or was killed accidentally or in a moribund condition during the termination period and was combined for statistical purposes with those killed at the end of the study.

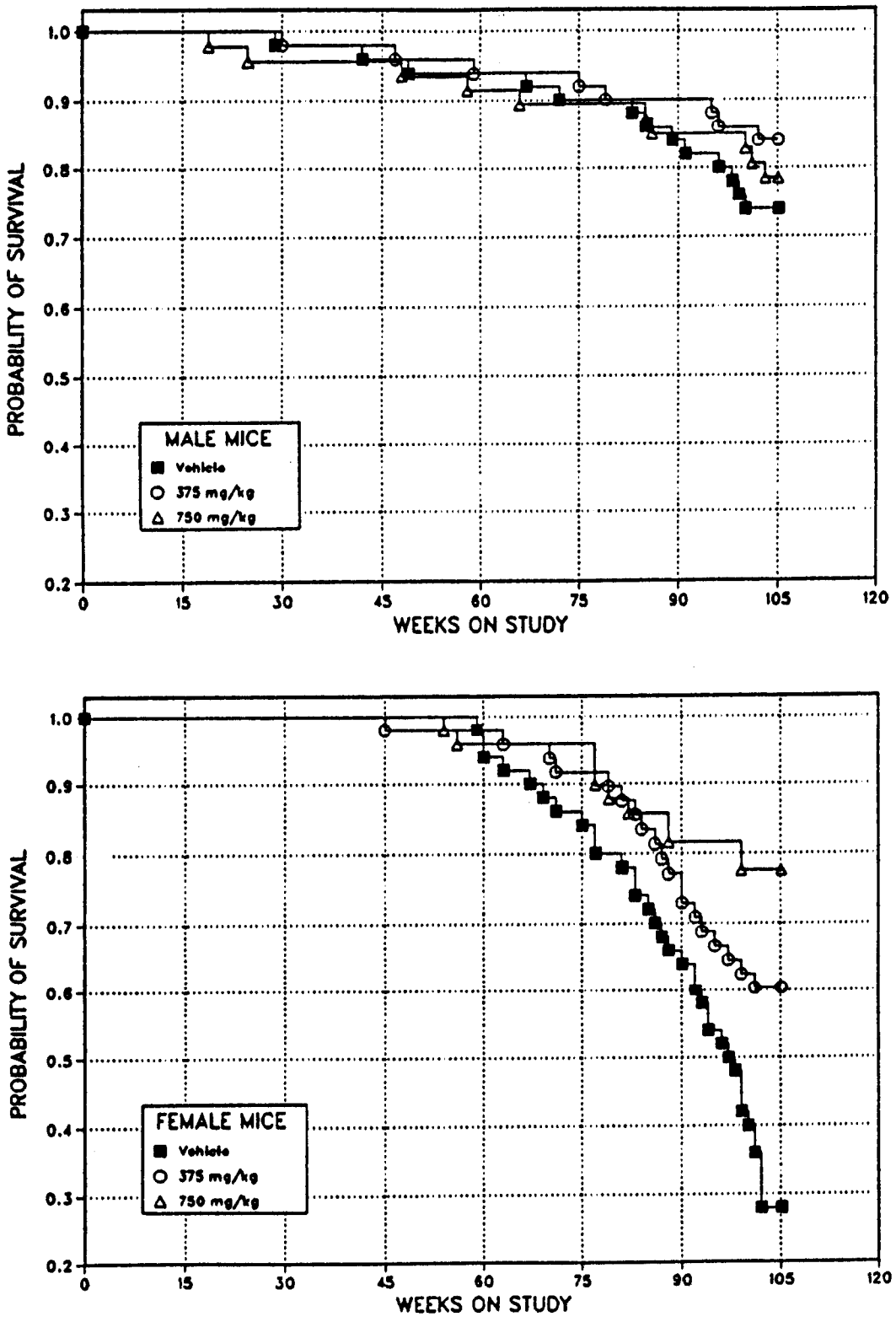


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED *d*-CARVONE IN CORN OIL BY GAVAGE FOR TWO YEARS

Nasal Cavity: Foreign material, presumably the corn oil vehicle, was observed in the nasal cavity of male and female mice in dosed and vehicle control groups. It consisted of accumulations of pale yellow, translucent, foamy, or vacuolated material that was sometimes surrounded by an inflammatory exudate of mucus and neutrophils. Several lesions occurred in male and female mice with dose-related increased incidences and/or severity. Atrophy of the olfactory epithelium and hyperplasia of the underlying Bowman's glands occurred together (Table 8). These lesions usually involved the mucosa along the dorsal meatus in the posterior region of the nose but extended to the septum and turbinates in the more severely affected animals. The olfactory epithelium was reduced in thickness because of the loss of the olfactory sensory epithelium and replacement by ciliated columnar cells. The Bowman's glands were dilated and consisted of tall, columnar cells similar to those replacing the sensory epithelium on the surface. Acute, multifocal inflammation was characterized by accumulations of neutrophils and cellular debris, primarily in the lumina of the Bowman's glands of the turbinates. Since evidence of the corn oil vehicle was seen in over 50% of the animals in all dosed and vehicle control groups, the Pathology Working Group felt that the lesions observed in the nasal mucosa were likely due to reflux of the gavage material into the nose after the gavage needle was withdrawn.

Subcutaneous Tissue: Fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) were observed with a negative trend in male mice, and the reduced incidence was significant in low dose male mice (vehicle control, 9/50; low dose, 1/50; high dose, 3/50).

Circulatory System: Three hemangiomas or hemangiosarcomas were observed in vehicle control male mice, but none was seen in dosed males; the difference was not significant.

Urogenital System: Abscesses of the ovary and the uterus occurred at a high incidence in vehicle control female mice and at much lower incidences in dosed female mice (ovary: vehicle control, 26/50; low dose, 9/48; high dose, 1/48; uterus: 10/50; 3/50; 0/50). The lesions were similar to those observed in other studies associated with Krebsiella sp. infections and are believed to be the cause of reduced survival in vehicle control female mice relative to that of dosed female mice.

TABLE 8. NUMBERS OF MICE WITH LESIONS OF THE NASAL CAVITY IN THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE

Site/Lesion	Male			Female		
	Vehicle Control	375 mg/kg	750 mg/kg	Vehicle Control	375 mg/kg	750 mg/kg
Number examined	50	50	49	49	49	50
Glands						
Hyperplasia	3	**42	**44	19	**45	**49
Olfactory epithelium						
Atrophy	11	**42	**44	25	**46	**49
Turbinate						
Multifocal acute inflammation	0	3	**27	5	**22	**39

**P<0.01 vs. vehicle controls

d-Carvone, at concentrations up to 333 µg/plate, was negative for induction of gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Mortelmans et al., 1986; Table F1). In cytogenetic tests with Chinese hamster ovary (CHO) cells, *d*-carvone induced both sister chromatid exchanges (Table F2) and chromosomal aberrations (Table F3) with and without Aroclor 1254-induced male Sprague Dawley rat liver S9. Although results were statistically positive in two of the three sister chromatid exchange (SCE) trials, there was no correlation of dose with response; this same phenomenon also occurred in the second trial of the chromosomal aberration test conducted without S9. No slowing of the cell cycle was noted in the CHO cells used for the SCE test, but in the chromosomal aberration test, the cells used in the trials conducted without S9 required delayed harvest to offset chemical-induced cell cycle delay. The methodology and full results are presented in Appendix F.

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

In the 13-week studies in mice, *d*-carvone was toxic at 1,500 mg/kg; at 750 mg/kg, it did not affect body weight, survival, or histopathology, but relative liver weights were increased.

Throughout the 2-year studies in mice, body weights of male and female mice administered 375 or 750 mg/kg *d*-carvone were similar to those of vehicle controls. Survival of dosed male mice was similar to that of vehicle controls; survival of dosed female mice was greater than that of vehicle controls. The low survival of female vehicle control mice was related to a high incidence (52%) of ovarian abscesses, a life-shortening condition (Rao et al., 1987). It is uncertain whether the reduced incidences of ovarian abscesses in dosed female mice were directly related to *d*-carvone administration.

No increases in tumor incidences were observed in mice administered *d*-carvone. In the current study, only nine primary neoplasms were seen in female vehicle control mice, each in a different animal. This low number may be related to the early deaths of female vehicle control mice. However, the incidences of male mice with primary neoplasms (vehicle control, 27/50; low dose, 15/50; high dose, 16/50; Table A3) and the total numbers of primary neoplasms (vehicle control, 38; low dose, 18; high dose, 20; Table A1) were significantly lower in dosed groups than in vehicle controls. It is not known if the low tumor yields are related to *d*-carvone administration.

Despite the absence of clearly chemically related toxic effects in the 2-year studies, the doses used were considered adequate for assessment of potential carcinogenicity because higher doses caused deaths in the 13-week studies.

The only lesions considered possibly related to *d*-carvone in the 2-year studies in mice were atrophy of the olfactory epithelium and hyperplasia of the underlying Bowman's glands. These may have been related to reflux of *d*-carvone into the nose after the gavage needle was withdrawn, because inflammatory exudate and "foreign material" were often found in the nasal passage of dosed animals.

The experimental and tabulated data for the NTP Technical Report on *d*-carvone were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix G, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of *d*-carvone for male or female B6C3F$_1$ mice administered *d*-carvone at 375 or 750 mg/kg, 5 days per week for 2 years.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 5.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 8-9.

V. REFERENCES

V. REFERENCES

1. Ames, B.N.; McCann, J.; Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

2. Andersen, K.E. (1978) Contact allergy to toothpaste flavors. Contact Dermatitis 4:195-198.

3. Armitage, P. (1971) Statistical Methods in Medical Research. New York: John Wiley & Sons, Inc., pp. 362-365.

4. Boorman, G.A.; Montgomery, C.A., Jr.; Eustis, S.L.; Wolfe, M.J.; McConnell, E.E.; Hardisty, J.F. (1985) Quality assurance in pathology for rodent carcinogenicity studies. Milman, H.; Weisburger, E., Eds.: Handbook of Carcinogen Testing. Park Ridge, NJ: Noyes Publications, pp. 345-357.

5. The Condensed Chemical Dictionary (1981) 10th ed. Hawley, G.G., Ed. New York: Van Nostrand Reinhold Company Inc., pp. 202-203.

6. Council of Europe (1974) Natural Flavouring Substances. Their Sources and Added Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. List 1, No. 146, p. 155.

7. Cox, D.R. (1972) Regression models and life tables. J. R. Stat. Soc. B34:187-220.

8. Dinse, G.E.; Haseman, J.K. (1986) Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. Fundam. Appl. Toxicol. 6:44-52.

9. Dinse, G.E.; Lagakos, S.W. (1983) Regression analysis of tumour prevalence data. J. R. Stat. Soc. C32:236-248.

10. Dunnett, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50:1096-1122.

11. Federal Register (Fed. Regist.) (1961) Food and Drugs; Food and Drug Administration, Department of Health, Education, and Welfare; food and food products; food additives; exemption of certain food additives from the requirement of tolerances; substances that are generally recognized as safe. 26:3991.

12. Fenaroli's Handbook of Flavor Ingredients (1975) 2nd ed., Vol. II. Furia, T.E.; Bellanca, N., Eds. Cleveland: CRC Press, Inc., p. 87.

13. Fischer, F.G.; Bielig, H.J. (1940) cited in Opdyke, D.L.J. (1973) *l*-Carvone. Food Cosmet. Toxicol. 11:1057-1058.

14. Flavoring Extract Manufacturers' Association (FEMA) (1965) Survey of flavoring ingredient usage levels. No. 2249. Food Technol. Champagne 19:155.

15. Florin, I.; Rutberg, L.; Curvall, M.; Enzell, C.R. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 15:219-232.

16. Food and Agriculture Organization/World Health Organization (FAO/WHO) (1967) Toxicological Evaluation of Some Flavouring Substances and Non-Nutritive Sweetening Agents. Report of the Joint FAO/WHO Expert Committee on Food Additives. FAO Ntr. Mtg. Rep. Ser. 44A. WHO/Food Add./68/33, p. 14.

17. Food Chemicals Codex (FCC) (1966) 1st ed. Washington, DC: National Academy of Sciences, pp. 741-742.

18. Food Chemicals Codex (FCC) (1972) 2nd ed. Washington, DC: Committee on Codex Specifications, National Academy of Sciences, pp. 176-177.

19. Food Chemicals Codex (FCC) (1981) 3rd ed. Washington, DC: Committee on Codex Specifications, National Academy of Sciences, pp. 362-363.

20. Fuhremann, T.W.; Lichtenstein, E.P. (1979) Insecticide toxicity and degradation in houseflies as affected by naturally occurring food plant components. J. Agric. Food Chem. 27:87-91.

21. Fuhremann, T.W.; Lichtenstein, E.P.; Stratman, F.W. (1978) Effects of naturally occurring food plant components on insecticide degradation in rats. J. Agric. Food Chem. 26:1068-1075.

22. Galloway, S.M.; Bloom, A.D.; Resnick, M.; Margolin, B.H.; Nakamura, F.; Archer, P.; Zeiger, E. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7:1-51.

23. Gart, J.J.; Chu, K.C.; Tarone, R.E. (1979) Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62:957-974.

24. Hagan, E.C.; Hansen, W.H.; Fitzhugh, O.G.; Jenner, P.M.; Jones, W.I.; Taylor, J.M.; Long, E.L.; Nelson, A.A.; Brouwer, J.B. (1967) Food flavorings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet. Toxicol. 5:141-157.

25. Hamalainen, J. (1912) cited in Williams, R.T. (1959) Detoxication Mechanisms, 2nd ed. New York: John Wiley & Sons, Inc., p. 527.

26. Haseman, J.K. (1984) Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58:385-392.

27. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. Suppl. 1:3-142.

28. Hildebrandt, H. (1902) cited in Williams, R.T. (1959) Detoxication Mechanisms, 2nd ed. New York: John Wiley & Sons, Inc., pp. 524-525.

29. Jenner, P.M.; Hagen, E.C.; Taylor, J.M.; Cook, E.L.; Fitzhugh, O.G. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2:327-343.

30. Kaplan, E.L.; Meier, P. (1958) Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53:457-481.

31. Kirk-Othmer Encyclopedia of Chemical Technology (1981) 3rd ed., Vol. 22. New York: John Wiley & Sons, Inc., p. 745.

32. Kligman, A.M. (1976) Report to RIFM, 9 April. Cited in Opdyke, D.L.J. (1978) *d*-Carvone. Food Cosmet. Toxicol. 16:673-674.

33. Levenstein, I. (1976) Report to RIFM, 18 August. Cited in Opdyke, D.L.J. (1978) *d*-Carvone. Food Cosmet. Toxicol. 16:673-674.

34. Lichtenstein, E.P.; Liang, T.T.; Schulz, K.R.; Schnoes, H.K.; Carter, G.T. (1974) Insecticidal and synergistic components isolated from dill plants. J. Agric. Food Chem. 22:658-664.

35. Longenecker, H.E.; Musulin, R.R.; Tully, R.H., III; King, C.G. (1939) An acceleration of vitamin C synthesis and excretion by feeding known organic compounds to rats. J. Biol. Chem. 129:445-453.

36. Marcus, C.; Lichtenstein, E.P. (1982) Interactions of naturally occurring food plant components with insecticides and pentobarbital in rats and mice. J. Agric. Food Chem. 30:563-568.

37. Maronpot, R.R.; Boorman, G.A. (1982) Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol. Pathol. 10:71-80.

38. McConnell, E.E.; Solleveld, H.A.; Swenberg, J.A.; Boorman, G.A. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76:283-289.

39. McKnight, B.; Crowley, J. (1984) Tests for differences in tumor incidence based on animal carcinogenesis experiments. J. Am. Stat. Assoc. 79:639-648.

40. The Merck Index (1968) 8th ed. Rahway, NJ: Merck & Co., Inc., p. 215.

41. The Merck Index (1983) 10th ed. Rahway, NJ: Merck & Co., Inc., p. 1856.

42. Mortelmans, K.; Haworth, S.; Lawlor, T.; Speck, W.; Tainer, B.; Zeiger, E. (1986) Salmonella mutagenicity tests. II. Results from the testing of 270 chemicals. Environ. Mutagen. 8(Suppl. 7):1-119.

V. REFERENCES

43. National Cancer Institute (NCI) (1976) Guidelines for Carcinogen Bioassay in Small Rodents. NCI Technical Report No. 1. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 65 p.

44. National Cancer Institute Executive Summary (NCI) (1979)

45. National Institutes of Health (NIH) (1978) Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

46. Opdyke, D.L.J. (1978) *d*-Carvone. Food Cosmet. Toxicol. 16:673-674.

47. Rao, G.N.; Hickman, R.L.; Seilkop, S.K.; Boorman, G.A. (1987) Utero-ovarian infection in aged B6C3F1 mice. Lab. Animal Sci. 37:153-158.

48. Ritz, N.D.; Samuels, L.T.; Addiss, G. (1940) The effect of salicylates and carvone on the ascorbic acid content of animal tissues. J. Pharmacol. Exp. Therap. 70:352-369.

49. Rockwell, P.; Raw, I. (1979) Mutagenic screening of various herbs, spices and food additives. Nutr. Cancer 1:10-15.

50. Sadtler Standard Spectra. IR No. 6626; UV No. 18291; NMR No. 10954M. Philadelphia: Sadtler Research Laboratories.

51. Shackelford, W.M.; Keith, L.H. (1976) Frequency of organic compounds indentified in water. U.S. Environmental Protection Agency, Athens, GA, p. 98.

52. SRI International (1977) 1977 Directory of Chemical Producers--USA Chemical Information Services. Menlo Park, CA: SRI International, p. 922.

53. Stoner, G.D.; Shimkin, M.B.; Kniazeff, A.J.; Weisburger, J.H.; Weisburger, E.K.; Gori, G.B. (1973) Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. 33:3069-3085.

54. Tarone, R.E. (1975) Tests for trend in life table analysis. Biometrika 62:679-682.

55. Zlatkis, A.; Bertsch, W.; Lichtenstein, H.A.; Tishbee, A.; Shunbo, F.; Liebich, H.M.; Coscia, A.M.; Fleischer, N. (1973) Profiles of volatile metabolites in urine by gas chromatography-mass spectrometry. Anal. Chem. 45:763-767.

APPENDIX A

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Esophagus	(50)	(50)	(49)
Periesophageal tissue, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Gallbladder	(38)	(36)	(43)
Carcinoma, metastatic, uncertain primary site			1 (2%)
Intestine large, cecum	(50)	(46)	(49)
Lymphoma malignant mixed			1 (2%)
Intestine small, ileum	(49)	(49)	(48)
Lymphoma malignant mixed			1 (2%)
Intestine small, jejunum	(48)	(48)	(48)
Peyer's patch, lymphoma malignant mixed		1 (2%)	
Liver	(50)	(50)	(49)
Hepatocellular carcinoma	5 (10%)	3 (6%)	3 (6%)
Hepatocellular adenoma	2 (4%)	4 (8%)	4 (8%)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	2 (4%)		1 (2%)
Pancreas	(50)	(50)	(48)
Carcinoma, metastatic, uncertain primary site			1 (2%)
Sarcoma, metastatic, lymph node	1 (2%)		
Salivary glands	(50)	(50)	(50)
Submandibular gland, lymphoma malignant lymphocytic	1 (2%)		
Stomach, forestomach	(48)	(48)	(47)
Papilloma squamous	1 (2%)	1 (2%)	
Stomach, glandular	(49)	(49)	(48)
Carcinoma, metastatic, uncertain primary site			1 (2%)
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland, medulla	(48)	(49)	(48)
Pheochromocytoma benign	1 (2%)		
Bilateral, lymphoma malignant lymphocytic	1 (2%)		
Bilateral, pheochromocytoma benign	1 (2%)		
Pituitary gland	(40)	(48)	(48)
Lymphoma malignant lymphocytic	1 (3%)		
Thyroid gland	(50)	(50)	(49)
Follicular cell, adenoma	1 (2%)		1 (2%)
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Epididymis	(50)	(50)	(50)
Lymphoma malignant lymphocytic	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(50)	(49)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	1 (2%)		
Lymph node	(50)	(50)	(49)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Renal, lymphoma malignant mixed			1 (2%)
Lymph node, mesenteric	(50)	(50)	(48)
Hemangioma	1 (2%)		
Lymphoma malignant mixed	1 (2%)	1 (2%)	1 (2%)
Sarcoma	1 (2%)		
Mandibular, lymphoma malignant histiocytic		1 (2%)	
Mandibular, lymphoma malignant lymphocytic	1 (2%)		
Spleen	(50)	(50)	(48)
Hemangiosarcoma	1 (2%)		
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	2 (4%)	1 (2%)	1 (2%)
Thymus	(44)	(42)	(44)
Lymphoma malignant mixed	1 (2%)		
INTEGUMENTARY SYSTEM			
Skin	*(50)	*(50)	*(50)
Back, subcutaneous tissue, fibroma	2 (4%)		1 (2%)
Prepuce, squamous cell carcinoma	1 (2%)		
Subcutaneous tissue, fibrosarcoma	1 (2%)		
Subcutaneous tissue, hemangioma	1 (2%)		
Subcutaneous tissue, neurofibrosarcoma	2 (4%)	1 (2%)	
Subcutaneous tissue, sarcoma	4 (8%)		2 (4%)
MUSCULOSKELETAL SYSTEM			
Skeletal muscle	*(50)	*(50)	*(50)
Fibrosarcoma, metastatic, skin	1 (2%)		
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Meninges, lymphoma malignant lymphocytic	1 (2%)		
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	4 (8%)	5 (10%)
Alveolar/bronchiolar carcinoma		1 (2%)	1 (2%)
Carcinoma, metastatic, uncertain primary site			1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	1 (2%)		
Nose	(50)	(50)	(49)
Lymphoma malignant lymphocytic	1 (2%)		
Trachea	(50)	(50)	(49)
Lymphoma malignant lymphocytic	1 (2%)		
SPECIAL SENSES SYSTEM			
Harderian gland	*(50)	*(50)	*(50)
Adenoma	1 (2%)	2 (4%)	1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
URINARY SYSTEM			
Kidney	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	1 (2%)		
Glomerulus, carcinoma, metastatic, uncertain primary site			1 (2%)
Renal tubule, adenoma			1 (2%)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Hemangioma	2 (4%)		
Lymphoma malignant mixed	4 (8%)	1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)		
Hemangiosarcoma	1 (2%)		
Lymphoma malignant histiocytic		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Dead	6	6	7
Terminal sacrifice	37	42	36
Moribund	7	2	3
Accident			4
TUMOR SUMMARY			
Total animals with primary neoplasms **	27	15	16
Total primary neoplasms	38	18	20
Total animals with benign neoplasms	17	10	10
Total benign neoplasms	18	11	13
Total animals with malignant neoplasms	17	7	7
Total malignant neoplasms	20	7	7
Total animals with secondary neoplasms ***	2		2
Total secondary neoplasms	2		10
Total animals with malignant neoplasms--uncertain primary site			1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE: VEHICLE CONTROL

WEEKS ON STUDY	029	042	049	067	072	083	085	089	091	096	098	099	100	105	115	105	105	105	105	105	105	105	105	105	105
CARCASS ID	081	091	092	082	052	101	094	021	111	144	255	155	244	233	232	411	555	611	711	721	755	844	893	955	102
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	+	M	+	+	M	M	+	A	+	+	+	+	+	+	M	+	+	+	M	M	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																X		X						X	
Hepatocellular adenoma																									
Lymphoma malignant lymphocytic					X																				
Lymphoma malignant mixed													X			X									
Mesentery		+																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, lymph node																							X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Submandibular gland, lymphoma malignant lymphocytic					X																				
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Bilateral, lymphoma malignant lymphocytic					X																				
Bilateral, pheochromocytoma benign																								X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	M	+	+	M	+	M	M	M	M	M	+	M	M	+	+	M	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M
Lymphoma malignant lymphocytic					X																				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic					X																				
Penis							+																		
Preputial gland																			+			+	+	+	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Seminal vesicle		+																							
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: VEHICLE CONTROL (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	105	013	022	031	034	035	042	043	045	051	054	083	103	012	033	044	053	062	063	064	065	073	074	085	104	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	38
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma											X							X								5
Hepatocellular adenoma									X			X														2
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										2
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, lymph node																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Submandibular gland, lymphoma malignant lymphocytic																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Papilloma squamous										X																1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARDIOVASCULAR SYSTEM																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign																						X				1
Bilateral, lymphoma malignant lymphocytic																										1
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	+	+	+	M	+	M	+	+	+	M	+	+	M	+	+	M	+	M	M	+	M	+	M	M	27
Pituitary gland	M	+	+	M	+	+	+	+	+	M	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	40
Lymphoma malignant lymphocytic																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																								X		1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Penis																										1
Preputial gland								+		+			M			+			+			+		+		10
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: VEHICLE CONTROL (Continued)

WEEKS ON STUDY	029	042	049	067	072	083	085	089	091	096	098	099	100	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	081	091	092	082	052	101	094	021	111	114	225	155	024	233	032	041	055	061	071	072	075	084	093	095	102
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic					X																				
Lymphoma malignant mixed													X												
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Lymphoma malignant mixed																					X				
Sarcoma																							X		
Mandibular, lymphoma malignant lymphocytic					X																				
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Lymphoma malignant lymphocytic					X																				
Lymphoma malignant mixed													X												
Thymus	+	+	+	+	+	+	+	+	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed													X												
INTEGUMENTARY SYSTEM																									
Mammary gland	M	+	+	+	M	M	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M
Skin	+		+		+		+		+	+	+	+	+	+	+					+		+	+	+	
Back, subcutaneous tissue, fibroma																						X			
Prepuce, squamous cell carcinoma																									
Subcutaneous tissue, fibrosarcoma							X																		
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, neurofibrosarcoma													X												
Subcutaneous tissue, sarcoma									X	X	X														
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle							+																		
Fibrosarcoma, metastatic, skin							X																		
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meninges, lymphoma malignant lymphocytic					X																				
Peripheral nerve																			+	+					+
Spinal cord																									
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X						X				X								X		X		
Lymphoma malignant lymphocytic					X																				
Lymphoma malignant mixed													X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic					X																				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic					X																				
SPECIAL SENSES SYSTEM																									
Harderian gland																						+			
Adenoma																						X			
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic					X																				
Lymphoma malignant mixed													X												
Ureter															+										
Urethra			+																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: VEHICLE CONTROL (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	105	013	022	031	034	035	042	043	045	051	054	083	103	012	033	044	053	062	063	064	065	073	074	085	104	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma	X																									1
Lymphoma malignant mixed																										1
Sarcoma																										1
Mandibular, lymphoma malignant lymphocytic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed												X														2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	44
Lymphoma malignant mixed																										1
INTEGUMENTARY SYSTEM																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	5
Skin		+		+	+					+		+	+	+	+		+			+				+	26
Back, subcutaneous tissue, fibroma				X																						2
Prepuce, squamous cell carcinoma												X														1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangioma		X																								1
Subcutaneous tissue, neurofibrosarcoma		X																								2
Subcutaneous tissue, sarcoma					X																					4
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Fibrosarcoma, metastatic, skin																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meninges, lymphoma malignant lymphocytic																										1
Peripheral nerve			+	+														+		+	+	+			+	10
Spinal cord																	+									1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma															X		X									7
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
SPECIAL SENSES SYSTEM																										
Harderian gland																										1
Adenoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Ureter																										1
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE: 375 mg/kg

WEEKS ON STUDY	030	047	059	075	079	095	096	102	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	221	225	211	271	241	284	295	275	215	232	234	235	242	255	272	281	282	283	291	293	303	305	213	214	222
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	A	+	M	+	A	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	M	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	M	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine small	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peyer's patch, lymphoma malignant mixed								X																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																						X			
Hepatocellular adenoma									X														X		
Lymphoma malignant histiocytic					X																				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																					X				
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	M	+	+	+	M	+	M	M	+	M	+	M	M	M	+	+	M	+	+	+	M	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland								+					+												
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed								X																	
Mandibular, lymphoma malignant histiocytic					X																				
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed								X																	
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+			+				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, neurofibrosarcoma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X				
Alveolar/bronchiolar carcinoma																									
Lymphoma malignant histiocytic					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland				+																			+		
Adenoma				X																			X		
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 375 mg/kg (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	231	243	245	253	261	262	264	265	274	292	301	212	223	224	233	244	251	252	254	263	273	285	294	302	304	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	M	+	+	+	+	M	M	+	M	+	+	+	+	+	+	M	M	36
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Peyer's patch, lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma															X								X			3
Hepatocellular adenoma							X							X												4
Lymphoma malignant histiocytic																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARDIOVASCULAR SYSTEM																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid gland	+	M	+	M	M	+	M	M	+	+	M	+	+	M	+	M	+	+	+	+	M	+	M	+	+	27
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland											+									+						4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																										1
Mandibular, lymphoma malignant histiocytic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																										1
Thymus	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	M	+	+	+	42
INTEGUMENTARY SYSTEM																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Subcutaneous tissue, neurofibrosarcoma						X																				1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma															X				X		X					4
Alveolar/bronchiolar carcinoma											X															1
Lymphoma malignant histiocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland																										2
Adenoma																										2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE: 750 mg/kg

WEEKS ON STUDY	0 0 1	0 0 1	0 0 1	0 1 9	0 2 5	0 4 8	0 5 8	0 6 6	0 7 2	0 8 5	0 8 6	1 0 0	1 0 1	1 0 3	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5
CARCASS ID	4 4 1	4 6 1	4 7 1	4 1 5	4 4 2	4 2 1	4 8 1	4 2 2	4 3 1	4 2 3	5 0 1	4 9 1	4 7 3	4 3 5	4 2 4	4 2 5	4 3 2	4 4 4	4 5 3	4 5 4	4 7 4	4 8 2	4 9 2	4 9 5	5 0 2
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Periesophageal tissue, alveolar/bronchiolar carcinoma, metastatic, lung													X												
Gallbladder	A	+	M	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uncertain primary site																									
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed												X													
Intestine large, colon	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	M	+	+	+	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed												X													
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																X									
Hepatocellular adenoma									X																
Lymphoma malignant mixed												X													
Pancreas	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uncertain primary site																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	A	+	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uncertain primary site																									
CARDIOVASCULAR SYSTEM																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung													X												
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	M	+	M	+	M	M	M	M	+	+	M	M	+	+	M	+	+	+	+	M	+	M	
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 750 mg/kg (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	5044	4122	4144	4333	4443	4552	4555	4633	4644	4755	4883	4933	5055	4111	4113	4334	4455	4511	4622	4655	4722	4844	4855	4944	5033	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Periesophageal tissue, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Gallbladder	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	43
Carcinoma, metastatic, uncertain primary site		X																								1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant mixed																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	M	+	+	+	M	+	+	M	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma		X																		X						3
Hepatocellular adenoma														X	X							X				4
Lymphoma malignant mixed																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, uncertain primary site		X																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, uncertain primary site		X																								1
CARDIOVASCULAR SYSTEM																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung																										1
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	M	+	M	+	+	+	M	M	M	+	+	M	M	M	+	M	+	M	+	+	+	+	+	26
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma						X																				1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+			+													+									3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 750 mg/kg (Continued)

WEEKS ON STUDY	001	001	001	019	025	048	058	066	072	085	086	100	101	103	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	441	461	471	415	442	421	481	422	431	423	501	491	473	435	424	425	432	444	453	454	474	482	492	495	502
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung													X												
Renal, lymphoma malignant mixed												X													
Lymph node, mesenteric	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Lymphoma malignant mixed												X													
Spleen	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed												X													
Thymus	+	+	+	+	M	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	M	M	+	I	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin						+	+			+	+			+		+						+			
Back, subcutaneous tissue, fibroma																									
Subcutaneous tissue, sarcoma										X															
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+																						
RESPIRATORY SYSTEM																									
Larynx						+																			
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X				X				
Alveolar/bronchiolar carcinoma													X												
Carcinoma, metastatic, uncertain primary site																									
Hepatocellular carcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland																	+								
Adenoma																	X								
URINARY SYSTEM																									
Kidney	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung													X												
Glomerulus, carcinoma, metastatic, uncertain primary site																									
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 750 mg/kg
(Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
CARCASS ID	504	412	414	433	443	452	455	463	464	475	483	493	505	411	413	434	445	451	462	465	472	484	485	494	503	TOTAL: TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Renal, lymphoma malignant mixed																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	44
INTEGUMENTARY SYSTEM																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	6
Skin					+			+			+						+		+	+		+	+			15
Back, subcutaneous tissue, fibroma																						X				1
Subcutaneous tissue, sarcoma											X															2
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord			+																							4
RESPIRATORY SYSTEM																										
Larynx																										1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X												X									X			5
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, uncertain primary site		X																								1
Hepatocellular carcinoma, metastatic, liver		X																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSES SYSTEM																										
Harderian gland																										1
Adenoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Glomerulus, carcinoma, metastatic, uncertain primary site		X																								1
Renal tubule, adenoma																X										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
Liver: Hepatocellular Adenoma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	4/49 (8%)
Adjusted Rates (b)	5.4%	9.5%	10.5%
Terminal Rates (c)	2/37 (5%)	4/42 (10%)	3/36 (8%)
Day of First Observation	729	729	498
Life Table Tests (d)	P=0.255	P=0.397	P=0.321
Logistic Regression Tests (d)	P=0.244	P=0.397	P=0.311
Cochran-Armitage Trend Test (d)	P=0.265		
Fisher Exact Test (d)		P=0.339	P=0.329
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	5/50 (10%)	3/50 (6%)	3/49 (6%)
Adjusted Rates (b)	13.5%	7.1%	8.3%
Terminal Rates (c)	5/37 (14%)	3/42 (7%)	3/36 (8%)
Day of First Observation	729	729	729
Life Table Tests (d)	P=0.289N	P=0.288N	P=0.370N
Logistic Regression Tests (d)	P=0.289N	P=0.288N	P=0.370N
Cochran-Armitage Trend Test (d)	P=0.292N		
Fisher Exact Test (d)		P=0.357N	P=0.369N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	7/50 (14%)	7/50 (14%)	7/49 (14%)
Adjusted Rates (b)	18.9%	16.7%	18.7%
Terminal Rates (c)	7/37 (19%)	7/42 (17%)	6/36 (17%)
Day of First Observation	729	729	498
Life Table Tests (d)	P=0.535	P=0.513N	P=0.589
Logistic Regression Tests (d)	P=0.511	P=0.513N	P=0.565
Cochran-Armitage Trend Test (d)	P=0.541		
Fisher Exact Test (d)		P=0.613N	P=0.597
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	7/50 (14%)	4/50 (8%)	5/50 (10%)
Adjusted Rates (b)	17.0%	9.5%	13.9%
Terminal Rates (c)	4/37 (11%)	4/42 (10%)	5/36 (14%)
Day of First Observation	337	729	729
Life Table Tests (d)	P=0.331N	P=0.208N	P=0.405N
Logistic Regression Tests (d)	P=0.342N	P=0.264N	P=0.410N
Cochran-Armitage Trend Test (d)	P=0.314N		
Fisher Exact Test (d)		P=0.262N	P=0.380N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	7/50 (14%)	5/50 (10%)	6/50 (12%)
Adjusted Rates (b)	17.0%	11.9%	16.2%
Terminal Rates (c)	4/37 (11%)	5/42 (12%)	5/36 (14%)
Day of First Observation	337	729	707
Life Table Tests (d)	P=0.459N	P=0.308N	P=0.522N
Logistic Regression Tests (d)	P=0.476N	P=0.379N	P=0.536N
Cochran-Armitage Trend Test (d)	P=0.439N		
Fisher Exact Test (d)		P=0.380N	P=0.500N
Subcutaneous Tissue: Sarcoma			
Overall Rates (e)	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted Rates (b)	9.7%	0.0%	5.1%
Terminal Rates (c)	1/37 (3%)	0/42 (0%)	1/36 (3%)
Day of First Observation	637		592
Life Table Tests (d)	P=0.236N	P=0.057N	P=0.362N
Logistic Regression Tests (d)	P=0.223N	P=0.063N	P=0.349N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Test (d)		P=0.059N	P=0.339N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
Subcutaneous Tissue: Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (e)	7/50 (14%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	16.4%	2.4%	5.1%
Terminal Rates (c)	2/37 (5%)	1/42 (2%)	1/36 (3%)
Day of First Observation	592	729	592
Life Table Tests (d)	P=0.041N	P=0.028N	P=0.100N
Logistic Regression Tests (d)	P=0.037N	P=0.033N	P=0.086N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Test (d)		P=0.030N	P=0.080N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (e)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	7.6%	0.0%	2.8%
Terminal Rates (c)	2/37 (5%)	0/42 (0%)	1/36 (3%)
Day of First Observation	592		729
Life Table Tests (d)	P=0.181N	P=0.107N	P=0.320N
Logistic Regression Tests (d)	P=0.184N	P=0.121N	P=0.323N
Cochran-Armitage Trend Test (d)	P=0.176N		
Fisher Exact Test (d)		P=0.121N	P=0.309N
Subcutaneous Tissue: Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (e)	9/50 (18%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	21.2%	2.4%	7.9%
Terminal Rates (c)	4/37 (11%)	1/42 (2%)	2/36 (6%)
Day of First Observation	592	729	592
Life Table Tests (d)	P=0.030N	P=0.008N	P=0.080N
Logistic Regression Tests (d)	P=0.028N	P=0.009N	P=0.072N
Cochran-Armitage Trend Test (d)	P=0.025N		
Fisher Exact Test (d)		P=0.008N	P=0.061N
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (e)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	8.1%	0.0%	0.0%
Terminal Rates (c)	3/37 (8%)	0/42 (0%)	0/36 (0%)
Day of First Observation	729		
Life Table Tests (d)	P=0.035N	P=0.100N	P=0.126N
Logistic Regression Tests (d)	P=0.035N	P=0.100N	P=0.126N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.121N	P=0.121N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (e)	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	12.5%	4.4%	2.6%
Terminal Rates (c)	3/37 (8%)	0/42 (0%)	0/36 (0%)
Day of First Observation	504	552	695
Life Table Tests (d)	P=0.065N	P=0.186N	P=0.115N
Logistic Regression Tests (d)	P=0.059N	P=0.225N	P=0.111N
Cochran-Armitage Trend Test (d)	P=0.060N		
Fisher Exact Test (d)		P=0.218N	P=0.102N
All Sites: Benign Tumors			
Overall Rates (e)	17/50 (34%)	10/50 (20%)	10/50 (20%)
Adjusted Rates (b)	42.1%	23.1%	26.8%
Terminal Rates (c)	14/37 (38%)	9/42 (21%)	9/36 (25%)
Day of First Observation	337	525	498
Life Table Tests (d)	P=0.075N	P=0.047N	P=0.107N
Logistic Regression Tests (d)	P=0.085N	P=0.077N	P=0.118N
Cochran-Armitage Trend Test (d)	P=0.066N		
Fisher Exact Test (d)		P=0.088N	P=0.088N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
All Sites: Malignant Tumors			
Overall Rates (e)	17/50 (34%)	7/50 (4%)	7/50 (14%)
Adjusted Rates (b)	39.3%	15.8%	17.7%
Terminal Rates (c)	11/37 (30%)	5/42 (12%)	4/36 (11%)
Day of First Observation	504	552	592
Life Table Tests (d)	P=0.014N	P=0.012N	P=0.030N
Logistic Regression Tests (d)	P=0.012N	P=0.016N	P=0.024N
Cochran-Armitage Trend Test (d)	P=0.009N		
Fisher Exact Test (d)		P=0.017N	P=0.017N
All Sites: All Tumors			
Overall Rates (e)	27/50 (54%)	15/50 (30%)	16/50 (32%)
Adjusted Rates (b)	61.1%	33.2%	39.8%
Terminal Rates (c)	20/37 (54%)	12/42 (29%)	12/36 (33%)
Day of First Observation	337	525	498
Life Table Tests (d)	P=0.027N	P=0.007N	P=0.043N
Logistic Regression Tests (d)	P=0.023N	P=0.011N	P=0.035N
Cochran-Armitage Trend Test (d)	P=0.016N		
Fisher Exact Test (d)		P=0.013N	P=0.021N

(a) Number of tumor-bearing animals/number of animals examined microscopically at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group than in vehicle controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined grossly at the site

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Esophagus	(50)	(50)	(49)
Perforation, focal		1 (2%)	
Periesophageal tissue, hemorrhage, focal			1 (2%)
Periesophageal tissue, inflammation, acute, diffuse			1 (2%)
Gallbladder	(38)	(36)	(43)
Infiltration cellular, lymphocytic, diffuse	4 (11%)	2 (6%)	1 (2%)
Infiltration cellular, lymphocytic, focal	1 (3%)	3 (8%)	
Infiltration cellular, lymphocytic, multifocal	1 (3%)	1 (3%)	
Inflammation, acute, diffuse	1 (3%)	4 (11%)	
Inflammation, acute, focal			1 (2%)
Intestine large, cecum	(50)	(46)	(49)
Lymphoid nodule, hyperplasia, lymphoid, focal		1 (2%)	
Lymphoid nodule, hyperplasia, lymphoid, multifocal		1 (2%)	
Intestine large, rectum	(48)	(45)	(44)
Inflammation, acute, focal	19 (40%)	15 (33%)	18 (41%)
Intestine small, duodenum	(48)	(47)	(46)
Peyer's patch, hyperplasia, lymphoid, multifocal			1 (2%)
Intestine small, jejunum	(48)	(48)	(48)
Peyer's patch, hyperplasia, lymphoid, focal		1 (2%)	2 (4%)
Liver	(50)	(50)	(49)
Basophilic focus	1 (2%)		
Cyst, multiple	1 (2%)		
Fatty change, multifocal		1 (2%)	1 (2%)
Infarct, focal	2 (4%)		1 (2%)
Infiltration cellular, lymphocytic, multifocal		3 (6%)	
Inflammation, acute, focal		1 (2%)	
Necrosis, coagulative, diffuse	1 (2%)		
Necrosis, coagulative, multifocal			2 (4%)
Bile duct, infiltration cellular, lymphocytic, multifocal	1 (2%)		
Mesentery	(1)		
Inflammation, acute, diffuse	1 (100%)		
Pancreas	(50)	(50)	(48)
Abscess, focal	1 (2%)		
Atrophy, focal	2 (4%)	3 (6%)	2 (4%)
Inflammation, chronic, focal	1 (2%)	7 (14%)	
Inflammation, chronic, multifocal	2 (4%)	2 (4%)	
Necrosis, focal	1 (2%)		
Vacuolization cytoplasmic, multifocal	1 (2%)	1 (2%)	
Artery, media, mineralization, multifocal			1 (2%)
Duct, inflammation, chronic, focal			1 (2%)
Salivary glands	(50)	(50)	(50)
Infiltration cellular, lymphocytic, focal	5 (10%)	10 (20%)	7 (14%)
Infiltration cellular, lymphocytic, multifocal	17 (34%)	25 (50%)	11 (22%)
Stomach, forestomach	(48)	(48)	(47)
Abscess, focal	4 (8%)	3 (6%)	
Abscess, multifocal	1 (2%)	3 (6%)	
Acanthosis, focal	2 (4%)	3 (6%)	5 (11%)
Acanthosis, multifocal	2 (4%)	2 (4%)	6 (13%)
Cyst epithelial inclusion, focal	1 (2%)		1 (2%)
Hyperkeratosis, focal		2 (4%)	
Inflammation, acute, focal		1 (2%)	1 (2%)
Inflammation, acute, multifocal			1 (2%)
Inflammation, chronic, focal	2 (4%)		6 (13%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
ALIMENTARY SYSTEM			
Stomach, forestomach (Continued)	(48)	(48)	(47)
Inflammation, chronic, multifocal	1 (2%)		1 (2%)
Serosa, inflammation, acute, diffuse	1 (2%)		
Stomach, glandular	(49)	(49)	(48)
Inflammation, acute, focal			2 (4%)
Inflammation, chronic, focal		1 (2%)	
Artery, adventitia, inflammation, chronic, multifocal	1 (2%)		
CARDIOVASCULAR SYSTEM			
Blood vessel	(49)	(50)	(50)
Mesenteric artery, aorta, adventitia, inflammation, chronic, multifocal	1 (2%)		
Mesenteric artery, aorta, media, hypertrophy, multifocal	1 (2%)		
Mesenteric artery, renal artery, adventitia, inflammation, chronic, multifocal	1 (2%)		
Mesenteric artery, renal artery, media, hypertrophy, diffuse	1 (2%)		
Heart	(50)	(50)	(50)
Artery, inflammation, chronic, diffuse	2 (4%)	1 (2%)	
Endocardium, inflammation, acute, focal			1 (2%)
Endocardium, inflammation, chronic, focal	1 (2%)		
Epicardium, inflammation, acute, diffuse	2 (4%)		1 (2%)
Epicardium, inflammation, acute, focal	1 (2%)		2 (4%)
Ventricle left, myocardium, hemorrhage, focal			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland, cortex	(50)	(50)	(50)
Hypertrophy, focal	1 (2%)	3 (6%)	2 (4%)
Bilateral, hypertrophy, multifocal		1 (2%)	
Bilateral, spindle cell, hyperplasia, diffuse		1 (2%)	
Bilateral, spindle cell, hyperplasia, focal	1 (2%)		
Bilateral, spindle cell, hyperplasia, multifocal	25 (50%)	30 (60%)	23 (46%)
Spindle cell, hyperplasia, diffuse			1 (2%)
Spindle cell, hyperplasia, focal	9 (18%)	8 (16%)	5 (10%)
Spindle cell, hyperplasia, multifocal	11 (22%)	6 (12%)	4 (8%)
Adrenal gland, medulla	(48)	(49)	(48)
Hyperplasia, focal	1 (2%)	1 (2%)	
Islets, pancreatic	(50)	(47)	(49)
Vacuolization cytoplasmic, diffuse	1 (2%)		
Parathyroid gland	(27)	(27)	(26)
Cyst, single		1 (4%)	
Infiltration cellular, lymphocytic, focal	1 (4%)		
Pituitary gland	(40)	(48)	(48)
Hypertrophy, focal	1 (3%)		
Craniopharyngeal duct, cyst	1 (3%)		1 (2%)
Thyroid gland	(50)	(50)	(49)
Hyperplasia, cystic, focal		1 (2%)	
Infiltration cellular, lymphocytic, focal	1 (2%)	1 (2%)	
Infiltration cellular, lymphocytic, multifocal	1 (2%)		
Ultimobranchial cyst	5 (10%)	9 (18%)	7 (14%)
GENERAL BODY SYSTEM			
None			

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
GENITAL SYSTEM			
Epididymis	(50)	(50)	(50)
Atypical cells, multifocal	1 (2%)		1 (2%)
Granuloma sperm, focal		1 (2%)	
Inflammation, chronic, focal	4 (8%)	2 (4%)	2 (4%)
Inflammation, chronic, multifocal	1 (2%)		
Spermatocele		1 (2%)	
Vacuolization cytoplasmic, diffuse	1 (2%)		2 (4%)
Vacuolization cytoplasmic, focal			1 (2%)
Bilateral, atypical cells, multifocal			4 (8%)
Bilateral, inflammation, chronic, multifocal		1 (2%)	
Bilateral, vacuolization cytoplasmic, diffuse	1 (2%)		1 (2%)
Bilateral, vacuolization cytoplasmic, multifocal	1 (2%)	1 (2%)	
Serosa, inflammation, acute, diffuse	1 (2%)		
Penis	(1)		
Inflammation, focal	1 (100%)		
Preputial gland	(10)	(4)	(3)
Abscess, focal	4 (40%)		1 (33%)
Abscess, multifocal	1 (10%)	1 (25%)	
Dilatation	1 (10%)	1 (25%)	1 (33%)
Inflammation, chronic, diffuse	3 (30%)	1 (25%)	1 (33%)
Inflammation, chronic, multifocal	1 (10%)		
Bilateral, abscess, multifocal		2 (50%)	
Bilateral, dilatation	1 (10%)		
Prostate	(49)	(49)	(49)
Abscess, multifocal	1 (2%)		
Infiltration cellular, lymphocytic, focal	3 (6%)	4 (8%)	2 (4%)
Infiltration cellular, lymphocytic, multifocal	2 (4%)	1 (2%)	1 (2%)
Inflammation, acute, diffuse	2 (4%)		
Seminal vesicle	(1)		
Abscess, multifocal	1 (100%)		
Testes	(50)	(50)	(50)
Bilateral, seminiferous tubule, degeneration, diffuse		1 (2%)	
Bilateral, seminiferous tubule, vacuolization cytoplasmic, diffuse	2 (4%)	3 (6%)	1 (2%)
Seminiferous tubule, degeneration, diffuse	1 (2%)	1 (2%)	1 (2%)
Seminiferous tubule, degeneration, focal	1 (2%)	1 (2%)	1 (2%)
Seminiferous tubule, mineralization, focal		1 (2%)	
Seminiferous tubule, mineralization, multifocal		1 (2%)	
Seminiferous tubule, vacuolization cytoplasmic, diffuse			2 (4%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(50)	(49)
Erythroid cell, hyperplasia, diffuse			1 (2%)
Myeloid cell, hyperplasia, diffuse	2 (4%)		
Lymph node	(50)	(50)	(49)
Axillary, hyperplasia, lymphoid, multifocal	2 (4%)		
Inguinal, hyperplasia, lymphoid, diffuse	1 (2%)		
Inguinal, hyperplasia, lymphoid, multifocal		1 (2%)	
Inguinal, pigmentation, diffuse	1 (2%)		
Inguinal, pigmentation, focal		1 (2%)	
Inguinal, pigmentation, multifocal			1 (2%)
Renal, hyperplasia, lymphoid, multifocal	1 (2%)		
Lymph node, mesenteric	(50)	(50)	(48)
Hematopoietic cell proliferation, diffuse	3 (6%)	7 (14%)	
Hematopoietic cell proliferation, focal	1 (2%)		
Hyperplasia, lymphoid, diffuse	12 (24%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid, focal	3 (6%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid, multifocal	11 (22%)	7 (14%)	10 (21%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
HEMATOPOIETIC SYSTEM			
Lymph node, mesenteric (Continued)	(50)	(50)	(48)
Hyperplasia, re cell, diffuse	2 (4%)		
Infiltration cellular, plasma cell, diffuse	1 (2%)		
Inflammation, acute, diffuse	1 (2%)		
Thrombus			1 (2%)
Mediastinal, hyperplasia, lymphoid, multifocal			1 (2%)
Spleen	(50)	(50)	(48)
Hematopoietic cell proliferation, diffuse	9 (18%)	3 (6%)	6 (13%)
Hyperplasia, lymphoid, diffuse	4 (8%)	2 (4%)	2 (4%)
Hyperplasia, lymphoid, focal	1 (2%)		
Hyperplasia, lymphoid, multifocal	12 (24%)	11 (22%)	10 (21%)
Pigmentation, diffuse	1 (2%)		
Thymus	(44)	(42)	(44)
Atrophy, diffuse	6 (14%)	8 (19%)	5 (11%)
Cyst, single	1 (2%)		
Hyperplasia, lymphoid, diffuse			1 (2%)
Hyperplasia, lymphoid, focal	1 (2%)		
Mineralization, multifocal		1 (2%)	
Mediastinum, abscess, focal	1 (2%)		
Mediastinum, hemorrhage, focal		1 (2%)	
INTEGUMENTARY SYSTEM			
Mammary gland	(5)	(4)	(6)
Inflammation, chronic, multifocal	1 (20%)		
Skin	(26)	(45)	(15)
Acanthosis, focal	4 (15%)	1 (2%)	
Acanthosis, multifocal	3 (12%)		5 (33%)
Cyst epithelial inclusion, single	1 (4%)		
Hyperkeratosis, focal	3 (12%)	1 (2%)	
Hyperkeratosis, multifocal	2 (8%)		1 (7%)
Inflammation, acute, multifocal	1 (4%)		
Inflammation, chronic, diffuse	3 (12%)		1 (7%)
Inflammation, chronic, focal	2 (8%)	2 (4%)	1 (7%)
Inflammation, chronic, multifocal	2 (8%)		
Ulcer, focal	5 (19%)		
Artery, subcutaneous tissue, adventitia, inflammation, chronic, focal	1 (4%)		
Artery, subcutaneous tissue, adventitia, inflammation, chronic, multifocal	1 (4%)		
Back, ulcer, focal			1 (7%)
Hair follicle, atrophy, diffuse	3 (12%)		1 (7%)
Hair follicle, atrophy, focal			3 (20%)
Hair follicle, inflammation, chronic, multifocal	1 (4%)		
Neck, edema, diffuse			1 (7%)
Prepuce, acanthosis, diffuse			1 (7%)
Prepuce, cyst epithelial inclusion, focal	1 (4%)		
Prepuce, inflammation, chronic, diffuse			1 (7%)
Subcutaneous tissue, edema, diffuse		2 (4%)	
Subcutaneous tissue, head, granuloma, focal	1 (4%)		
MUSCULOSKELETAL SYSTEM			
None			
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Bilateral, thalamus, mineralization, multifocal	23 (46%)	20 (40%)	22 (44%)
Cerebellum, hemorrhage, multifocal			1 (2%)
Lateral ventricle, meninges, abscess, multifocal	1 (2%)		
Olfactory lobe, mineralization, focal			1 (2%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
NERVOUS SYSTEM			
Brain (Continued)	(50)	(50)	(50)
Thalamus, mineralization, focal	2 (4%)	1 (2%)	
Thalamus, mineralization, multifocal	5 (10%)	9 (18%)	7 (14%)
RESPIRATORY SYSTEM			
Larynx			(1)
Hemorrhage			1 (100%)
Lung	(50)	(50)	(50)
Congestion, diffuse			1 (2%)
Foreign body			1 (2%)
Hemorrhage, focal	1 (2%)	2 (4%)	
Hemorrhage, multifocal		2 (4%)	3 (6%)
Thrombus, diffuse	1 (2%)		
Alveolar epithelium, hyperplasia	2 (4%)		1 (2%)
Alveolar epithelium, hypertrophy, focal		4 (8%)	1 (2%)
Alveolus, foreign body, multifocal			1 (2%)
Peribronchial, infiltration cellular, lymphocytic, multifocal	29 (58%)	32 (64%)	21 (42%)
Peribronchiolar, infiltration cellular, lymphocytic, multifocal	1 (2%)		
Perivascular, infiltration cellular, lymphocytic, multifocal	4 (8%)	5 (10%)	1 (2%)
Pleura, inflammation, acute, diffuse	1 (2%)		
Pleura, inflammation, acute, multifocal	1 (2%)		
Pleura, inflammation, chronic, focal		1 (2%)	
Nose	(50)	(50)	(49)
Foreign body	25 (50%)	23 (46%)	26 (53%)
Glands, hyperplasia	3 (6%)	42 (84%)	44 (90%)
Nasolacrimal duct, inflammation, chronic, diffuse			1 (2%)
Nasolacrimal duct, inflammation, chronic, focal	1 (2%)		
Olfactory epithelium, atrophy	11 (22%)	42 (84%)	44 (90%)
Turbinate, congestion, diffuse			1 (2%)
Turbinate, exudate	9 (18%)	10 (20%)	8 (16%)
Turbinate, foreign body			1 (2%)
Turbinate, hemorrhage, diffuse			6 (12%)
Turbinate, hemorrhage, focal		1 (2%)	
Turbinate, inflammation, acute, diffuse			1 (2%)
Turbinate, inflammation, acute, focal		1 (2%)	2 (4%)
Turbinate, inflammation, acute, multifocal		3 (6%)	27 (55%)
Turbinate, inflammation, chronic, diffuse	1 (2%)	6 (12%)	1 (2%)
Turbinate, inflammation, chronic, focal	6 (12%)	2 (4%)	
Turbinate, inflammation, chronic, multifocal	9 (18%)	3 (6%)	6 (12%)
Trachea	(50)	(50)	(49)
Inflammation, chronic, diffuse	10 (20%)	8 (16%)	
Inflammation, chronic, focal	1 (2%)	2 (4%)	
Inflammation, chronic, multifocal	10 (20%)	5 (10%)	11 (22%)
Peritracheal tissue, foreign body, multifocal			1 (2%)
Peritracheal tissue, hemorrhage, diffuse		1 (2%)	1 (2%)
Peritracheal tissue, hemorrhage, multifocal			1 (2%)
Peritracheal tissue, inflammation, acute, multifocal			1 (2%)
Peritracheal tissue, inflammation, chronic, focal			1 (2%)
Peritracheal tissue, inflammation, chronic, multifocal	1 (2%)		
SPECIAL SENSES SYSTEM			
None			

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
URINARY SYSTEM			
Kidney	(50)	(50)	(49)
Bacterium, multifocal	1 (2%)		
Casts protein, diffuse	1 (2%)		
Cyst, multiple			1 (2%)
Cyst, single	1 (2%)	4 (8%)	1 (2%)
Hydronephrosis	1 (2%)		
Inflammation, acute, multifocal	1 (2%)		
Inflammation, chronic, focal	2 (4%)	5 (10%)	7 (14%)
Inflammation, chronic, multifocal	3 (6%)	3 (6%)	3 (6%)
Bilateral, cyst, multiple			1 (2%)
Bilateral, inflammation, acute, focal	1 (2%)		
Bilateral, inflammation, chronic, focal	1 (2%)		
Bilateral, inflammation, chronic, multifocal	29 (58%)	25 (50%)	19 (39%)
Bilateral, artery, inflammation, chronic, multifocal	1 (2%)		
Bilateral, renal tubule, casts protein, multifocal	1 (2%)		
Bilateral, renal tubule, concretion, multifocal	1 (2%)	2 (4%)	
Bilateral, renal tubule, degeneration, multifocal	1 (2%)	2 (4%)	1 (2%)
Bilateral, renal tubule, regeneration, multifocal	2 (4%)	2 (4%)	2 (4%)
Bilateral, renal tubule, vacuolization cytoplasmic, multifocal	1 (2%)		
Interstitial tissue, inflammation, chronic, diffuse	1 (2%)		
Renal tubule, casts protein, diffuse	1 (2%)		
Renal tubule, concretion, multifocal	1 (2%)		
Renal tubule, degeneration, focal	1 (2%)	4 (8%)	1 (2%)
Renal tubule, degeneration, multifocal	2 (4%)		1 (2%)
Renal tubule, regeneration, focal		2 (4%)	3 (6%)
Renal tubule, regeneration, multifocal	2 (4%)	4 (8%)	
Renal tubule, vacuolization cytoplasmic, multifocal		1 (2%)	
Ureter	(1)		
Dilatation	1 (100%)		
Urethra	(1)		
Mucosa, inflammation, acute, diffuse	1 (100%)		
Urinary bladder	(50)	(47)	(48)
Dilatation	1 (2%)		1 (2%)
Infiltration cellular, lymphocytic, focal	3 (6%)	3 (6%)	3 (6%)
Infiltration cellular, lymphocytic, multifocal	13 (26%)	6 (13%)	3 (6%)
Inflammation, acute, focal	1 (2%)		
Transitional epithelium, hyperplasia, diffuse	1 (2%)		
Transitional epithelium, vacuolization cytoplasmic, diffuse	1 (2%)		

APPENDIX B

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Gallbladder	(31)	(34)	(42)
Lymphoma malignant mixed		1 (3%)	1 (2%)
Intestine large, cecum	(46)	(44)	(46)
Lymphoma malignant mixed		1 (2%)	
Intestine large, colon	(48)	(47)	(47)
Lymphoma malignant mixed		1 (2%)	
Intestine small, jejunum	(41)	(40)	(47)
Lymphoma malignant mixed		1 (3%)	
Liver	(50)	(50)	(50)
Hepatocellular carcinoma		2 (4%)	1 (2%)
Hepatocellular adenoma	1 (2%)		
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed		3 (6%)	3 (6%)
Sarcoma, metastatic, skeletal muscle			1 (2%)
Mesentery	*(50)	*(50)	*(50)
Lymphoma malignant mixed		1 (2%)	
Sarcoma, metastatic, skin		1 (2%)	
Fat, sarcoma, metastatic, skeletal muscle			1 (2%)
Pancreas	(49)	(49)	(49)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed		1 (2%)	
Parenchyma, sarcoma, metastatic, skin		1 (2%)	
Parenchyma, sarcoma, metastatic, skeletal muscle			1 (2%)
Stomach, forestomach	(47)	(47)	(49)
Papilloma squamous		3 (6%)	
Squamous cell carcinoma		1 (2%)	
CARDIOVASCULAR SYSTEM			
None			
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(49)	(50)
Capsule, sarcoma, metastatic, skin		1 (2%)	
Adrenal gland, cortex	(50)	(49)	(50)
Sarcoma, metastatic, skeletal muscle			1 (2%)
Pituitary gland	(46)	(49)	(48)
Adenoma	1 (2%)		1 (2%)
Pars distalis, adenoma			1 (2%)
Thyroid gland	(49)	(46)	(50)
Adenoma			1 (2%)
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Ovary	(50)	(48)	(48)
Adenoma, papillary	1 (2%)		
Lymphoma malignant mixed		1 (2%)	
Uterus	(50)	(50)	(50)
Leiomyosarcoma			1 (2%)
Polyp			2 (4%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
HEMATOPOIETIC SYSTEM			
Lymph node	(47)	(47)	(48)
Mediastinal, lymphoma malignant mixed		1 (2%)	
Mediastinal, sarcoma			1 (2%)
Pancreatic, leiomyosarcoma, metastatic, uterus			1 (2%)
Lymph node, mesenteric	(46)	(47)	(48)
Lymphoma malignant mixed		3 (6%)	2 (4%)
Sarcoma, metastatic			1 (2%)
Sarcoma, metastatic, skin		1 (2%)	
Renal, mediastinal, lymphoma malignant lymphocytic	1 (2%)		
Spleen	(50)	(49)	(50)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	1 (2%)	4 (8%)	4 (8%)
Capsule, sarcoma, metastatic, skin		1 (2%)	
Capsule, sarcoma, metastatic, skeletal muscle			1 (2%)
Thymus	(35)	(42)	(46)
Lymphoma malignant mixed	1 (3%)	1 (2%)	
INTEGUMENTARY SYSTEM			
Mammary gland	(48)	(46)	(42)
Adenocarcinoma	1 (2%)		1 (2%)
Lymphoma malignant mixed		1 (2%)	
Skin	*(50)	*(50)	*(50)
Subcutaneous tissue, sarcoma		1 (2%)	
MUSCULOSKELETAL SYSTEM			
Skeletal muscle	*(50)	*(50)	*(50)
Osteosarcoma, metastatic, uncertain primary site		1 (2%)	
Back, sarcoma			1 (2%)
Diaphragm, sarcoma, metastatic, skeletal muscle			1 (2%)
Thigh, sarcoma			1 (2%)
NERVOUS SYSTEM			
None			
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	6 (12%)	3 (6%)
Lymphoma malignant mixed		1 (2%)	1 (2%)
Osteosarcoma, metastatic, uncertain primary site		1 (2%)	
Parenchyma, sarcoma, metastatic, skeletal muscle			1 (2%)
Trachea	(49)	(50)	(50)
Peritracheal tissue, lymphoma malignant mixed		1 (2%)	
SPECIAL SENSES SYSTEM			
Harderian gland	*(50)	*(50)	*(50)
Adenoma	2 (4%)		
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed		1 (2%)	2 (4%)
Bilateral, capsule, sarcoma, metastatic, skin		1 (2%)	
Renal tubule, adenoma		1 (2%)	

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
URINARY SYSTEM (Continued)			
Urinary bladder	(46)	(46)	(48)
Lymphoma malignant mixed			1 (2%)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Lymphoma malignant mixed	1 (2%)	4 (8%)	4 (8%)
Lymphoma malignant lymphocytic	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Moribund	23	10	4
Terminal sacrifice	14	28	38
Dead	13	10	7
Accident		2	1
TUMOR SUMMARY			
Total animals with primary neoplasms **	9	15	14
Total primary neoplasms	9	18	18
Total animals with benign neoplasms	6	8	7
Total benign neoplasms	6	10	8
Total animals with malignant neoplasms	3	9	8
Total malignant neoplasms	3	8	10
Total animals with secondary neoplasms ***		2	2
Total secondary neoplasms		8	9
Total animals with malignant neoplasms--uncertain primary site		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE: VEHICLE CONTROL

WEEKS ON STUDY	059	060	060	063	067	069	071	075	077	077	081	083	083	085	086	087	088	090	092	092	093	094	094	096	097
CARCASS ID	141	151	131	171	152	121	201	191	161	163	111	153	132	172	173	142	192	133	193	181	154	182	122	165	194
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	A	A	A	+	A	+	+	+	A	M	A	+	+	+	+	M	M	M
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	A	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	A	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	A	A	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	A	A	A	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+	A	A	A	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Lymphoma malignant lymphocytic																									X
Mesentery	+			+	+			+	+	+					+	+	+			+	+	+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Lymphoma malignant lymphocytic																									X
Salivary glands	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, *forestomach*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	M	+	+	+
Stomach, glandular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+
CARDIOVASCULAR SYSTEM																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+
Parathyroid gland	M	+	M	M	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	M
Adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, papillary																									
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	M	M	+	+
Lymph node, mandibular						+															+				
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	M	M	+	+
Renal, mediastinal, lymphoma malignant lymphocytic																									X
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									X
Lymphoma malignant mixed																									
Thymus	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	M	M	M	M
Lymphoma malignant mixed																									
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Adenocarcinoma																									
Skin						+		+					+				+	+	+				+		+
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																							+		
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx																	+								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye																							A		
Harderian gland								+															+		
Adenoma								X															X		
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									X
Urinary bladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	A	+	+

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: VEHICLE CONTROL (Continued)

WEEKS ON STUDY	0 9 8	0 9 9	0 9 9	0 9 9	1 0 0	1 0 1	1 0 1	1 0 2	1 0 2	1 0 2	1 0 2	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	TOTAL:
CARCASS ID	1 8 4	1 4 3	1 7 5	2 0 5	1 4 4	1 6 4	2 0 3	1 7 4	1 9 5	1 4 5	2 0 2	1 1 2	1 1 5	1 2 4	1 6 2	1 8 3	1 1 3	1 3 4	1 5 5	2 0 4	1 1 4	1 2 3	1 2 5	1 3 5	1 8 5	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	M	M	A	M	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	31
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, jejunum	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma					X																					1
Lymphoma malignant lymphocytic																										1
Mesentery		+	+	+		+	+		+	+	+															20
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant lymphocytic																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CARDIOVASCULAR SYSTEM																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid gland	+	M	+	+	+	+	M	+	M	M	+	M	+	M	+	+	+	+	+	M	+	M	+	M	+	33
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	46
Adenoma											X															1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, papillary												X														1
Oviduct											M															
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mandibular							+							+	+											7
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Renal, mediastinal, lymphoma malignant lymphocytic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																		X								1
Thymus	+	+	+	M	M	M	M	M	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	35
Lymphoma malignant mixed																		X								1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma														X												1
Skin	+			+			+							+				+				+	+	+		16
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx																										1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSES SYSTEM																										
Eye	+						+																			2
Harderian gland																										2
Adenoma																										2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Urinary bladder	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE: 375 mg/kg

WEEKS ON STUDY	001	045	055	063	070	071	079	081	083	084	086	087	088	090	090	092	093	095	097	099	101	105	105	105	105
CARCASS ID	36 1	33 1	38 1	39 1	34 1	34 2	32 1	31 1	31 2	39 2	39 5	35 1	32 2	40 1	35 2	32 3	34 3	35 3	39 3	32 5	38 4	31 3	31 4	31 5	33 5
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	M	A	+	M	+	+	M	+	+	A	+	+	+	M	+	+	A	+	M	M	+	+	+	+
Lymphoma malignant mixed									X																
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	A	+	+	A	+	+	+	A	A	+	A	+	A	+	+	+	+	+	+	+	+
Lymphoma malignant mixed									X																
Intestine large, colon	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+
Lymphoma malignant mixed									X																
Intestine large, rectum	+	M	+	+	+	M	+	+	+	+	+	+	+	+	M	+	A	+	+	+	+	+	+	+	+
Intestine small	+	A	+	+	A	+	+	+	+	+	+	A	A	+	A	+	A	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	A	+	A	+	+	A	+	A	A	A	A	A	A	+	A	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	A	+	+	+	+	+	+	A	A	+	A	+	A	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	A	+	+	A	+	A	+	A	A	A	A	+	A	+	A	+	+	+	+	+	+
Lymphoma malignant mixed									X																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma								X																	
Lymphoma malignant mixed									X				X												
Mesentery		+			+		+					+	+	+	+					+	+	+			
Lymphoma malignant mixed													X												
Sarcoma, metastatic, skin																									
Pancreas	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed									X																
Parenchyma, sarcoma, metastatic, skin																									
Salivary glands	+	M	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	M	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	M	+
Papilloma squamous																									
Squamous cell carcinoma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Capsule, sarcoma, metastatic, skin																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	M	M	+	+	M	M	+	+	+	+	M	+	+	+	M	+	+	+	+	M	M	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	M	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Ovary	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed													X												
Oviduct		+																							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 375 mg/kg (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	344	345	355	382	383	402	404	324	332	334	354	364	365	375	385	403	333	362	363	371	372	373	374	394	405	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	49
Gallbladder	+	M	M	+	+	M	M	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	34
Lymphoma malignant mixed																										1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Lymphoma malignant mixed																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymphoma malignant mixed																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	45
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																X										2
Lymphoma malignant mixed			X																							3
Mesentery				+									+													12
Lymphoma malignant mixed																										1
Sarcoma, metastatic, skin				X																						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant mixed																										1
Parenchyma, sarcoma, metastatic, skin				X																						1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Papilloma squamous																			X	X		X				3
Squamous cell carcinoma																				X						1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARDIOVASCULAR SYSTEM																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Capsule, sarcoma, metastatic, skin				X																						1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	M	+	M	+	M	M	M	M	M	+	+	+	M	M	M	+	M	+	+	+	+	M	+	+	M	27
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	46
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 375 mg/kg (Continued)

WEEKS ON STUDY	001	045	055	063	070	071	079	081	083	084	086	087	088	090	090	092	093	095	097	099	101	105	105	105
CARCASS ID	361	331	381	391	341	342	321	311	312	392	395	351	322	401	352	323	343	353	393	325	384	313	314	315	335
HEMATOPOIETIC SYSTEM																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+
Mediastinal, lymphoma malignant mixed									X																
Lymph node, mandibular										+							+								
Lymph node, mesenteric	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed									X				X												
Sarcoma, metastatic, skin																									
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed									X				X												
Capsule, sarcoma, metastatic, skin																									
Thymus	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	+
Lymphoma malignant mixed													X												
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+
Lymphoma malignant mixed									X																
Skin			+														+	+	+		+	+	+		+
Subcutaneous tissue, sarcoma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle				+																					
Osteosarcoma, metastatic, uncertain primary site				X																					
NERVOUS SYSTEM																									
Brain	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+																								
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X					X			
Lymphoma malignant mixed																									
Osteosarcoma, metastatic, uncertain primary site				X																					
Nose	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peritracheal tissue, lymphoma malignant mixed									X																
SPECIAL SENSES SYSTEM																									
None																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																									
Bilateral, capsule, sarcoma, metastatic, skin																									
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	A	+	+	+	+	+	+	M	+	+	+	I	A	+	+	+	+	+	+	+	+

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 375 mg/kg (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	344	345	355	382	383	402	404	324	332	334	354	364	365	375	385	403	333	362	363	371	372	373	374	394	405	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood					+																					1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Mediastinal, lymphoma malig. mixed																										1
Lymph node, mandibular		+	+		+		+		+	+	+				+				+	+	+	+			+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymphoma malignant mixed																						X				3
Sarcoma, metastatic, skin				X																						1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant mixed			X																			X				4
Capsule, sarcoma, metastatic, skin				X																						1
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	42
Lymphoma malignant mixed																										1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	46
Lymphoma malignant mixed																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	34
Subcutaneous tissue, sarcoma				X																						1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Osteosarcoma, metastatic, uncertain primary site																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spinal cord																										1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X											X				X	X			6
Lymphoma malignant mixed			X																							1
Osteosarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peritracheal tissue, lymphoma malignant mixed																										1
SPECIAL SENSES SYSTEM																										
None																										
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																						X				1
Bilateral, capsule, sarcoma, metastatic, skin				X																						1
Renal tubule, adenoma																						X				1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE: 750 mg/kg

WEEKS ON STUDY	001	054	056	077	077	077	079	082	088	088	099	099	105	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	6001	5441	5111	5112	5212	5381	5331	5711	5551	5772	5775	5225	5222	5224	5334	5444	5445	5535	5555	5563	5646	5822	5883	5995	6003
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	+	+	M	+	M	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	M
Lymphoma malignant mixed																									
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Intestine large, colon	+	M	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	M	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+
Intestine small	A	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Lymphoma malignant mixed													X												
Sarcoma, metastatic, skeletal muscle				X																					
Mesentary				+			+																		
Fat, sarcoma, metastatic, skeletal muscle				X																					
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parenchyma, sarcoma, metastatic, skeletal muscle				X																					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle				X																					
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	I	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	M	M	+	+	M	M	M	+	+	+	+	+	M	M	+	+	M	M	M	M	+	M	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Pars distalis, adenoma																									X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																			X						
Polyp																		X			X				

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 750 mg/kg (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	513	514	515	523	533	565	574	584	585	593	602	604	605	532	535	542	543	552	554	561	562	573	591	592	594	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Lymphoma malignant mixed											X															1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma					X																					1
Lymphoma malignant mixed					X						X															3
Sarcoma, metastatic, skeletal muscle																										1
Mesentery																										2
Fat, sarcoma, metastatic, skeletal muscle																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parenchyma, sarcoma, metastatic, skeletal muscle																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARDIOVASCULAR SYSTEM																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skeletal muscle																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	M	+	M	M	M	+	M	M	+	M	M	M	M	+	M	M	+	M	+	M	M	M	+	M	+	19
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	48
Adenoma																										1
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X																					1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Polyp																										2

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 750 mg/kg (Continued)

WEEKS ON STUDY	001	054	056	077	077	077	079	082	088	088	099	099	105	105	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	601	541	511	512	521	581	531	571	551	572	575	525	522	524	534	544	545	553	555	563	564	582	583	595	603
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, sarcoma				X																					
Pancreatic, leiomyosarcoma, metastatic, uterus																			X						
Lymph node, mesenteric	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed													X				X								
Sarcoma, metastatic				X																					
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed													X				X								
Capsule, sarcoma, metastatic, skeletal muscle				X																					
Thymus	+	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	I	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+
Adenocarcinoma																									
Skin						+					+		+		+	+	+	+	+						+
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle				+					+																
Back, sarcoma				X																					
Diaphragm, sarcoma, metastatic, skeletal muscle				X																					
Thigh, sarcoma									X																
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+																								
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X														
Lymphoma malignant mixed																									
Parenchyma, sarcoma, metastatic, skeletal muscle				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
None																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																									
Ureter		+																							
Urinary bladder	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 750 mg/kg (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	513	514	515	523	533	565	574	584	585	593	602	604	605	532	535	542	543	552	554	561	562	573	591	592	594	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mediastinal, sarcoma																										1
Pancreatic, leiomyosarcoma, metastatic, uterus																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										2
Sarcoma, metastatic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed					X						X															4
Capsule, sarcoma, metastatic, skeletal muscle																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	42
Adenocarcinoma		X																								1
Skin	+		+	+														+	+							14
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Back, sarcoma																										1
Diaphragm, sarcoma, metastatic, skeletal muscle																										1
Thigh, sarcoma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma														X								X				3
Lymphoma malignant mixed											X															1
Parenchyma, sarcoma, metastatic, skeletal muscle																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
None																										
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed					X						X															2
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed											X															1

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	1/50 (2%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	3.1%	19.7%	7.6%
Terminal Rates (c)	0/14 (0%)	5/29 (17%)	2/38 (5%)
Day of First Observation	638	645	690
Life Table Tests (d)	P=0.515N	P=0.190	P=0.561
Logistic Regression Tests (d)	P=0.417	P=0.089	P=0.375
Cochran-Armitage Trend Test (d)	P=0.274		
Fisher Exact Test (d)		P=0.056	P=0.309
Forestomach: Squamous Cell Papilloma			
Overall Rates (e)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	0.0%	10.3%	0.0%
Terminal Rates (c)	0/14 (0%)	3/29 (10%)	0/38 (0%)
Day of First Observation		729	
Life Table Tests (d)	P=0.380N	P=0.274	(f)
Logistic Regression Tests (d)	P=0.380N	P=0.274	(f)
Cochran-Armitage Trend Test (d)	P=0.640		
Fisher Exact Test (d)		P=0.121	(f)
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (e)	2/50 (4%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	10.7%	11.5%	10.5%
Terminal Rates (c)	1/14 (7%)	2/29 (7%)	4/38 (11%)
Day of First Observation	676	580	729
Life Table Tests (d)	P=0.529N	P=0.546	P=0.589N
Logistic Regression Tests (d)	P=0.365	P=0.364	P=0.593
Cochran-Armitage Trend Test (d)	P=0.274		
Fisher Exact Test (d)		P=0.339	P=0.339
All Sites: Benign Tumors			
Overall Rates (e)	6/50 (12%)	8/50 (16%)	7/50 (14%)
Adjusted Rates (b)	24.4%	26.4%	17.9%
Terminal Rates (c)	1/14 (7%)	7/29 (24%)	6/38 (16%)
Day of First Observation	522	645	690
Life Table Tests (d)	P=0.140N	P=0.447N	P=0.219N
Logistic Regression Tests (d)	P=0.471N	P=0.486	P=0.607N
Cochran-Armitage Trend Test (d)	P=0.443		
Fisher Exact Test (d)		P=0.387	P=0.500
All Sites: Malignant Tumors			
Overall Rates (e)	3/50 (6%)	9/50 (18%)	8/50 (16%)
Adjusted Rates (b)	17.6%	24.8%	19.5%
Terminal Rates (c)	2/14 (14%)	5/29 (17%)	6/38 (16%)
Day of First Observation	676	435	535
Life Table Tests (d)	P=0.470	P=0.236	P=0.512
Logistic Regression Tests (d)	P=0.111	P=0.067	P=0.161
Cochran-Armitage Trend Test (d)	P=0.093		
Fisher Exact Test (d)		P=0.061	P=0.100
All Sites: All Tumors			
Overall Rates (e)	9/50 (18%)	15/50 (30%)	14/50 (28%)
Adjusted Rates (b)	38.5%	42.2%	33.8%
Terminal Rates (c)	3/14 (21%)	10/29 (34%)	11/38 (29%)
Day of First Observation	522	435	535
Life Table Tests (d)	P=0.246N	P=0.542	P=0.301N
Logistic Regression Tests (d)	P=0.236	P=0.151	P=0.300
Cochran-Armitage Trend Test (d)	P=0.150		
Fisher Exact Test (d)		P=0.121	P=0.171

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

(a) Number of tumor-bearing animals/number of animals examined microscopically at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group than in vehicle controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined grossly at the site
(f) No P value is reported because no tumors were observed in the dosed and vehicle control groups.

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE

	Vehicle Control	375 mg/kg	750 mg/kg
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Esophagus	(49)	(49)	(49)
Perforation, focal		1 (2%)	1 (2%)
Mucosa, muscularis, inflammation, acute, diffuse	1 (2%)		
Periesophageal tissue, abscess, focal	1 (2%)		
Periesophageal tissue, abscess, multifocal	2 (4%)		
Periesophageal tissue, inflammation, chronic		1 (2%)	
Gallbladder	(31)	(34)	(42)
Infiltration cellular, lymphocytic, diffuse	1 (3%)	1 (3%)	1 (2%)
Infiltration cellular, lymphocytic, focal		2 (6%)	2 (5%)
Infiltration cellular, lymphocytic, multifocal	2 (6%)	1 (3%)	1 (2%)
Inflammation, acute, diffuse	1 (3%)		1 (2%)
Inflammation, acute, focal		3 (9%)	1 (2%)
Intestine large	(50)	(49)	(50)
Ulcer, diffuse		1 (2%)	
Intestine large, cecum	(46)	(44)	(46)
Lymphoid nodule, hyperplasia, lymphoid, focal			1 (2%)
Lymphoid nodule, hyperplasia, lymphoid, multifocal			1 (2%)
Wall, abscess, multifocal		1 (2%)	
Intestine large, rectum	(47)	(45)	(45)
Inflammation, acute, focal	5 (11%)	15 (33%)	22 (49%)
Intestine small, jejunum	(41)	(40)	(47)
Peyer's patch, hyperplasia, lymphoid, diffuse			1 (2%)
Peyer's patch, hyperplasia, lymphoid, focal			2 (4%)
Peyer's patch, hyperplasia, lymphoid, multifocal		1 (3%)	
Liver	(50)	(50)	(50)
Angiectasis, multifocal			1 (2%)
Fatty change, diffuse			1 (2%)
Hematopoietic cell proliferation, multifocal	18 (36%)	12 (24%)	1 (2%)
Hypertrophy, focal			1 (2%)
Infiltration cellular, lymphocytic, focal	3 (6%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocytic, multifocal	2 (4%)	4 (8%)	4 (8%)
Inflammation, acute, multifocal	1 (2%)		2 (4%)
Bile duct, infiltration cellular, lymphocytic, multifocal	3 (6%)	4 (8%)	5 (10%)
Bile duct, mineralization, multifocal		1 (2%)	
Hepatocyte, focal cellular change			1 (2%)
Serosa, abscess, focal	1 (2%)		
Serosa, inflammation, acute, focal		1 (2%)	
Serosa, inflammation, acute, multifocal	2 (4%)		
Serosa, inflammation, chronic, focal	1 (2%)		
Mesentery	(20)	(12)	(2)
Abscess, focal	1 (5%)		
Abscess, multifocal	19 (95%)	8 (67%)	
Cyst, single		1 (8%)	
Inflammation, acute, multifocal			1 (50%)
Inflammation, granulomatous, multifocal		1 (8%)	
Pigmentation, diffuse		1 (8%)	
Fat, necrosis, multifocal			1 (50%)
Pancreas	(49)	(49)	(49)
Abscess, multifocal	3 (6%)	1 (2%)	
Atrophy, diffuse	1 (2%)		1 (2%)
Atrophy, focal	1 (2%)		3 (6%)
Atrophy, multifocal	2 (4%)		
Inflammation, acute, diffuse	4 (8%)	1 (2%)	
Inflammation, acute, focal		1 (2%)	
Inflammation, acute, multifocal	2 (4%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
ALIMENTARY SYSTEM			
Pancreas (Continued)	(49)	(49)	(49)
Inflammation, chronic, diffuse	1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic, focal	1 (2%)	5 (10%)	2 (4%)
Inflammation, chronic, multifocal	4 (8%)	1 (2%)	1 (2%)
Necrosis, focal			1 (2%)
Necrosis, multifocal	1 (2%)		
Duct, cyst, single			2 (4%)
Duct, dilatation	1 (2%)		1 (2%)
Duct, inflammation, chronic, focal		1 (2%)	2 (4%)
Duct, inflammation, chronic, multifocal		1 (2%)	3 (6%)
Salivary glands	(49)	(45)	(50)
Infiltration cellular, lymphocytic, focal	4 (8%)	5 (11%)	9 (18%)
Infiltration cellular, lymphocytic, multifocal	11 (22%)	8 (18%)	17 (34%)
Stomach, forestomach	(47)	(47)	(49)
Abscess, focal	1 (2%)	1 (2%)	1 (2%)
Abscess, multifocal			1 (2%)
Acanthosis, focal	5 (11%)	2 (4%)	7 (14%)
Acanthosis, multifocal	1 (2%)		
Cyst epithelial inclusion, focal		1 (2%)	1 (2%)
Hyperkeratosis, focal	1 (2%)	3 (6%)	1 (2%)
Hyperplasia, basal cell		1 (2%)	
Inflammation, acute, focal	1 (2%)		1 (2%)
Inflammation, chronic, focal	5 (11%)	3 (6%)	1 (2%)
Stomach, glandular	(48)	(49)	(50)
Inflammation, acute, focal	1 (2%)	1 (2%)	1 (2%)
Inflammation, acute, multifocal		1 (2%)	
Inflammation, chronic, focal	1 (2%)		
CARDIOVASCULAR SYSTEM			
Blood vessel	(50)	(49)	(50)
Aorta, media, mineralization, diffuse	1 (2%)		
Heart	(50)	(50)	(50)
Artery, inflammation, chronic, focal	1 (2%)	3 (6%)	
Atrium left, bacterium, focal			1 (2%)
Atrium left, thrombus		1 (2%)	1 (2%)
Epicardium, inflammation, acute, diffuse	1 (2%)		
Epicardium, inflammation, acute, focal	1 (2%)		
Epicardium, inflammation, acute, multifocal	3 (6%)		
Epicardium, inflammation, chronic, diffuse	1 (2%)		
Epicardium, inflammation, chronic, focal		2 (4%)	
Epicardium, inflammation, chronic, multifocal	1 (2%)		
Myocardium, inflammation, acute, diffuse		1 (2%)	
Myocardium, inflammation, acute, focal	1 (2%)		
Myocardium, inflammation, chronic, focal			2 (4%)
Myocardium, inflammation, chronic, multifocal		1 (2%)	
Pericardium, inflammation, chronic, focal		1 (2%)	
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(49)	(50)
Capsule, inflammation, chronic, multifocal		1 (2%)	
Adrenal gland, cortex	(50)	(49)	(50)
Cyst, multiple			1 (2%)
Cyst, single	1 (2%)		1 (2%)
Hematopoietic cell proliferation, diffuse	2 (4%)		
Hematopoietic cell proliferation, focal	1 (2%)	1 (2%)	
Hematopoietic cell proliferation, multifocal	2 (4%)	1 (2%)	1 (2%)
Hypertrophy, focal		1 (2%)	1 (2%)
Bilateral, hematopoietic cell proliferation, diffuse	1 (2%)	1 (2%)	

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
ENDOCRINE SYSTEM			
Adrenal gland, cortex (Continued)	(50)	(49)	(50)
Bilateral, hematopoietic cell proliferation, multifocal	9 (18%)	2 (4%)	
Bilateral, vacuolization cytoplasmic, diffuse	2 (4%)	1 (2%)	1 (2%)
Bilateral, spindle cell, hyperplasia, diffuse	32 (64%)	33 (67%)	39 (78%)
Bilateral, spindle cell, hyperplasia, multifocal	16 (32%)	10 (20%)	7 (14%)
Spindle cell, hyperplasia, diffuse	1 (2%)	4 (8%)	3 (6%)
Spindle cell, hyperplasia, multifocal	1 (2%)	1 (2%)	
Adrenal gland, medulla	(49)	(48)	(50)
Hematopoietic cell proliferation, focal		1 (2%)	
Pituitary gland	(46)	(49)	(48)
Hypertrophy, focal	1 (2%)	1 (2%)	1 (2%)
Craniopharyngeal duct, cyst		1 (2%)	
Thyroid gland	(49)	(46)	(50)
Granuloma, focal	1 (2%)		
Hyperplasia, multifocal		1 (2%)	
Inflammation, acute, focal	1 (2%)		
Inflammation, chronic, focal	1 (2%)	1 (2%)	
Ultimobranchial cyst	13 (27%)	18 (39%)	4 (8%)
Follicle, cyst	1 (2%)	2 (4%)	1 (2%)
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Ovary	(50)	(48)	(48)
Abscess, focal	4 (8%)		
Abscess, multifocal	16 (32%)	7 (15%)	
Cyst, multiple	1 (2%)		1 (2%)
Cyst, single	7 (14%)	4 (8%)	10 (21%)
Mineralization, focal		2 (4%)	
Bilateral, abscess, multifocal	6 (12%)	2 (4%)	1 (2%)
Bilateral, cyst, multiple	2 (4%)	1 (2%)	1 (2%)
Germinal epithelium, hyperplasia, focal		1 (2%)	
Periovarian tissue, inflammation, chronic, diffuse		1 (2%)	
Oviduct		(1)	
Inflammation, acute, diffuse		1 (100%)	
Uterus	(50)	(50)	(50)
Abscess, focal	1 (2%)	1 (2%)	
Abscess, multifocal	9 (18%)	2 (4%)	
Dilatation	5 (10%)	7 (14%)	14 (28%)
Inflammation, acute, diffuse	3 (6%)	3 (6%)	
Endometrium, edema, diffuse		1 (2%)	
Endometrium, hyperplasia, cystic, diffuse	14 (28%)	26 (52%)	27 (54%)
Endometrium, hyperplasia, cystic, multifocal	1 (2%)	1 (2%)	1 (2%)
Lumen, exudate	1 (2%)		
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(50)	(50)
Hypoplasia, diffuse	1 (2%)		
Myelofibrosis, focal	2 (4%)		
Myeloid cell, hyperplasia, diffuse	6 (12%)	4 (8%)	1 (2%)
Lymph node	(47)	(47)	(48)
Bronchial, abscess, focal	1 (2%)		
Bronchial, hyperplasia, lymphoid, diffuse	1 (2%)		
Bronchial, infiltration cellular, plasma cell, diffuse	1 (2%)		
Iliac, hyperplasia, lymphoid, multifocal	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
HEMATOPOIETIC SYSTEM			
Lymph node (Continued)	(47)	(47)	(48)
Iliac, infiltration cellular, plasma cell, diffuse	4 (9%)	1 (2%)	
Inguinal, infiltration cellular, plasma cell, diffuse	2 (4%)		
Mediastinal, abscess, focal	1 (2%)		
Mediastinal, abscess, multifocal		1 (2%)	
Mediastinal, hyperplasia, lymphoid, diffuse		1 (2%)	
Mediastinal, infiltration cellular, plasma cell, diffuse	1 (2%)		
Renal, infiltration cellular, plasma cell, diffuse	4 (9%)	1 (2%)	
Lymph node, mandibular	(7)	(15)	
Hyperplasia, lymphoid, diffuse	1 (14%)		
Hyperplasia, lymphoid, focal		1 (7%)	
Hyperplasia, lymphoid, multifocal	2 (29%)	8 (53%)	
Infiltration cellular, plasma cell, diffuse	4 (57%)	7 (47%)	
Sinus, ectasia, diffuse	1 (14%)		
Lymph node, mesenteric	(46)	(47)	(48)
Hematopoietic cell proliferation, multifocal	1 (2%)	1 (2%)	
Hyperplasia, lymphoid, diffuse	1 (2%)	1 (2%)	2 (4%)
Hyperplasia, lymphoid, focal	2 (4%)		
Hyperplasia, lymphoid, multifocal	2 (4%)	3 (6%)	14 (29%)
Hyperplasia, re cell, diffuse	7 (15%)	1 (2%)	1 (2%)
Infiltration cellular, plasma cell, diffuse	2 (4%)	1 (2%)	
Inflammation, acute, focal	1 (2%)		
Necrosis, caseous, diffuse	1 (2%)		
Spleen	(50)	(49)	(50)
Depletion lymphoid, diffuse	1 (2%)		
Hematopoietic cell proliferation, diffuse	32 (64%)	18 (37%)	7 (14%)
Hyperplasia, lymphoid, diffuse	4 (8%)	3 (6%)	16 (32%)
Hyperplasia, lymphoid, multifocal	1 (2%)	2 (4%)	3 (6%)
Hyperplasia, re cell, multifocal			1 (2%)
Pigmentation, diffuse	6 (12%)	3 (6%)	3 (6%)
Artery, trabecula, amyloid deposition, multifocal	1 (2%)		
Capsule, inflammation, acute, diffuse	1 (2%)		
Capsule, inflammation, acute, multifocal	1 (2%)		
Capsule, inflammation, chronic, diffuse	1 (2%)		
Capsule, inflammation, chronic, focal		2 (4%)	
Thymus	(35)	(42)	(46)
Abscess, focal		1 (2%)	
Abscess, multifocal	1 (3%)		
Atrophy, diffuse	17 (49%)	13 (31%)	6 (13%)
Hyperplasia, lymphoid, focal		1 (2%)	2 (4%)
Hyperplasia, lymphoid, multifocal	2 (6%)		
Hyperplasia, re cell, focal			1 (2%)
Mediastinum, abscess, focal	1 (3%)		
Mediastinum, abscess, multifocal		1 (2%)	
INTEGUMENTARY SYSTEM			
Mammary gland	(48)	(46)	(42)
Hyperplasia, diffuse	16 (33%)	8 (17%)	3 (7%)
Inflammation, acute		1 (2%)	
Skin	(16)	(34)	(14)
Acanthosis, diffuse		2 (6%)	1 (7%)
Acanthosis, focal	1 (6%)		2 (14%)
Acanthosis, multifocal	1 (6%)	3 (9%)	8 (57%)
Hyperkeratosis, diffuse		1 (3%)	1 (7%)
Hyperkeratosis, focal		1 (3%)	
Hyperkeratosis, multifocal		2 (6%)	

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
INTEGUMENTARY SYSTEM			
Skin (Continued)	(16)	(34)	(14)
Inflammation, acute, multifocal			1 (7%)
Inflammation, chronic, diffuse		5 (15%)	2 (14%)
Inflammation, chronic, focal		1 (3%)	
Inflammation, chronic, multifocal		1 (3%)	1 (7%)
Ulcer, focal			1 (7%)
Back, subcutaneous tissue, abscess, focal	1 (6%)		
Hair follicle, acanthosis, diffuse			1 (7%)
Hair follicle, atrophy, diffuse	3 (19%)	3 (9%)	6 (43%)
Hair follicle, atrophy, focal	1 (6%)		
Hair follicle, atrophy, multifocal			1 (7%)
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(50)	(50)
Femur, fibrous osteodystrophy, diffuse	3 (6%)		
Femur, fracture healed		1 (2%)	
Skeletal muscle	(1)	(1)	(2)
Inflammation, acute, diffuse	1 (100%)		
NERVOUS SYSTEM			
Brain	(50)	(49)	(50)
Bilateral, thalamus, mineralization, multifocal	12 (24%)	17 (35%)	15 (30%)
Meninges, abscess, focal	1 (2%)		
Meninges, inflammation, acute, focal	1 (2%)		
Meninges, cerebrum, abscess, multifocal		1 (2%)	
Thalamus, hemorrhage, multifocal		1 (2%)	
Thalamus, mineralization, focal	3 (6%)	3 (6%)	4 (8%)
Thalamus, mineralization, multifocal	5 (10%)	6 (12%)	6 (12%)
Ventricle, abscess, multifocal	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Abscess, multifocal	1 (2%)	2 (4%)	
Bacterium, multifocal	1 (2%)		
Congestion, multifocal	1 (2%)		
Hemorrhage, diffuse		1 (2%)	
Hemorrhage, focal	2 (4%)		
Hemorrhage, multifocal	2 (4%)		
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	
Alveolus, inflammation, acute, focal			1 (2%)
Artery, adventitia, inflammation, chronic, multifocal	1 (2%)		
Artery, media, hypertrophy, multifocal	4 (8%)		
Peribronchial, infiltration cellular, lymphocytic, diffuse		1 (2%)	1 (2%)
Peribronchial, infiltration cellular, lymphocytic, multifocal	30 (60%)	28 (56%)	35 (70%)
Peribronchiolar, infiltration cellular, lymphocytic, multifocal	2 (4%)		2 (4%)
Perivascular, infiltration cellular, lymphocytic, diffuse	1 (2%)		1 (2%)
Perivascular, infiltration cellular, lymphocytic, multifocal	16 (32%)	23 (46%)	8 (16%)
Pleura, foreign body, focal	1 (2%)		1 (2%)
Pleura, granuloma, focal	1 (2%)		
Pleura, inflammation, acute, diffuse	1 (2%)		
Pleura, inflammation, acute, multifocal			1 (2%)
Pleura, inflammation, chronic, diffuse		1 (2%)	

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
RESPIRATORY SYSTEM (Continued)			
Nose	(49)	(49)	(50)
Foreign body	35 (71%)	35 (71%)	33 (66%)
Hyperplasia, lymphoid, focal			1 (2%)
Glands, hyperplasia	19 (39%)	45 (92%)	49 (98%)
Nasolacrimal duct, exudate, diffuse	1 (2%)		
Olfactory epithelium, atrophy	25 (51%)	46 (94%)	49 (98%)
Turbinate, exudate	15 (31%)	22 (45%)	17 (34%)
Turbinate, fungus, focal			1 (2%)
Turbinate, fungus, multifocal	1 (2%)		
Turbinate, hemorrhage, diffuse		2 (4%)	
Turbinate, hemorrhage, focal	1 (2%)		
Turbinate, hemorrhage, multifocal		1 (2%)	
Turbinate, inflammation, acute, diffuse	2 (4%)		2 (4%)
Turbinate, inflammation, acute, focal			1 (2%)
Turbinate, inflammation, acute, multifocal	5 (10%)	22 (45%)	39 (78%)
Turbinate, inflammation, chronic, diffuse	1 (2%)	10 (20%)	3 (6%)
Turbinate, inflammation, chronic, focal	1 (2%)	4 (8%)	2 (4%)
Turbinate, inflammation, chronic, multifocal	29 (59%)	17 (35%)	16 (32%)
Turbinate, ulcer, focal	1 (2%)		
Trachea	(49)	(50)	(50)
Inflammation, acute, focal			1 (2%)
Inflammation, chronic, diffuse	3 (6%)	3 (6%)	1 (2%)
Inflammation, chronic, focal	2 (4%)	5 (10%)	1 (2%)
Inflammation, chronic, multifocal	6 (12%)	7 (14%)	10 (20%)
Peritracheal tissue, abscess, multifocal	1 (2%)		
Peritracheal tissue, foreign body, focal			1 (2%)
Peritracheal tissue, hemorrhage, focal			1 (2%)
Peritracheal tissue, inflammation, acute, focal	1 (2%)		
Peritracheal tissue, inflammation, chronic, diffuse		1 (2%)	
SPECIAL SENSES SYSTEM			
None			
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Cyst, multiple	1 (2%)		1 (2%)
Inflammation, chronic, focal	1 (2%)	2 (4%)	4 (8%)
Inflammation, chronic, multifocal	2 (4%)	10 (20%)	7 (14%)
Bilateral, abscess, multifocal	1 (2%)		
Bilateral, glomerulosclerosis, diffuse	2 (4%)	1 (2%)	
Bilateral, hematopoietic cell proliferation, diffuse		1 (2%)	
Bilateral, inflammation, chronic, diffuse	1 (2%)		
Bilateral, inflammation, chronic, multifocal	33 (66%)	21 (42%)	21 (42%)
Bilateral, cortex, abscess, multifocal		1 (2%)	
Bilateral, pelvis, bacterium, multifocal	1 (2%)		
Bilateral, renal tubule, bacterium, multifocal		1 (2%)	
Bilateral, renal tubule, casts protein, multifocal		1 (2%)	
Bilateral, renal tubule, mineralization, multifocal			1 (2%)
Bilateral, renal tubule, pigmentation, multifocal		2 (4%)	
Bilateral, renal tubule, regeneration, multifocal		1 (2%)	
Capsule, interstitial tissue, inflammation, chronic		1 (2%)	
Glomerulus, renal tubule, amyloid deposition, diffuse	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-CARVONE (Continued)

	Vehicle Control	375 mg/kg	750 mg/kg
URINARY SYSTEM			
Kidney (Continued)	(50)	(50)	(50)
Interstitial tissue, renal tubule, pigmentation, multifocal	1 (2%)		
Left, hydronephrosis	1 (2%)		
Pelvis, bacterium, focal	1 (2%)		
Pelvis, bacterium, multifocal	1 (2%)	1 (2%)	1 (2%)
Pelvis, concretion, multifocal	1 (2%)		
Renal tubule, bacterium, multifocal	1 (2%)		
Renal tubule, casts protein, multifocal	1 (2%)		
Renal tubule, degeneration, focal	3 (6%)		
Renal tubule, degeneration, multifocal		1 (2%)	
Renal tubule, regeneration, focal	1 (2%)	1 (2%)	
Renal tubule, regeneration, multifocal	2 (4%)		
Ureter			(1)
Dilatation			1 (100%)
Urinary bladder	(46)	(46)	(48)
Abscess, focal	1 (2%)	1 (2%)	
Infiltration cellular, lymphocytic, focal	4 (9%)	6 (13%)	5 (10%)
Infiltration cellular, lymphocytic, multifocal	16 (35%)	22 (48%)	19 (40%)
Serosa, inflammation, chronic, multifocal		1 (2%)	

APPENDIX C

SENTINEL ANIMAL PROGRAM

APPENDIX C. SENTINEL ANIMAL PROGRAM

Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen B6C3F$_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Data from animals surviving 24 months were collected from 5/50 randomly selected vehicle control animals of each sex. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the antibody titers. The following tests were performed:

Hemagglutination Inhibition	Complement Fixation	ELISA
PVM (pneumonia virus of mice)	M. Ad. (mouse adenovirus) (12,18,24 mo)	MHV (mouse hepatitis virus) (12,18,24 mo)
Reo 3 (reovirus type 3)	MHV (6 mo)	GD VII (24 mo)
GDVII (Theiler's encephalomyelitis virus) (6,12,18 mo)	LCM (lymphocytic choriomeningitis virus)	*M. pul.* (*Mycoplasma pulmonis*) (12 mo)
Poly (polyoma virus)		
MVM (minute virus of mice)		
Ectro (infectious ectromelia)		
Sendai		

Immunofluorescence Assay
MHV (24 mo)

Results

Results are presented in Table C1.

TABLE C1. MURINE ANTIBODY DETERMINATIONS FOR MICE IN THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE (a)

Interval (months)	Number of Animals	Positive Serologic Reaction for
6	(b)	None positive
12	1/10	Sendai
	8/10	MHV
18	8/10	MHV
24	9/10	MHV

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from vehicle control animals just before they were killed; samples were sent to Microbiological Associates, Inc. (Bethesda, MD) for determination of antibody titers.
(b) No positive antibody titers were observed for any of the 10 mice tested.

APPENDIX D

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Pellet Diet: May 1982 to May 1984

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

TABLE D1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NCI, 1976; NIH, 1978
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE D2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE D3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (percent by weight)	23.19 ± 1.08	21.3-26.3	25
Crude fat (percent by weight)	5.11 ± 0.55	3.3-5.7	25
Crude fiber (percent by weight)	3.48 ± 0.51	2.9-5.6	25
Ash (percent by weight)	6.62 ± 0.38	5.7-7.3	25
Amino Acids (percent of total diet)			
Arginine	1.320 ± 0.072	1.310-1.390	5
Cystine	0.319 ± 0.088	0.218-0.400	5
Glycine	1.146 ± 0.063	1.060-1.210	5
Histidine	0.571 ± 0.026	0.531-0.603	5
Isoleucine	0.914 ± 0.030	0.881-0.944	5
Leucine	1.946 ± 0.056	1.850-1.990	5
Lysine	1.280 ± 0.067	1.200-1.370	5
Methionine	0.436 ± 0.165	0.306-0.699	5
Phenylalanine	0.938 ± 0.158	0.665-1.050	5
Threonine	0.855 ± 0.035	0.824-0.898	5
Tryptophan	0.277 ± 0.221	0.156-0.671	5
Tyrosine	0.618 ± 0.086	0.564-0.769	5
Valine	1.108 ± 0.043	1.050-1.170	5
Essential Fatty Acids (percent of total diet)			
Linoleic	2.290 ± 0.313	1.83-2.52	5
Linolenic	0.258 ± 0.040	0.210-0.308	5
Vitamins			
Vitamin A (IU/kg)	12,584 ± 4,585	4,100-24,000	25
Vitamin D (IU/kg)	4,450 ± 1,382	3,000-6,300	4
α-Tocopherol (ppm)	43.58 ± 6.92	31.1-48.00	5
Thiamine (ppm)	17.16 ± 3.58	12.0-27.0	25
Riboflavin (ppm)	7.6 ± 0.85	6.10-8.20	5
Niacin (ppm)	97.8 ± 31.68	65.0-150.0	5
Pantothenic acid (ppm)	30.06 ± 4.31	23.0-34.0	5
Pyridoxine (ppm)	7.68 ± 1.31	5.60-8.80	5
Folic acid (ppm)	2.62 ± 0.89	1.80-3.70	5
Biotin (ppm)	0.254 ± 0.053	0.19-0.32	5
Vitamin B_{12} (ppb)	24.21 ± 12.66	10.6-38.0	5
Choline (ppm)	3,122 ± 416.8	2,400-3,430	5
Minerals			
Calcium (percent)	1.28 ± 0.12	1.11-1.54	25
Phosphorus (percent)	0.97 ± 0.05	0.89-1.10	25
Potassium (percent)	0.900 ± 0.098	0.772-0.971	3
Chloride (percent)	0.513 ± 0.114	0.380-0.635	5
Sodium (percent)	0.323 ± 0.043	0.258-0.371	5
Magnesium (percent)	0.167 ± 0.012	0.151-0.181	5
Sulfur (percent)	0.304 ± 0.064	0.268-0.420	5
Iron (ppm)	410.3 ± 94.04	262.0-523.0	5
Manganese (ppm)	90.29 ± 7.15	81.7-99.4	5
Zinc (ppm)	52.78 ± 4.94	46.1-58.2	5
Copper (ppm)	10.72 ± 2.76	8.09-15.39	5
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.85 ± 0.25	1.44-2.09	5
Cobalt (ppm)	0.681 ± 0.14	0.490-0.780	4

TABLE D4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.52 ± 0.15	0.17-0.77	25
Cadmium (ppm) (a)	<0.10		25
Lead (ppm)	0.76 ± 0.64	0.33-3.37	25
Mercury (ppm) (a)	<0.05		25
Selenium (ppm)	0.32 ± 0.06	0.13-0.42	25
Aflatoxins (ppb)	<5.0		25
Nitrate nitrogen (ppm) (b)	9.03 ± 4.76	0.10-22.0	25
Nitrite nitrogen (ppm) (b)	1.66 ± 1.76	0.10-7.10	25
BHA (ppm) (c)	4.08 ± 4.76	2.00-17.0	25
BHT (ppm) (c)	2.68 ± 2.55	1.00-12.0	25
Aerobic plate count (CFU/g) (d)	44,080 ± 33,191	6,600-130,000	25
Coliform (MPN/g) (e)	49.20 ± 125	3.00-460	25
E. coli (MPN/g) (e)	<3.00		25
Total nitrosamines (ppb) (f)	5.43 ± 5.85	1.8-30.9	25
N-Nitrosodimethylamine (ppb) (f)	4.39 ± 5.85	0.80-30.0	25
N-Nitrosopyrrolidine (ppb) (f)	1.04 ± 0.25	0.81-1.70	25
Pesticides (ppm)			
α-BHC (a,g)	<0.01		25
β-BHC (a)	<0.02		25
γ-BHC-Lindane (a)	<0.01		25
δ-BHC (a)	<0.01		25
Heptachlor (a)	<0.01		25
Aldrin (a)	<0.01		25
Heptachlor epoxide (a)	<0.01		25
DDE (a)	<0.01		25
DDD (a)	<0.01		25
DDT (a)	<0.01		25
HCB (a)	<0.01		25
Mirex (a)	<0.01		25
Methoxychlor (a)	<0.05		25
Dieldrin (a)	<0.01		25
Endrin (a)	<0.01		25
Telodrin (a)	<0.01		25
Chlordane (a)	<0.05		25
Toxaphene (a)	<0.1		25
Estimated PCBs (a)	<0.2		25
Ronnel (a)	<0.01		25
Ethion (a)	<0.02		25
Trithion (a)	<0.05		25
Diazinon (a)	<0.1		25
Methyl parathion (a)	<0.02		25
Ethyl parathion (a)	<0.02		25
Malathion (h)	0.10 ± 0.09	0.05-0.45	25
Endosulfan I (a)	<0.01		25
Endosulfan II (a)	<0.01		25
Endosulfan sulfate (a)	<0.03		25

(a) All values were less than the detection limit, given in the table as the mean.
(b) Sources of contamination: alfalfa, grains, and fish meal
(c) Sources of contamination: soy oil and fish meal
(d) CFU = colony-forming unit
(e) MPN = most probable number
(f) All values were corrected for percent recovery.
(g) BHC is hexachlorocyclohexane or benzene hexachloride
(h) Fourteen lots contained more than 0.05 ppm.

APPENDIX E

CHEMICAL CHARACTERIZATION, ANALYSIS, AND DOSE PREPARATION OF *d*-CARVONE FOR THE TOXICOLOGY STUDIES

APPENDIX E. CHEMICAL CHARACTERIZATION

Procurement and Characterization of *d*-Carvone

d-Carvone was obtained as a clear, pale yellow liquid in one lot (lot no. K-332) from Fritzsche, Dodge, and Olcott, Inc. (New York, NY), with the purity indicated on the label as food grade (Food Chemicals Codex, purity of not less than 95%). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on analyses performed in support of the *d*-carvone studies are on file at the National Institute of Environmental Health Services.

The study chemical was identified as *d*-carvone by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy and by optical rotation. All spectra (Figures E1 and E2) were consistent with those in the literature (Sadtler Standard Spectra). The specific rotation, +60.3°, was consistent with a literature value (Merck, 1968) and confirmed that the sample was the *d*-isomer.

Purity of *d*-carvone was determined to be approximately 96% by elemental analysis, Karl Fischer water analysis, back-titration of excess base with hydrochloric acid after the carbonyl group was reacted with hydroxylamine hydrochloride dissolved in alcoholic potassium hydroxide (FCC, 1966), thin-layer chromatography, and gas chromatography. Thin-layer chromatography was performed on silica gel plates with *n*-hexane:1,4-dioxane (9:1), saturated, under nitrogen, and in the dark (solvent system 1) or ethyl acetate:hexanes (3:7), saturated, under nitrogen, and in the dark (solvent system 2). *trans*-Cinnamaldehyde was the reference standard, and visualization was with a spray of 5% (w/v) vanillin in concentrated sulfuric acid, followed by heating until maximum color development occurred. Gas chromatographic analysis was performed with flame ionization detection, nitrogen as the carrier, and either a 10% DEGS-PS column and a carrier flow rate of 45 ml/minute (system 1) or a 20% SP2100/0.1% Carbowax 1500 column and a carrier flow rate of 46 ml/minute (system 2).

Results of elemental analysis for lot no. K-332 indicated that carbon and hydrogen were slightly low. Karl Fischer analysis indicated 0.88% water. Titration of the carbonyl group indicated a purity of 98.0%. Thin-layer chromatographic solvent system 1 detected a major spot followed by two trace impurities and preceded by one trace and three slight trace impurities. Thin-layer chromatographic solvent system 2 indicated a major spot followed by one trace impurity and preceded by one trace and three slight trace impurities. Gas chromatographic system 1 detected a major peak preceded by 16 impurities and followed by 6 impurities, with a total relative area of 4.6%. Eight impurities, seven preceding and one following the major peak, had individual relative areas greater than 0.1% and totaled 4.1%. Gas chromatographic system 2 detected a major peak preceded by 11 impurities and followed by 8 impurities, with a total relative area of 4.2%. Eight impurities, five preceding and three following the major peak, had relative areas greater than 0.1% and totaled 4.0%.

Stability studies performed by high-performance liquid chromatography (Varian 5000) indicated that *d*-carvone was stable as a bulk chemical when stored in the dark in glass containers with Teflon®-lined caps at temperatures up to 25° C for 2 weeks. Samples kept at 60° C had about 3% decomposition. During the 2-year studies, *d*-carvone was stored under a nitrogen headspace at 0° C. Confirmation of the stability of the bulk chemical during the 2-year studies was obtained by gas chromatography and titration of the carbonyl group. No deterioration of *d*-carvone was seen throughout the studies. The identity of the chemical at the study laboratory was confirmed by infrared analysis throughout the studies.

The complete battery of Food Chemicals Codex tests (FCC, 1981) for *d*-carvone was performed after storage for 5 years and indicated that lot no. K-332, a synthetic *d*-carvone, met the Food Chemicals Codex specifications that were in effect at the time of purchase (FCC, 1972). Under present requirements, results of the titrimetric assay (96.5%) met the specification for natural *d*-carvone (95.0%) but were slightly lower than that required for the synthetic material (97.0%).

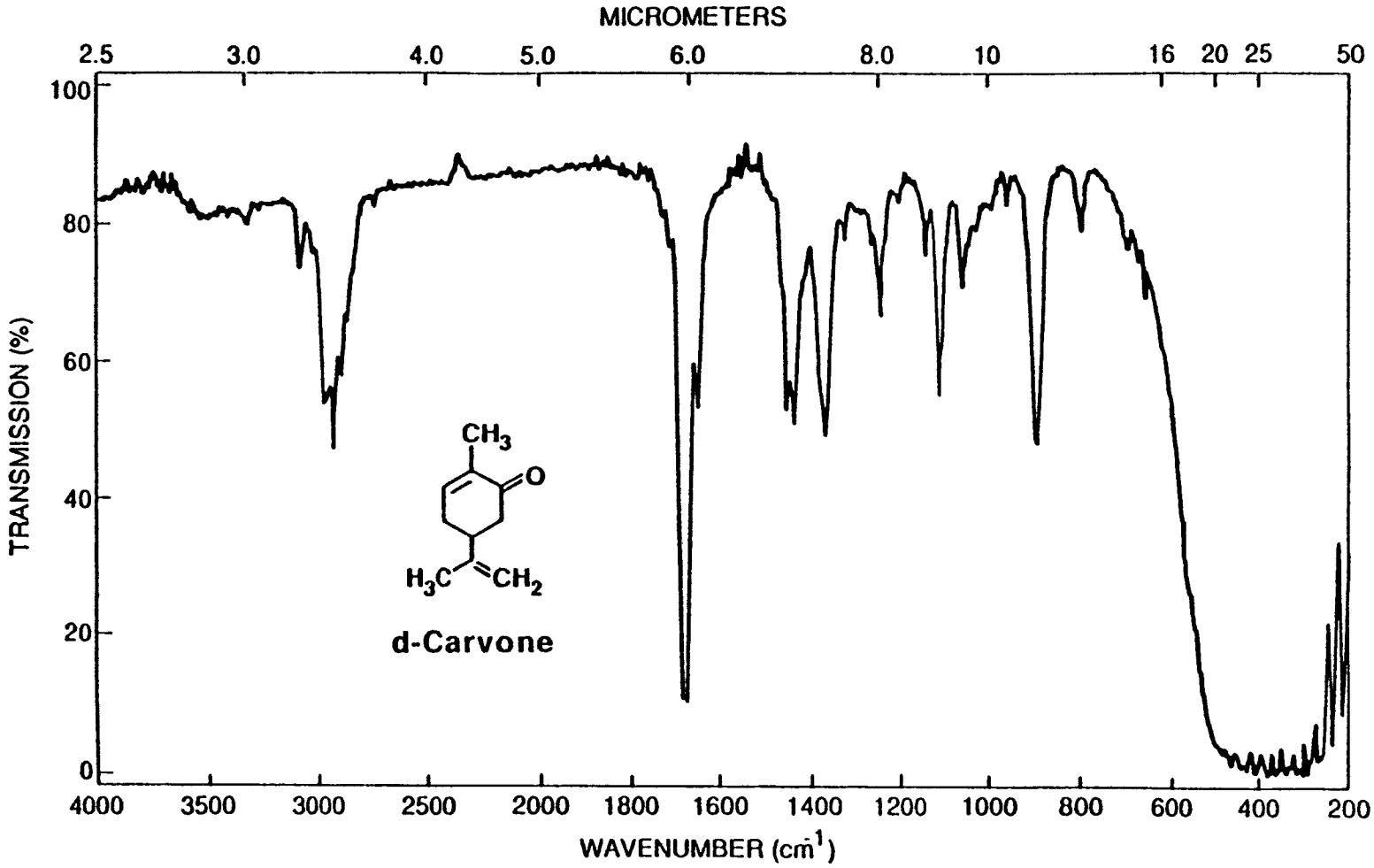


FIGURE E1. INFRARED ABSORPTION SPECTRUM OF *d*-CARVONE (LOT NO. K-332)

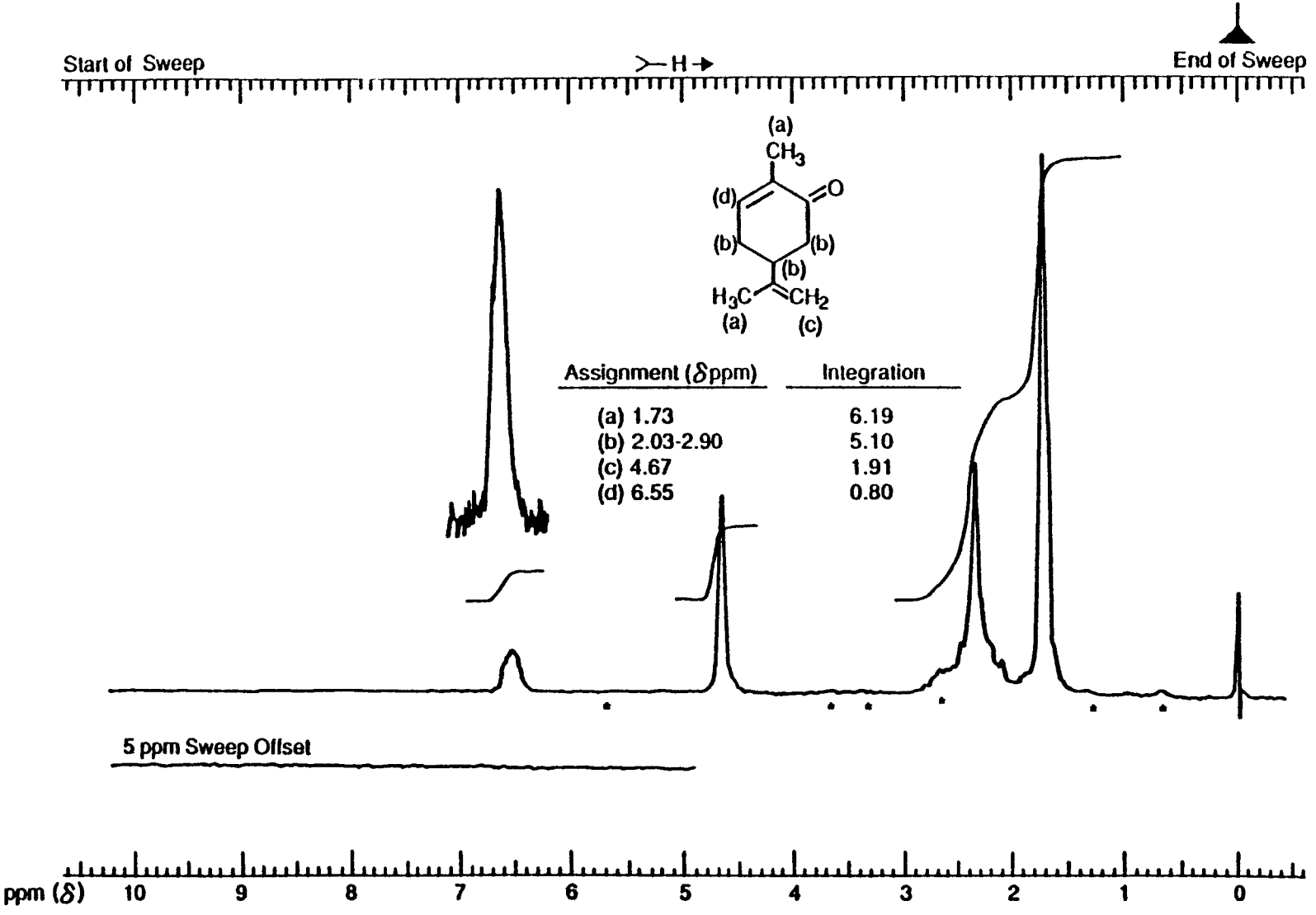


FIGURE E2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF *d*-CARVONE (LOT NO. K-332)

Preparation and Characterization of Dose Mixtures

The stability of *d*-carvone mixed with NIH 07 Rat and Mouse Ration at 1% and stored at temperatures varying from – 20° C to room temperature for 2 weeks was determined. The feed mixtures were extracted with acetonitrile and analyzed by gas chromatography with a 10% Carbowax 2M column and flame ionization detection. The study found that more than 4% of the *d*-carvone was lost during the feed blending process. Formulated diets stored open to air and light under simulated animal exposure conditions lost 5.5%, 11.2%, and 22.6% of the study chemical after 1, 3, and 7 days, respectively. The same feed stored in the dark for 2 weeks in sealed containers lost 0%, 2%, and 3% of the study chemical at – 20° C, 5° C, and room temperature.

Because the feed blends of *d*-carvone were found to be unstable under the feed blending and simulated dosing conditions and because *d*-carvone is insoluble in water, corn oil gavage was selected as the route of administration for these studies. The stability of *d*-carvone in corn oil at 0.5% (5 mg/g) stored at room temperature or at 5° C for 21 days was determined. The corn oil solutions were extracted with methanol and analyzed by high-performance liquid chromatography with a Brownlee RP-18 column and ultraviolet detection at 229 nm. The *d*-carvone/corn oil solutions were found to be stable for at least 21 days when stored in the dark at room temperature or at 5° C. The corn oil solutions were also stable under simulated dosing conditions for at least 3 hours.

The appropriate amount of *d*-carvone was mixed (w/v) with corn oil to give the desired concentrations (Table E1). Periodic analyses of formulated *d*-carvone/corn oil dose mixtures were conducted at the study laboratory and the analytical chemistry laboratory. Dose mixtures were analyzed before the start of, and once during, the 13-week studies. During the 13-week studies, concentrations of *d*-carvone in corn oil were determined by gas chromatography with a 10% Carbowax column and flame ionization detection. During the 13-week studies, all dose mixtures were found to be within ± 10% of the target concentrations by the study laboratory (Table E2). The referee laboratory analyzed one dose mixture.

During the 2-year studies, the dose mixtures were analyzed at approximately 8-week intervals by extraction with methanol, followed by analysis with ultraviolet detection at 236 nm. For the *d*-carvone studies, all 46 mixtures that were analyzed were formulated within ± 10% of the target concentrations (Table E3). Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results from the study laboratory (Table E4).

TABLE E1. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE GAVAGE STUDIES OF *d*-CARVONE

Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation		
Appropriate weight of *d*-carvone was dissolved in corn oil to a specified volume in a graduated cylinder. Mixture was stored under nitrogen.	Same as 16-d studies	*d*-Carvone was dissolved in corn oil in a beaker and transferred to a volumetric flask. Solution was diluted to appropriate volume, transferred to a Nalgene® container, and manually shaken until homogeneous.
Maximum Storage Time		
1 wk	1 wk	3 wk
Storage Conditions		
70° F	Room temperature	Room temperature

TABLE E2. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE THIRTEEN-WEEK GAVAGE STUDIES OF *d*-CARVONE

Date Mixed	Concentration of *d*-Carvone in Corn Oil (mg/ml)		Determined as a Percent of Target
	Target	Determined (a)	
07/31/81			
	9.3	9.3	100
	18.7	18.5	99
	37.5	36.5	97
	75.0	74.2	99
	150.0	148	99
09/11/81			
	9.3	8.7	94
	18.7	17.6	94
	37.5	36.3	97
	75.0	76.2	102
	150.0	154	104

(a) Results of duplicate analysis

TABLE E3. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE

Date Mixed	Concentration of *d*-Carvone in Corn Oil for Target Concentration (mg/ml) (a)	
	37.5 mg/ml	75 mg/ml
06/23/82	37.8	74.8
09/29/82	37.7	75.3
11/17/82	36.7	73.6
11/24/82	37.7	76.1
12/01/82		75.6
01/05/83	37.7	75.2
	37.9	74.4
02/09/83	38.3	75.8
03/30/83	37.6	76.0
05/11/83	38.6	75.3
05/18/83	36.5	73.0
06/15/83	38.2	
	38.3	
06/29/83	36.8	75.2
10/17/83	36.1	74.2
10/31/83	38.2	77.4
12/12/83	37.8	
	37.9	76.9
	38.0	75.8
02/06/84	38.1	75.8
02/20/84	38.1	76.6
04/02/84	38.1	75.5
04/16/84	38.6	76.1
05/29/84	38.2	76.3
06/11/84	38.2	75.6
Mean (mg/ml)	37.8	75.5
Standard deviation	0.68	1.03
Coefficient of variation (percent)	1.8	1.4
Range (mg/ml)	36.1-38.6	73.0-77.4
Number of samples	24	22

(a) Results of duplicate analysis

TABLE E4. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF *d*-CARVONE

Date Mixed	Target Concentration (mg/ml)	Determined Concentration (mg/ml)	
		Study Laboratory (a)	Referee Laboratory (b)
12/01/82	75.0	75.6	73.1
05/29/84	37.5	38.2	38.2

(a) Results of duplicate analysis
(b) Results of triplicate analysis

APPENDIX F

GENETIC TOXICOLOGY

OF *d*-CARVONE

METHODS

Salmonella Protocol: Testing was performed as reported by Ames et al. (1975) with modifications listed below and described in greater detail by Haworth et al. (1983). Chemicals were sent to the laboratory as coded aliquots from Radian Corporation (Austin, TX). The study chemical was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours.

Chemicals were tested in four strains. Each test consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the study chemical. The high dose was limited by toxicity or solubility but did not exceed 333 mg/plate. All negative assays were repeated, and all positive assays were repeated under the conditions that elicited the positive response.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants which was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

Chinese Hamster Ovary Cytogenetics Assays: Testing was performed as reported by Galloway et al. (1985) and is described briefly below. Chemicals were sent to the laboratory as coded aliquots from Radian Corporation (Austin, TX). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued for 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; incubation proceeded for an additional 26 hours, with colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the chromosomal aberration test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours; colcemid was added, and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberration test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal

aberration test was based on the cell cycle information obtained in the SCE test; if cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 100 or 200 first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCEs, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P<0.003$) trend test or a significantly increased dose point ($P<0.05$) was sufficient to indicate a chemical effect.

RESULTS

d-Carvone, at concentrations up to 333 µg/plate, was negative for induction of gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Mortelmans et al., 1986; Table F1). In cytogenetic tests with CHO cells, *d*-carvone induced both sister chromatid exchanges (Table F2) and chromosomal aberrations (Table F3) with and without Aroclor 1254-induced male Sprague Dawley rat liver S9. Although results were statistically positive in two of the three SCE trials, there was no correlation of dose with response; this same phenomenon also occurred in the second trial of the chromosomal aberration test conduced without S9. No slowing of the cell cycle was noted in the CHO cells used for the SCE test, but in the chromosomal aberration test, the cells used in the trials conducted without S9 required delayed harvest to offset chemical-induced cell cycle delay.

TABLE F1. MUTAGENICITY OF *d*-CARVONE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	97 ± 2.1	89 ± 4.6	145 ± 13.1	121 ± 12.1	156 ± 12.5	133 ± 11.7
	3.3	108 ± 13.3	84 ± 7.6	144 ± 6.1	119 ± 9.9	121 ± 3.8	94 ± 14.1
	10	91 ± 1.5	75 ± 4.0	131 ± 9.4	113 ± 9.6	137 ± 6.5	105 ± 6.7
	33	105 ± 3.2	89 ± 4.1	143 ± 3.8	107 ± 6.7	123 ± 3.8	108 ± 2.2
	100	95 ± 4.5	73 ± 7.4	121 ± 5.7	104 ± 3.0	115 ± 6.5	100 ± 5.0
	333	71 ± 4.4	48 ± 9.0	104 ± 9.9	78 ± 8.4	94 ± 6.0	65 ± 11.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		492 ± 75.2	224 ± 7.0	2,162 ± 99.9	3,096 ± 178.2	1,631 ± 121.6	3,091 ± 157.4
TA1535	0	5 ± 1.5	4 ± 1.5	8 ± 1.2	6 ± 1.5	11 ± 1.9	8 ± 3.0
	3.3	6 ± 1.3	3 ± 0.9	5 ± 0.3	5 ± 1.3	7 ± 2.1	5 ± 0.3
	10	5 ± 1.0	3 ± 0.7	3 ± 0.3	4 ± 0.9	10 ± 1.5	5 ± 1.2
	33	5 ± 1.0	3 ± 0.3	3 ± 0.7	3 ± 0.7	6 ± 0.7	4 ± 1.5
	100	1 ± 0.6	2 ± 1.0	7 ± 0.3	5 ± 0.6	4 ± 0.3	3 ± 0.6
	333	2 ± 0.6	1 ± 0.6	7 ± 0.3	4 ± 0.6	10 ± 0.9	3 ± 2.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		260 ± 13	122 ± 11.1	192 ± 13.3	41 ± 5.8	109 ± 8.1	76 ± 13.4
TA1537	0	4 ± 0.9	3 ± 0.7	6 ± 1.0	6 ± 0.6	5 ± 0.6	6 ± 1.3
	3.3	3 ± 0.3	3 ± 0.6	3 ± 0.6	5 ± 0.7	4 ± 0.3	5 ± 1.2
	10	2 ± 0.3	2 ± 0.0	5 ± 0.0	5 ± 1.2	4 ± 0.9	8 ± 1.2
	33	2 ± 0.7	3 ± 1.2	8 ± 0.3	2 ± 0.7	5 ± 0.9	7 ± 1.7
	100	Toxic	3 ± 1.0	3 ± 0.3	4 ± 0.7	6 ± 1.5	8 ± 2.7
	333	Toxic	3 ± 0.6	4 ± 0.3	6 ± 1.2	1 ± 0.3	6 ± 0.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		543 ± 68	1,041 ± 154.2	125 ± 16.2	261 ± 12.0	65 ± 2.3	211 ± 24.1
TA98	0	16 ± 2.9	14 ± 1.9	27 ± 2.8	25 ± 1.2	25 ± 2.6	21 ± 3.8
	3.3	12 ± 2.1	16 ± 2.8	36 ± 1.0	23 ± 5.0	29 ± 0.9	20 ± 3.2
	10	18 ± 3.2	15 ± 0.3	27 ± 1.2	23 ± 1.2	25 ± 0.9	15 ± 3.2
	33	15 ± 2.3	11 ± 1.2	29 ± 1.2	25 ± 2.5	26 ± 1.2	22 ± 0.9
	100	17 ± 2.0	13 ± 0.9	21 ± 0.7	23 ± 2.0	25 ± 1.0	24 ± 1.5
	333	15 ± 1.8	10 ± 2.1	22 ± 0.7	17 ± 4.0	27 ± 2.7	22 ± 2.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		305 ± 47	359 ± 21.4	1,815 ± 76.3	1,969 ± 56.9	1,741 ± 264.3	973 ± 201.3

(a) Study performed at Case Western Reserve University. The detailed protocol is presented by Mortelmans et al. (1986). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE F2. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY *d*-CARVONE (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/ Chromosome (percent) (b)
−S9 (c)								
Trial 1--Summary: Positive								
Dimethyl sulfoxide		50	1,050	333	0.31	6.7	25.8	
d-Carvone	0.167	50	1,049	542	0.51	10.8	25.8	*62.92
	0.502	50	1,050	423	0.40	8.5	25.8	*27.03
	5	50	1,046	536	0.51	10.7	25.8	*61.58
Mitomycin C	0.001	50	1,050	610	0.58	12.2	25.8	83.18
	0.01	5	105	196	1.86	39.2	25.8	488.59
Trend test: P<0.001								
Trial 2--Summary: Positive								
Dimethyl sulfoxide		25	521	279	0.53	11.2	25.8	
d-Carvone	5	25	519	326	0.62	13.0	25.8	17.30
	15	25	517	394	0.76	15.8	25.8	*42.31
	20	25	514	389	0.75	15.6	25.8	*41.33
Mitomycin C	0.001	25	522	368	0.70	14.7	25.8	31.65
	0.01	5	105	259	2.46	51.8	25.8	360.63
Trend test: P<0.001								
+S9 (d)--Summary: Positive								
Dimethyl sulfoxide		50	1,050	350	0.33	7.0	25.8	
d-Carvone	50.2	50	1,049	371	0.35	7.4	25.8	6.10
	167.3	50	1,048	490	0.46	9.8	25.8	*40.27
	501.8	50	1,049	427	0.40	8.5	25.8	*22.12
Cyclophosphamide	0.4	50	1,048	616	0.58	12.3	25.8	76.34
	2	5	105	181	1.72	36.2	25.8	417.15
Trend test: P<0.001								

(a) Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as described in (c) and (d) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air dried, and stained.
(b) Percentage change in the value of SCEs/chromosome for exposed culture compared with solvent control culture. An increase of 20% or more was considered to be a significant response.
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.
*P<0.01

TABLE F3. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY *d*-CARVONE (a)

Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9 (b)				
Trial 1--Harvest time: 20.2 h (d)				
Dimethyl sulfoxide				
	200	0	0	0.0
d-Carvone				
5	200	3	0.02	1.5
12.5	200	16	0.08	*6.5
25.1	200	21	0.11	*8.0
Summary: Positive				
Mitomycin C				
0.05	200	37	0.19	16.5
0.08	25	24	0.96	60.0
Trend test: P<0.001				
Trial 2--Harvest time: 20.1 h (d)				
Dimethyl sulfoxide				
	100	0	0.00	0.0
d-Carvone				
25	100	1	0.01	1.0
31.3	100	9	0.09	*7.0
37.5	100	4	0.04	3.0
43.8	100	1	0.01	1.0
Summary: Equivocal				
Mitomycin C				
0.05	100	56	0.56	33.0
0.08	25	25	1.00	68.0
Trend test: P=0.154				

Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
+S9 (c)				
Trial 1--Harvest time: 12 h				
Dimethyl sulfoxide				
	200	1	0.01	0.5
d-Carvone				
75	200	4	0.02	2.0
100	200	3	0.02	1.5
250	200	13	0.07	*5.5
Summary: Weakly positive				
Cyclophosphamide				
7.5	200	17	0.09	7.0
37.5	25	20	0.80	52.0
Trend test: P<0.001				
Trial 2--Harvest time: 12.0 h				
Dimethyl sulfoxide				
	100	1	0.01	1.0
d-Carvone				
250	100	2	0.02	2.0
325	100	1	0.01	1.0
400	100	22	0.22	*19.0
Summary: Weakly positive				
Cyclophosphamide				
7.5	100	4	0.04	4.0
37.5	25	23	0.92	56.0
Trend test: P<0.001				

(a) Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.
(d) Because of significant chemical-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened to provide sufficient metaphases at harvest.
*P<0.05

APPENDIX G

AUDIT SUMMARY

APPENDIX G. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and draft (May 1989) of the Technical Report for the 2-year studies of *d*-carvone in mice were audited for the National Institute of Environmental Health Sciences (NIEHS) at the National Toxicology Program (NTP) Archives quality assurance contractors. The audit included review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All inlife records including protocol, correspondence, animal identification, animal husbandry, environmental conditions, dosing, external masses, mortality, and serology.
(3) Body weight and clinical observation data; all data were scanned before individual data for a random 10% sample of animals in each study group were reviewed in detail.
(4) All study chemistry records.
(5) All postmortem records for individual animals concerning date of death, disposition code, condition code, tissue accountability, correlation of masses or clinical signs recorded at or near the last inlife observation with gross observations and microscopic diagnoses, consistency of data entry on necropsy record forms, and correlation between gross observations and microscopic diagnoses.
(6) Inventory for wet tissue bags for all animals and residual wet tissues from a random 20% sample of animals in each study group, plus other relevant cases, to evaluate the integrity of individual animal identity and the thoroughness of necropsy and trimming procedure performance.
(7) Blocks and slides of tissues from a random 20% sample of animals from each study group, plus animals with less than complete or correct identification, to examine for proper inventory, labeling, matching of tissue sections, and preservation.
(8) All microscopic diagnoses for a random 10% sample of animals, plus 100% of the changes in diagnoses made to preliminary pathology tables, to verify their incorporation into the final pathology tables.
(9) The extent of correlation between the data, factual information, and procedures for the 2-year studies as presented in the draft Technical Report and the study records available at the NTP Archives.

Procedures and events for the exposure phase of the studies were documented adequately, with the exception that records needed to document part or all of the following were not at the Archives: room air change rate, room light cycle, type of cage and filters, and dosing regimen followed during week 103. Review of the study records indicated that protocol-specified procedures for animal care were followed adequately. Records that documented the preparation, analysis, and administration of doses to animals were complete and accurate. Recalculation of approximately 30% of the group mean body weight values in the Technical Report showed all 24 to be correct.

Data entries on necropsy forms were made appropriately, and condition codes were consistent with other observations recorded at necropsy. The correlation between observations of external masses recorded both at the last inlife observation and at necropsy was good (all 36 correlated). The date of animal removal correlated with the date of necropsy for all 105 unscheduled-death animals. The reason for animal removal recorded during life correlated with the disposition code recorded at necropsy for 289/300 mice; none of the discrepancies influenced the overall survival values presented in the Technical Report, but 9 of them (1 vehicle control male, 2 low dose male, 4 high dose male, 1 low dose female, and 1 high dose female) were suggestive of dosing accident rather than natural death or moribund kill, which could influence the survival-adjusted statistical analyses, but only slightly.

Individual animal identifiers (feet) were present and correct in the residual tissue bags for 84/91 mice examined. Review of the entire data trial for the seven mice with less than complete and correct identifiers indicated that the integrity of individual-animal identity had been maintained. A total of 16

untrimmed potential lesions were found in the wet tissues of 91 mice examined; 8 of these involved the forestomach (2 vehicle control male, 2 low dose male, 1 high dose male, 1 low dose female, and 2 high dose female), and the other 8 were scattered among different organs and groups. Intestinal segments were incompletely opened for 55/91 mice examined; however, no apparent untrimmed potential lesions were evident by external examination of residual tissues. Each gross observation made at necropsy had a corresponding microscopic diagnosis, except for 10, 6 of which involved some of the untrimmed potential lesions of the forestomach referred to above. Blocks and slides were present and labeled correctly; corresponding tissue sections in blocks and on slides matched each other properly. All post-Pathology Working Group changes in diagnoses had been incorporated into the final pathology tables.

This summary describes general audit findings and the extent to which the data and factual information presented in the Technical Report are supported by records at the NTP archives. Full details are presented in audit reports that are on file at the NIEHS.

☆ U.S. GOVERNMENT PRINTING OFFICE : 1990 0 - 258-617