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TOXICOLOGY AND CARCINOGENESIS STUDIES OF *d*-LIMONENE

(CAS NO. 5989-27-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

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

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF *d*-LIMONENE

(CAS NO. 5989-27-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

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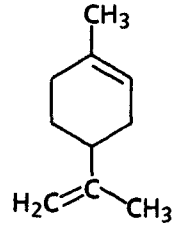

***d*-LIMONENE**

CAS No. 5989-27-5

$C_{10}H_{16}$ Molecular weight 136.2

Synonyms: Cyclohexene, 4-isopropenyl-1-methyl; 1-methyl-4-(1-methylethenyl)cyclohexene; *p*-mentha-1,8-diene; carvene; cinene; cajeputene

ABSTRACT

Toxicology and carcinogenesis studies of *d*-limonene, a naturally occurring monoterpene found in many volatile oils, especially in citrus oils, were conducted because of its widespread use as a flavor and fragrance additive for food and household cleaning products and its increasing use as an industrial solvent. The *d*-limonene used in these studies was more than 99% pure and was administered in corn oil by gavage. Short-term studies were conducted in F344/N rats and B6C3F$_1$ mice to identify toxic effects and affected sites and to help establish doses for the 2-year studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

The doses selected for the 16-day studies ranged from 413 to 6,600 mg/kg for both rats and mice; deaths and reduction in body weight gain occurred at the two highest doses. No compound-related clinical signs or histopathologic lesions were observed in any of the surviving dose groups.

In the 13-week studies, doses of *d*-limonene ranged from 150 to 2,400 mg/kg for rats and from 125 to 2,000 mg/kg for mice. Deaths occurred in the high dose group of each species and sex. Greater than 10% reductions in body weight gain were observed in the two highest dose groups of male rats and male mice and the high dose female rats. Rough hair coats and decreased activity were observed at the two highest doses in both rats and mice. There were no chemical-related histopathologic lesions in female rats or in mice of either sex. A compound-related increased severity of nephropathy was observed in the kidney of male rats. This lesion was characterized by degeneration of epithelial cells in the convoluted tubules, granular casts in the outer stripe of the outer medulla, and epithelial regeneration. These lesions have been described as reasonably characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated $\alpha_{2\mu}$-globulin in the cytoplasm of tubular epithelial cells.

Two-year studies of *d*-limonene were conducted by administering 0, 75, or 150 mg/kg *d*-limonene in corn oil by gavage to groups of 50 F344/N male rats, 5 days per week for 103 weeks; groups of 50 female F344/N rats were administered 0, 300, or 600 mg/kg. These doses were selected based on compound-related, potentially life-threatening kidney lesions observed in males at 300 mg/kg and higher and on the large number of deaths of female rats at 2,400 mg/kg. Groups of 50 male B6C3F$_1$ mice were administered 0, 250, or 500 mg/kg according to the same schedule; groups of 50 female B6C3F$_1$ mice were administered 0, 500, or 1,000 mg/kg. These doses were selected based on the deaths

observed for both male and female mice at 2,000 mg/kg during the 13-week studies and the body weight depression in male mice at 1,000 mg/kg and higher.

Mean body weights of rats dosed with *d*-limonene were similar to those of vehicle controls throughout the studies. Survival of the high dose female rats after week 39 and of the vehicle control male rats after week 81 was significantly reduced (survival at week 104--male: vehicle control, 29/50; low dose, 33/50; high dose, 40/50; female: 42/50; 40/50; 26/50). Mean body weights of dosed and vehicle control male mice were similar throughout the studies. Mean body weights of high dose female mice were notably lower than those of the vehicle controls after week 28. Survival of the low dose group of male mice was significantly lower than that of vehicle controls at the end of the study (33/50; 24/50; 39/50). No difference in survival was observed between vehicle control and dosed female mice (43/50; 44/50; 43/50).

In the 2-year studies, the kidney was confirmed as the primary target organ for chemically related lesions. No lesions were observed in female rats. For males, the nonneoplastic lesions included exacerbation of the age-related nephropathy, linear deposits of mineral in the renal medulla and papilla, and focal hyperplasia of the transitional epithelium overlying the renal papilla. Uncommon tubular cell adenomas and adenocarcinomas of the kidney also occurred in dosed male rats, and this effect was supported by a dose-related increased incidence of tubular cell hyperplasia, as shown in the table below.

In subsequent 21-day studies, male and female F344/N rats were administered *d*-limonene at doses ranging from 75 to 1,200 mg/kg. Microscopic examination of the kidney sections from these rats indicated a compound-related increase in intracytoplasmic granules in the proximal convoluted tubules of dosed male rats but not of female rats. The granules were shown to contain $\alpha_{2\mu}$-globulin by an immunohistochemical stain. $\alpha_{2\mu}$-Globulin was shown to be increased in kidney homogenates from dosed male rats by an ELISA test.

In mice, no chemically related increases in neoplasms were observed. The incidence of neoplasms of the anterior pituitary gland in high dose female mice was lower than that in vehicle controls (adenomas or carcinomas, combined: vehicle control, 12/49; high dose, 2/48). Cells with an abnormal number of nuclei (8/49; 32/50) and cytomegaly (23/49; 38/50) were observed in the liver of high dose male mice.

Genetic Toxicology: *d*-Limonene was not mutagenic in four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537), did not significantly increase the number of trifluorothymidine (Tft)-resistant cells in the mouse L5178Y/TK $^{+/-}$ assay, and did not induce chromosomal aberrations or sister chromatid exchanges (SCEs) in cultured CHO cells. All assays were conducted in the presence and absence of exogenous metabolic activation.

INCIDENCES OF MALE RATS WITH RENAL LESIONS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

Site/Lesion	Vehicle Control	75 mg/kg	150 mg/kg
Renal papilla			
Mineralization	7/50	43/50	48/50
Epithelial hyperplasia	0/50	35/50	43/50
Kidney			
Tubular cell hyperplasia	0/50	4/50	7/50
Tubular cell adenoma	0/50	4/50	8/50
Tubular cell adenocarcinoma	0/50	4/50	3/50

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of *d*-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was *no evidence of carcinogenic activity* of *d*-limonene for female F344/N rats that received 300 or 600 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for male B6C3F$_1$ mice that received 250 or 500 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for female B6C3F$_1$ mice that received 500 or 1,000 mg/kg.

An increased severity of spontaneous nephropathy, increased incidences of linear mineralization of the renal medulla and papilla, and hyperplasia of the transitional epithelium of the renal papilla were present in dosed male rats.

SUMMARY OF THE TWO-YEAR GAVAGE AND GENETIC TOXICOLOGY STUDIES OF *d*-LIMONENE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 75, or 150 mg/kg *d*-limonene in corn oil by gavage, 5 d/wk	0, 300, or 600 mg/kg *d*-limonene in corn oil by gavage, 5 d/wk	0, 250, or 500 mg/kg *d*-limonene in corn oil by gavage, 5 d/wk	0, 500, or 1,000 mg/kg *d*-limonene in corn oil by gavage, 5 d/wk
Body weights in the 2-year study			
Approximately 5% reduction in high dose group	Approximately 5% reduction in high dose group	No effect	10% reduction in high dose group by end of study
Survival rates in the 2-year study			
29/50; 33/50; 40/50	42/50; 40/50; 26/50	33/50; 24/50; 39/50	43/50; 44/50; 43/50
Nonneoplastic effects			
Mineralization (7/50; 43/50; 48/50) and epithelial hyperplasia (0/50; 35/50; 43/50) of the renal papilla; renal tubular cell hyperplasia (0/50; 4/50; 7/50)	None	None	None
Neoplastic effects			
Renal tubular cell adenomas (0/50; 4/50; 8/50) and adenocarcinomas (0/50; 4/50; 3/50)	None	None	None
Level of evidence of carcinogenic activity			
Clear evidence	No evidence	No evidence	No evidence
Genetic toxicology assays			
S. typhimurium (gene mutation)	Mouse L5178Y/TK$^{+/-}$ (Tft-resistance)	CHO Cells in Vitro	
		SCE	Aberration
Negative with and without S9	Negative with and without S9	Negative with and without S9	Negative with and without S9

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 9-10.

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*-Limonene is based on the 13-week studies that began in January 1980 and ended in April 1980 and on the 2-year studies that began in February 1981 and ended in February 1983 at Microbiological Associates (Bethesda, Maryland).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on *d*-limonene on April 18, 1988, 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.

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

On April, 18, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of *d*-limonene 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 (NIEHS), Research Triangle Park, North Carolina.

Dr. C.W. Jameson, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clear evidence of carcinogenic activity for male rats, no evidence of carcinogenic activity for female rats or for male or female mice).

Dr. Jameson presented results from a short-term in vivo study that used a range of *d*-limonene doses given by gavage to groups of male and female F344/N rats in 14 daily doses over a 3-week period. Based on microscopic examination of kidney sections from these rats, *d*-limonene was shown to cause a dose-related increase in hyaline droplets in tubular epithelial cells in male rats. This effect was not seen in the kidney of female rats. Sections of kidney stained by immunohistochemical techniques revealed that the hyaline droplets contained $\alpha_{2\mu}$-globulin in male and, to a lesser extent, in female rats. These data are included in the Technical Report.

Dr. Popp, a principal reviewer, agreed with the conclusions for female rats and for male and female mice but thought that the conclusion for male rats should be changed to some evidence of carcinogenic activity, based on the hyaline droplet nephropathy and its likely relationship to renal tubular cell neoplasms in male rats. He asked that the Discussion section mention that humans have not been shown to have $\alpha_{2\mu}$-globulin and that the recent findings on this protein be added to the Abstract.

Dr. Lijinsky, the second principal reviewer, agreed with the conclusions. He considered there to be far too much discussion regarding the presence of hyaline droplets and the associated $\alpha_{2\mu}$-globulin proteins and their relationship with renal tubular cell neoplasms in male rats and noted that the mechanism of carcinogenesis is still not known for this type of tumor or for any compound. He indicated that this association was a research hypothesis and that much work remains to be done.

Dr. Ashby, the third principal reviewer, agreed with the conclusions and opined that discussion of the putative mechanism of carcinogenic action was appropriate as part of the hazard-definition process and could be mentioned briefly in the Abstract. He commented on the well-defined nongenotoxicity of *d*-limonene.

Dr. Mirer, the fourth principal reviewer, was unable to attend the meeting. Dr. L. Hart, NIEHS, read his review into the record. Dr. Mirer agreed with the proposed conclusions. He argued that the hypothesis that the carcinogenic effect is secondary to renal toxicity has not been proven. He thought inclusion of a review of instances with other chemicals where kidney lesions were present and neoplasia absent, or where neoplasia was present and toxicity not observed, was certainly warranted. Because of the apparent lack of any overt toxicity, body weight decreases, or reduced survival, Dr. Mirer noted that male mice might have tolerated a higher dose. Dr. Jameson noted increases in nonneoplastic effects in high dose male mice, which indicated a toxic response in the liver.

Dr. J. Swenberg, Chemical Industry Institute of Toxicology, made a presentation concerning *d*-limonene, $\alpha_{2\mu}$-globulin-associated nephropathy, and carcinogenesis. He stated that it was likely, but not proven, that this mechanism of induction of neoplasia was unique to male rats. Dr. Swenberg showed

data on the binding of trimethylpentane and components of unleaded gasoline to $\alpha_{2\mu}$-globulin in the kidney of male rats and concomitant cell proliferation. Dr. J. Huff, NIEHS, said that the new NTP experimental data would be added to the Technical Report.

In the ensuing discussion, Dr. Hooper and Dr. Perera said that they accepted the association of $\alpha_{2\mu}$-globulin with chemically induced nephropathy but felt that the evidence was more circumstantial for association with tumorigenesis. Dr. Perera thought that the current Technical Report was objectively balanced in the Discussion section, as well as in the Abstract, but said that the potential relevance of $\alpha_{2\mu}$-globulin to human risk of cancer should not be noted. She commented that the incidence of kidney tumors in humans is considerably greater in males than in females. Dr. Gallo stated that this fact should be cited in the Report. There was a consensus among Panel members that the statement already in the Abstract was adequate and should be retained: "These lesions have been described as reasonably characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated $\alpha_{2\mu}$-globulin in the cytoplasm of tubular epithelial cells." Also, there was general agreement that a summary of the new data regarding hyaline droplets and $\alpha_{2\mu}$-globulin should be included in the Results and highlighted in the Abstract.

Dr. Perera moved that the Technical Report on *d*-limonene be accepted as written with revisions discussed, with no mention of the uniqueness to male rats of the $\alpha_{2\mu}$-globulin-associated nephropathy, with inclusion of the recent short-term in vivo results as described, and with a statement about the greater incidence of human kidney tumors in males than in females. Dr. Hooper seconded the motion, and it was approved by nine votes to one (Dr. Popp).

I. INTRODUCTION

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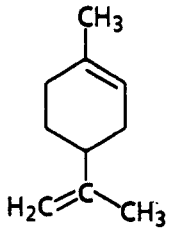

***d*-LIMONENE**

CAS No. 5989-27-5

$C_{10}H_{16}$ Molecular weight 136.2

Synonyms: Cyclohexene, 4-isopropenyl-1-methyl; 1-methyl-4-(1-methylethenyl)cyclohexene; *p*-mentha-1,8-diene; carvene; cinene; cajeputene

Physical Properties and Purity

d-Limonene is a liquid with a pleasant, lemon-like odor and a fresh citrus taste; it is practically insoluble in water but is miscible in alcohol. *d*-Limonene has a boiling point of 176° C at 760 mm mercury, a density of 0.8402 between 4° C and 20° C, an index of refraction of 1.4743 at 21° C, absorption maxima in isooctane at 220 nm and 250 nm, and a specific optical rotation of +123.8° at 20° C (Merck, 1983).

Production, Use, and Exposure

d-Limonene is a natural component of a variety of foods and beverages and is found in many fruits (especially citrus fruits), vegetables, meats, spices, and other food items (Van Straten and Maarse, 1983). *d*-Limonene is obtained commercially by alkali treatment and steam distillation of citrus peel and pulp remaining after production of juice and cold-pressed oils or from deterpenation of citrus oils; it is sometimes redistilled (Fenaroli, 1971). U.S. consumption patterns in 1976 indicated that 68,000 kg was produced for use as a fragrance ingredient (CEH, 1976), and in 1984, 254,000 kg was consumed in the United States.

d-Limonene is the most widely distributed optically active terpene and is closely related to isoprene. It occurs naturally in both the dextrorotatory and levorotatory forms; the racemic mixture of the two isomers is known as dipentene. It is found naturally in orange juice at an average concentration of 100 ppm. The major use of *d*-limonene is as a lemon fragrance in soap and perfume and as a flavoring agent in foods, beverages, and chewing gum. *d*-Limonene is used as a flavoring ingredient for citrus flavor in artificial oils and can be found in nonalcoholic beverages (31 ppm), ice cream and ices (68 ppm), candy (49 ppm), baked goods (120 ppm), gelatins and puddings (48-400 ppm), and chewing gum (2,300 ppm). It is also used as a chemical intermediate in the production of *l*-carvone, in terpene resin manufacturing as a wetting and dispersing agent, and in the preparation of sulfurized terpene lubricating oil additives. *d*-Limonene has found increased use as an industrial solvent in degreasing operations, replacing chlorinated hydrocarbons.

The U.S. Environmental Protection Agency reported that an unspecified isomer of limonene was detected in water at concentrations up to 0.03 µg/liter (Shackelford and Keith, 1976) and that the chemical was also detected in the air over Houston, Texas, at levels up to 5.7 ppb (Bertsch et al., 1974).

Acute Toxicity

d-Limonene is a skin irritant and sensitizer which is rated as moderately toxic (Gosselin et al., 1976), with a probable lethal dose in humans of 0.5-5.0 g/kg (between 1 fluid ounce and 1 pint for a 150-pound adult). No toxicity was reported after humans were given a single dose of 20 g *d*-limonene in an attempt to dissolve gallstones (Igimi et al., 1976).

The oral LD_{50} values in mice ranged between 5.6 and 6.6 g/kg, and the intraperitoneal LD_{50} value was reported as 1.3 g/kg (Tsuji et al., 1975a). When administered to dogs at 1.2-3.6 ml/kg per day for 6 months, *d*-limonene induced frequent vomiting and nausea and decrements in body weight and in blood sugar and blood cholesterol concentrations. Histopathologic lesions in dogs were restricted to the kidney (Tsuji et al., 1975b).

Absorption, Distribution, and Metabolism

Studies with [^{14}C]*d*-limonene in humans and animals have shown that 75%-95% of the orally administered radioactivity was excreted in the urine and less than 10% in the feces within 2-3 days (Kodama et al., 1976). The major urinary metabolites of *d*-limonene were identified as perillic acid-8,9-diol (M-IV) in rats and rabbits, perillyl-β-D-glucopyranosiduronic acid (M-IX) in hamsters, *p*-menth-1-ene-8,9-diol (M-II) in dogs, and 8-hydroxy-*p*-menth-1-en-9-yl-β-D-glucopyranosiduronic (M-VI) acid in guinea pigs and humans (Figure 1). Kodama and coworkers isolated five new metabolites from dog and rat urine after oral administration of radiolabeled *d*-limonene: 2-hydroxy-*p*-menth-8-en-7-oic acid (M-VII), perillylglycine (M-VIII), perillyl-β-D-glucopyranosiduronic acid (M-IX), *p*-mentha-1,8-diene-6-ol (M-X), and probably *p*-menth-1-ene-6,8,9-triol (M-XI). In vitro incubation with rat liver microsomes resulted in metabolism of *d*-limonene to *d*-limonene-1,2-diol and *d*-limonene-8,9-diol; intermediate products were identified as *d*-limonene-1,2-epoxide and *d*-limonene-8,9-epoxide (Watabe et al., 1980, 1981).

Reproductive Effects

Rabbit dams were administered oral doses of 250 or 1,000 mg/kg *d*-limonene (Kodama et al., 1977a). Decrements in feed intake and body weight gain and deaths in 6/21 animals were observed in the high dose group. These effects were not caused at 250 mg/kg *d*-limonene; no teratogenic effects were observed.

Pregnant rats were given 2,869 mg/kg *d*-limonene orally from day 9 to 15 of gestation (Tsuji et al., 1975c). Body weight gain of the dams was decreased, and a prolongation of the ossification of metacarpals and proximal phalanges was observed in the fetuses. Oral administration of 2,363 mg/kg *d*-limonene to mice between days 7 and 12 of gestation also caused maternal body weight decrements and increased incidences of abnormal bone formation in the fetuses (Kodama et al., 1977b).

Biochemical Effects

Inhibition of cholesterol biosynthesis occurred in the small intestine of rats after administration of *d*-limonene for 7 days, but no significant effect on the secretion of radiolabeled cholesterol into bile and feces was observed (Ariyoshi et al., 1979). *d*-Limonene increased the perfusion pressure of the sphincter of Oddi in dogs when injected intravenously or directly into the common bile duct (Tsuji et al., 1975d). *d*-Limonene has also been used successfully for the postoperative dissolution of retained cholesterol gallstones (Igimi et al., 1976).

Genetic Toxicity

Based on the results from National Toxicology Program (NTP) tests and one brief communication in the literature, *d*-limonene appears to be a nonmutagen in vitro. The compound was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in a preincubation protocol in both the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Haworth et al., 1983; Table E1). Watabe et al. (1980) investigated the mutagenicity, with and without induced S9, of *d*-limonene and two presumed intermediate metabolites (the 1,2- and 8,9-epoxides, which are in turn converted to the corresponding glycols) in *S. typhimurium*, and they also observed no increase in revertants. *d*-Limonene did not increase the number of trifluorothymidine (Tft)-resistant cells in an NTP mouse lymphoma L5178Y/TK$^{+/-}$ assay conducted with and without Aroclor 1254-induced male F344 rat liver S9 (Table E2). Treatment of Chinese hamster ovary cells with *d*-limonene, in the presence and absence of S9, did not induce sister chromatid exchanges or chromosomal aberrations (Tables E3 and E4).

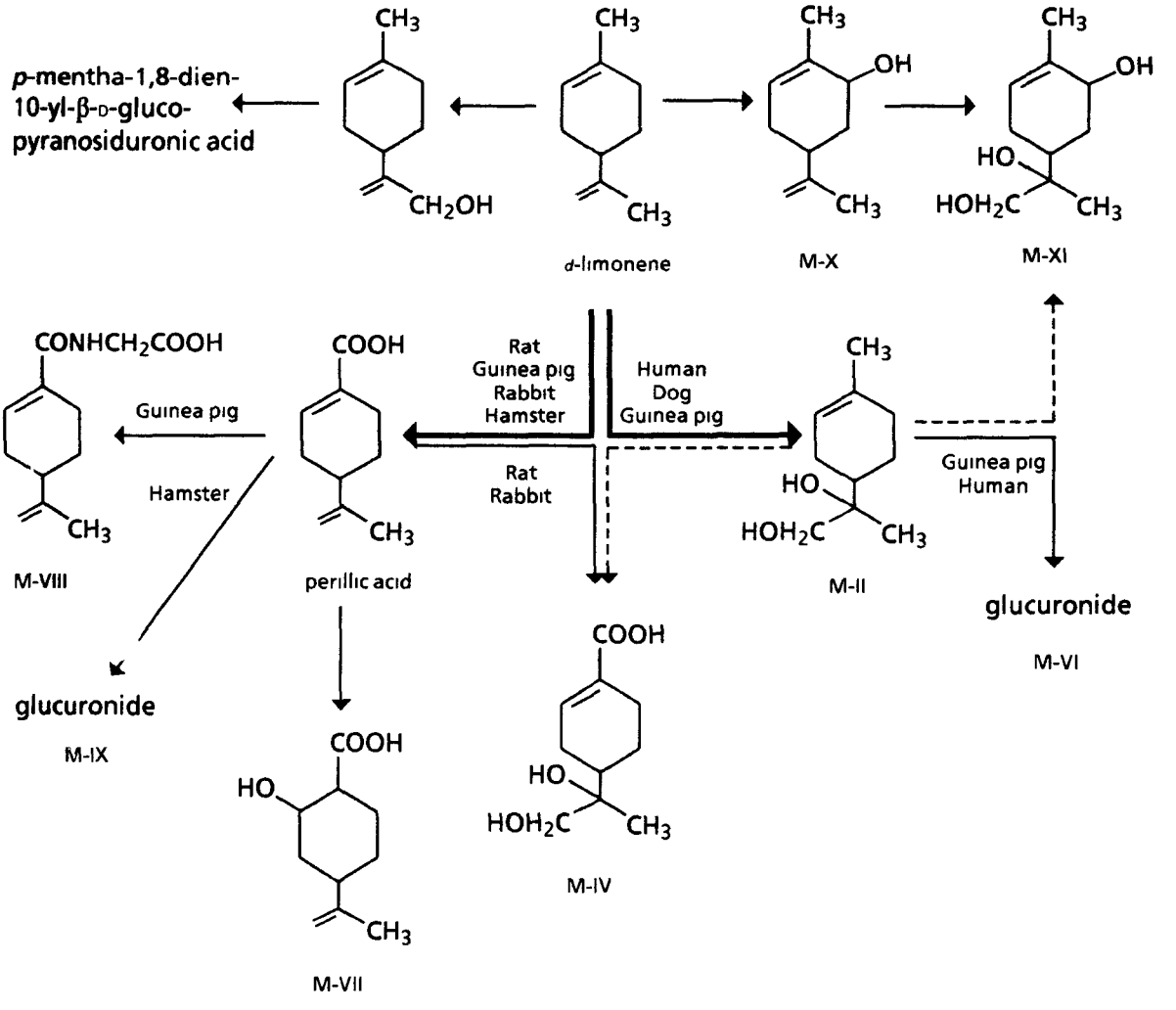

FIGURE 1. POSSIBLE METABOLIC PATHWAYS OF *d*-LIMONENE
(adapted from Kodama et al., 1976)

Carcinogenicity

Data from a variety of model systems suggested that *d*-limonene acts as a tumor inhibitory factor. Homburger et al. (1971) reported that *d*-limonene inhibited induction of subcutaneous tumors in C57BL/6 Jax mice by benzo[*rst*]pentaphene. *d*-Limonene was negative in the Strain A mouse lung tumor model system after intraperitoneal injections three times per week for 8 weeks (total doses of 4.8 or 12.0 g/kg) and termination of the study 24 weeks after the first dose (Stoner et al., 1973). In a cocarcinogenesis study, benzo[*a*]pyrene-induced skin tumors were inhibited by orange peel oil, which contains *d*-limonene as a major constituent (Van Duuren and Goldschmidt, 1976). Wattenberg (1983) presented data that suggested orange oil acted as an "antipromoter" of 7,12-dimethylbenz[*a*]anthracene (DMBA) or DMBA-induced mammary carcinomas. All of these studies were in contrast to the original data on orange oil by Roe and Peirce (1960), who reported that both this product and the "terpene fraction" promoted the formation of DMBA-initiated mouse skin tumors. Both these authors and Boutwell (1974) suggested that the tumor promoter in orange oil was *d*-limonene, since it represented up to 95% of the terpene content. To resolve this question, Elegbede et al. (1986a) compared orange peel oil and DMBA in a two-stage skin carcinogenesis model with female CD-1 mice and confirmed that topically

applied orange peel oil was a very weak promoter of both skin papillomas and carcinomas but that minor terpene components, and not topically applied *d*-limonene, possessed the promoter activity. Although Elegbede et al. reported that neither orange peel oil nor *d*-limonene exhibited promoter activity when fed in the diets, these results are questionable as *d*-limonene has been found by the NTP to be unstable when mixed with rodent feed (Materials and Methods section).

Elegbede et al. (1984) determined that female Sprague Dawley rats fed diets containing 1,000 or 10,000 ppm *d*-limonene from 1 week before to 27 weeks after a single oral administration of DMBA at a dose of 65 mg/kg had a significant reduction in DMBA-induced mammary gland tumors. The authors reported a significant reduction in mammary gland carcinogenesis in both dose groups at 27 weeks post-DMBA administration, which was mainly due to an increase in the latency period, although at selected intervals there was greater tumor regression in the dosed groups than in the controls.

A subsequent study (Elegbede et al., 1986b) followed the appearance of mammary gland tumors induced by DMBA in female (W/Fu × F344)F_2 rats in a paired feed study, with diets containing either 10% *d*-limonene or 10% cellulose. The investigators found that *d*-limonene caused the regression of chemically induced, primary, differentiated, in situ mammary gland tumors and that the formation of secondary or subsequent de novo breast tumors was inhibited.

Study Rationale

d-Limonene was nominated by the National Cancer Institute for toxicity and carcinogenicity testing because of widespread human exposure in food products and cosmetics and possibly in water and air. The instability of *d*-limonene in diet mixtures necessitated a gavage route of administration for both the short-term and 2-year studies.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *d*-LIMONENE

PREPARATION AND 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

TWENTY-ONE-DAY STUDIES

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *d*-LIMONENE

d-Limonene was obtained in one lot (lot no. 1F57A) from SCM Corporation (Jacksonville, Florida). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, Missouri). MRI reports on analyses performed in support of the *d*-limonene studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot no. 1F57A was obtained as a clear, colorless liquid with a boiling point of 177° C (an endotherm was observed at 177°-183° C with a shoulder at 175°-176° C), a density (at 21° C) of 0.84207 g/ml, and an optical rotation (at 25° C) of +122.34°. The study chemical was identified as *d*-limonene by its physical properties and by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Infrared and nuclear magnetic resonance spectra (Figures 2 and 3) were consistent with spectra in the literature (Sadtler Standard Spectra). The ultraviolet/visible spectrum was consistent with that expected for the structure of *d*-limonene.

The purity of *d*-limonene lot no. 1F57A was determined by elemental analysis, water analysis, the Food Chemicals Codex (1972) test for peroxide content, and gas chromatography. Cumulative data indicated that this lot of *d*-limonene was greater than 99% pure. The result of the elemental analysis for carbon was in agreement with the theoretical value; that for hydrogen was slightly low. Water content by Karl Fischer titration was 0.031%. The peroxide value was 2.01 mmol/liter in the original analysis and 1.88 mmol/liter at the completion of the studies. The values are within the limits specified by the Food Chemical Codex (2.0 mmol/liter and 5 mmol/liter for the 2nd and 3rd editions, 1972 and 1981, respectively). Gas chromatography with a 10% SP2100 column, a nitrogen carrier at a flow rate of 70 ml/minute, and flame ionization detection indicated one impurity with a peak area of 0.02% relative to the major peak area; three impurities with relative areas totaling 0.26% were detected with a 10% Carbowax 20M-TPA column.

Analysis by gas chromatography or high-performance liquid chromatography was conducted to determine the concentrations in the study material of the pesticides most commonly used on citrus fruits in the southeastern United States. None of the selected pesticides was detected in this lot of *d*-limonene (Table 1).

TABLE 1. DETECTION LIMIT FOR SELECTED PESTICIDES IN *d*-LIMONENE (LOT NO. 1F57A)

Pesticide	Limiting Concentration (ppb) (a)
Ethyl parathion (b)	<10
Ethion (b)	<10
Carbophenothion (b)	<10
Guthion (b)	<10
Benomyl (c)	<670
Captafol (d)	<100
Dicofol (d)	<100
Chlorobenzilate (d)	<2,000

(a) Values are based on spiked levels of each pesticide in *d*-limonene which could be detected and quantitated.
(b) *d*-Limonene in hexane was extracted with acetonitrile; the extract was analyzed by gas chromatography with a 1.5% SP2250/1.95% SP2401 column, a nitrogen carrier at a flow rate of 70 ml/minute, and a thermionic specific-phosphorus detector.
(c) Acetonitrile extracts of *d*-limonene were acid hydrolyzed to produce 2-benzimidazole carbamate from benomyl. 2-Benzimidazole carbamate was extracted with ethyl acetate and quantitated by high-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of 50% methanol in 5 mM phosphate aqueous buffer, pH 6.5, at a flow rate of 1 ml/minute and ultraviolet detection at 280 nm.
(d) *d*-Limonene was extracted with acetonitrile; the extract was analyzed by gas chromatography with a 1.5% SP2250/1.95% SP2401 column and a pulsed ^{63}Ni electron capture detector.

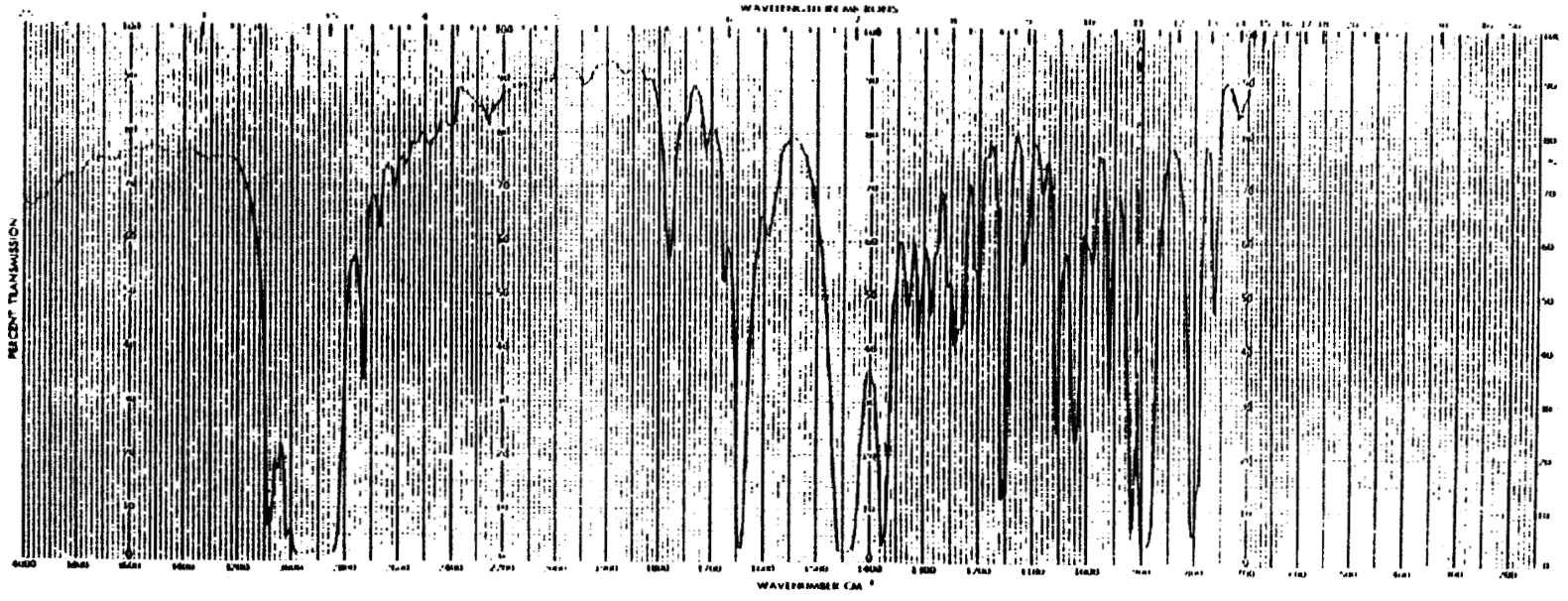

FIGURE 2. INFRARED ABSORPTION SPECTRUM OF *d*-LIMONENE (LOT NO. 1F57A)

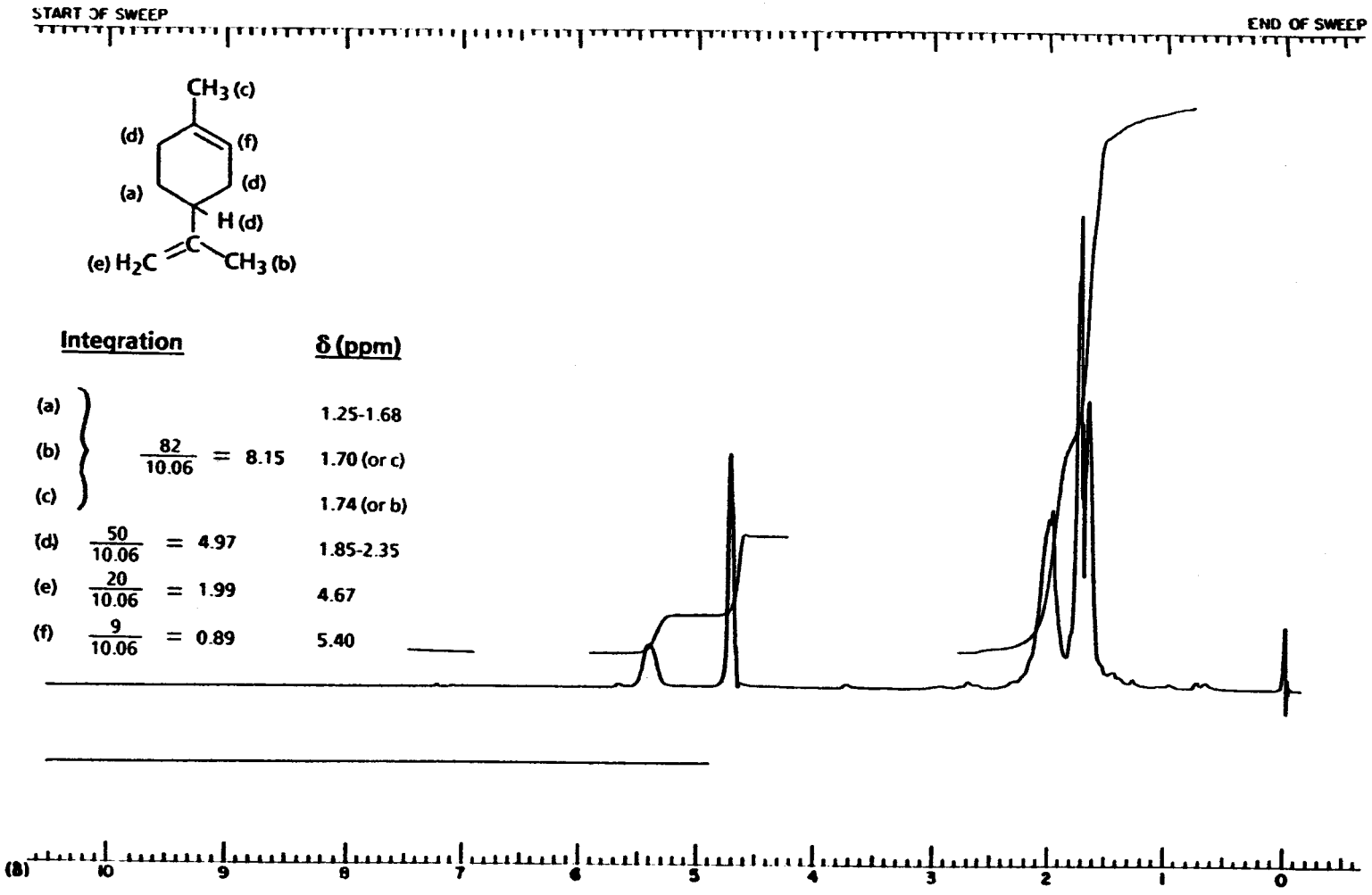

FIGURE 3. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF *d*-LIMONENE (LOT NO. 1F57A)

Stability studies performed by gas chromatography with a 10% Carbowax 20M-TPA column, a nitrogen carrier at a flow rate of 70 ml/minute, and flame ionization detection indicated that *d*-limonene was stable as a bulk chemical for 2 weeks at temperatures up to 60° C. Further confirmation of the stability of the bulk chemical during the toxicity studies (storage at room temperature) was obtained by determination of the peroxide content and analysis by the same gas chromatographic system but with a helium carrier at a flow rate of 40-50 ml/minute. No degradation was seen over the course of the studies. The identity of the chemical at the study laboratory was confirmed by infrared spectroscopy.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

The stability of *d*-limonene at 40,000 ppm in feed was determined by extraction with methanol followed by gas chromatographic analysis of the extract with a 6-foot 3% OV-17 glass column, a nitrogen carrier at a flow rate of 30 ml/minute, and a flame ionization detector. Formulated diets containing *d*-limonene were not stable when stored for 2 weeks at temperatures above −20° C (Table 2). Because the *d*-limonene/feed blends were determined to be unstable, corn oil was chosen as the vehicle for the gavage studies of *d*-limonene.

TABLE 2. TWO-WEEK STABILITY OF *d*-LIMONENE/FEED MIXTURES

Storage Temperature (degrees centigrade)	Percent Recovery
−20	98.5
5	58.9
25	46.1
45	30.3

The appropriate amounts of *d*-limonene and corn oil were mixed to give the desired concentrations (Table 3). Stability of *d*-limonene in corn oil was evaluated at the study and analytical chemistry laboratories after extraction of dose mixtures with methanol and quantitation by gas chromatography with a 3% OV-17 column, a helium or nitrogen carrier at a flow rate of 30 ml/minute, and flame ionization detection. At the analytical chemistry laboratory, *d*-limonene in corn oil (40 mg/ml) was found to be stable for up to 7 days at room temperature. Stability study results at the study laboratory showed that *d*-limonene in corn oil (12.5 and 240 mg/ml) was stable for at least 14 days at room temperature. In the 13-week studies, *d*-limonene/corn oil mixtures were stored at room temperature for no more than 15 days. In the 2-year studies, the dose mixtures were stored at room temperature for no longer than 2 weeks.

Periodic analysis for *d*-limonene in dose preparations by the same methanol extraction and gas chromatographic quantitation step was performed by the study and analytical chemistry laboratories to determine if the dose mixtures contained the correct concentrations of *d*-limonene. Dose preparations were analyzed once during the 13-week studies. The results ranged from 101% to 109% of the target concentrations (Table 4).

During the 2-year studies, the dose preparations were analyzed approximately every 8 weeks with concentrations varying from 87% to 110% of the target value (Table 5). Because 85/91 dose mixtures analyzed in the 2-year studies were within 10% of the target concentrations, it is estimated that dose mixtures were prepared within specifications 93% of the time. Referee analyses were performed periodically by the analytical chemistry laboratory. Good agreement was generally found between the study and the analytical chemistry laboratories (Table 6).

TABLE 3. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE GAVAGE STUDIES OF *d*-LIMONENE

Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation		
Appropriate amount of *d*-limonene was weighed into a 100-ml volumetric flask; corn oil added to volume; the flask was stoppered and shaken until the contents thoroughly mixed.	Similar to 16-d studies	Similar to 16-d studies
Maximum Storage Time		
8 d	15 d	14 d
Storage Conditions		
Room temperature	Room temperature	Room temperature

TABLE 4. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE THIRTEEN-WEEK GAVAGE STUDIES OF *d*-LIMONENE (a)

Concentration of *d*-Limonene in Corn Oil (mg/ml)		Determined as a
Target	Determined	Percent of Target
12.5	13.1	104.8
25	25.4	101.6
30	31.0	103.3
50	50.5	101.0
60	63.1	105.2
100	108.8	108.8
120	124.2	103.5
200	201.6	100.8
240	251.8	104.9
480	501.4	104.5

(a) Date mixed: 3/7/80; results of duplicate analysis.

TABLE 5. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE

Date Mixed	Concentration of *d*-Limonene in Corn Oil for Target Concentration (mg/ml) (a)						
	15	25	30	50	60	100	120
02/05/81	14.2	24.7	30.0	47.6	56.8	98.8	123.4
04/02/81	16.4	26.4	33.1	52.9	62.8	97.4	113.2
05/28/81	13.7	(b) 21.9	29.1	44.4	61.8	(b) 84.2	124.0
07/23/81	13.9	24.0	28.3	(c) 37.4	(c) 44.3	(b) 82.5	(b) 99.5
08/03/81	--	--	--	(d) 47.5	(d) 60.1	--	--
09/17/81	14.6	24.7	30.1	50.8	60.7	100.4	122.9
11/12/81	15.2	24.5	28.9	47.7	61.4	107.8	128.8
01/07/82	14.7	24.2	27.7	48.8	59.9	101.8	122.3
03/04/82	15.1	27.3	32.1	51.6	61.4	101.3	119.6
04/29/82	14.5	25.9	31.2	50.2	62.0	104.9	115.9
06/24/82	15.9	23.5	29.8	46.4	63.7	103.0	119.9
08/19/82	14.1	23.5	28.0	45.6	55.3	95.0	111.7
10/14/82	15.3	26.1	31.8	51.2	62.9	105.3	127.0
12/09/82	14.7	24.1	31.0	50.2	62.1	103.1	124.3
Mean (mg/ml)	14.8	24.7	30.1	48.1	59.6	98.9	119.4
Standard deviation	0.80	1.44	1.68	4.07	5.19	7.69	7.82
Coefficient of variation (percent)	5.4	5.8	5.6	8.5	8.7	7.8	6.5
Range (mg/ml)	13.7-16.4	21.9-27.3	27.7-33.1	37.4-52.9	44.3-63.7	82.5-107.8	99.5-128.8
Number of samples	13	13	13	13	13	13	13

(a) Results of duplicate analysis
(b) Out of specifications; used in the studies.
(c) Out of specifications; not used in the studies.
(d) Remix; not included in the mean.

TABLE 6. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE

Date Mixed	Target Concentration (mg/ml)	Determined Concentration (mg/ml)	
		Study Laboratory (a)	Referee Laboratory (b)
02/05/81	120	123.4	127.3
09/17/81	30	30.1	29.3
04/29/82	15	14.5	15.4
10/14/82	60	62.9	59.8

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

II. MATERIALS AND METHODS

SIXTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and observed for 12 or 13 days before the studies began. The animals were 6-8 weeks old when placed on study.

Groups of five rats and five mice of each sex were administered 0, 413, 825, 1,650, 3,300, or 6,600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16-day period. Animals were housed five per cage. Water and feed were available ad libitum. Further experimental details are summarized in Table 7.

The rats and mice were observed twice per day and weighed once per week. A necropsy was performed on all animals. Tissues and groups examined are listed in Table 7.

THIRTEEN-WEEK STUDIES

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

Four- to six-week-old male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories, observed for 18 or 19 days, distributed to weight classes, and then assigned to cages according to a table of random numbers. Cages were assigned to dosed and vehicle control groups according to another table of random numbers.

Groups of 10 rats of each sex were administered 0, 150, 300, 600, 1,200, or 2,400 mg/kg *d*-limonene in corn oil by gavage, 5 days per week for 13 weeks. Groups of 10 mice of each sex were administered 0, 125, 250, 500, 1,000, or 2,000 mg/kg according to the same schedule. Rats and mice were housed five per cage in polycarbonate cages. Feed and water were available ad libitum. Animals were observed two times per day; moribund animals were killed. Individual animal weights were recorded once per week.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 7.

TWO-YEAR STUDIES

Study Design

Groups of 50 male rats were administered 0, 75, or 150 mg/kg *d*-limonene in corn oil by gavage, 5 days per week for 103 weeks; groups of 50 female rats were administered 0, 300, or 600 mg/kg. Groups of 50 male mice were administered 0, 250, or 500 mg/kg according to the same schedule; groups of 50 female mice were administered 0, 500, or 1,000 mg/kg.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 4-5 weeks of age and mice at 5-6 weeks. The animals were quarantined at the study facility for 19 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were placed on study at 7-8 weeks of age and the mice at 8-9 weeks. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line

TABLE 7. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF *d*-LIMONENE

Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 males and 5 females of each species	10 males and 10 females of each species	50 males and 50 females of each species
Doses		
0, 413, 825, 1,650, 3,300, or 6,600 mg/kg *d*-limonene in corn oil by gavage; dose vol--10 ml/kg	Rats--0, 150, 300, 600, 1,200, or 2,400 mg/kg *d*-limonene in corn oil by gavage; mice--0, 125, 250, 500, 1,000, or 2,000 mg/kg; dose vol--rats: 5 ml/kg; mice: 10 ml/kg	Rats--male. 0, 75, or 150 mg/kg *d*-limonene in corn oil by gavage; female: 0, 300, or 600 mg/kg; mice--male: 0, 250, or 500 mg/kg; female: 0, 500, or 1,000 mg/kg; dose vol--rats: 5 ml/kg; mice· 10 ml/kg
Date of First Dose		
Rats--9/18/79, mice--9/17/79	1/28/80	Rats--2/9/81, mice--2/16/81
Date of Last Dose		
Rats--10/3/79; mice--10/2/79	4/25/80	Rats--1/28/83, mice--2/4/83
Duration of Dosing		
5 d/wk (12 doses over 16 d)	5 d/wk for 13 wk	5 d/wk for 103 wk
Type and Frequency of Observation		
Observed 2 × d; weighed initially and 1 × wk thereafter	Observed 2 × d; weighed initially and 1 × wk thereafter	Observed 2 × d; weighed 1 × wk for 12 wk and 1 × mo thereafter
Necropsy and Histologic Examinations		
Necropsy performed on all animals; histologic exams performed on six mice and seven rats from survivors of highest dose groups	Necropsy performed on all animals; histologic exams performed on all vehicle control and high dose animals and all female rats in the 1,200 mg/kg group. Tissues examined include· adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Kidneys examined for all male rats	Necropsy performed on all animals; histologic exams performed on all animals dying during the studies, all vehicle controls, all low dose female rats, and all high dose animals. Tissues examined include: adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymis/seminal vesicles/tunica vaginalis/scrotal sac/prostate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder, and Zymbal gland. Tissues examined in low dose groups include adrenal glands, kidney, liver, spleen, and testis for male rats and liver for female mice
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)

TABLE 7. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF *d*-LIMONENE (Continued)

Sixteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Study Laboratory Microbiological Associates	Microbiological Associates	Microbiological Associates
Method of Animal Identification Ear punch	Ear punch	Ear tag
Time Held Before Study Rats--12 or 13 d; mice--13 d	Rats--18 or 19 d; mice--18 d	19 d
Age When Placed on Study Rats--6-7 wk; mice--6-8 wk	Rats--7-8 wk; mice--7-9 wk	Rats--7-8 wk; mice--8-9 wk
Age When Killed Rats--8-9 wk; mice--8-10 wk	Rats--20-21 wk; mice--21-23 wk	112-114 wk
Necropsy Dates Rats--10/4/79; mice--10/3/79	4/28/80-4/30/80	Rats--2/7/83-2/11/83; mice--2/14/83-2/17/83
Method of Animal Distribution Animals distributed to weight classes; assigned to cages and then to groups according to a table of random numbers	Same as 16-d studies	Same as 16-d studies
Feed Purina Lab Blox® (Chesapeake Feed Co., Beltsville, MD); available ad libitum	Same as 16-d studies or NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum
Bedding Rats--hardwood chips (P.J. Murphy Forest Products Co., NY); mice--Sani Chips (Northeastern Products Corp., 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 (Lab Products, Inc., Rochelle Park, NJ, and Hazleton Systems, 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--64°-76° F; hum--80%-90%; fluorescent light 12 h/d; 12-15 room air changes/h	Temp--60°-82° F; hum--35%-80%; fluorescent light 12 h/d; 12-15 room air changes/h	Temp--66°-84° F; hum--20%-78%; fluorescent light 12 h/d; 12-15 room air changes/h

from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hybrid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Cages were not rotated until the last 10 weeks of the studies. Further details of animal maintenance are given in Table 7.

Clinical Examinations and Pathology

All animals were observed two times per day. Body weights were recorded once per week for the first 12 weeks of the studies and 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, unless they 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. Histopathologic examination of tissues was performed according to an "inverse pyramid" design (McConnell, 1983a,b). That is, complete histopathologic examinations (Table 7) were performed on all high dose and vehicle control animals and on low dose animals dying before the end of the study. In addition, histopathologic examinations were performed on all grossly visible lesions in all dose groups. Potential target organs for chemically related neoplastic and nonneoplastic effects were identified from the short-term studies or the literature and were determined by examination of the pathology data; these target organs/tissues in the lower dose group were examined histopathologically. If mortality in the highest dose group exceeded that in the vehicle control group by 15%, complete histopathologic examinations were performed on all animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Carcinogenesis Bioassay Data 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, all potential target tissues, 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 and neoplasms 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 to be missing or 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 an incidental tumor 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, the proportions of tumor-bearing animals in dosed and vehicle control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined to obtain a single overall result.

In addition to incidental tumor analysis, 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.)

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

TWENTY-ONE-DAY STUDIES

After evaluation of the 2-year studies, a supplemental study was performed at NIEHS to evaluate the short-term effects of *d*-limonene specifically on the rat kidney. Eight-week-old male and female F344/N rats were obtained from Charles River Breeding Laboratories and held for 10 weeks before being placed on study. Groups of 12 rats of each sex were administered 0, 75, 150, 300, 600, or 1,200 mg/kg *d*-limonene in corn oil by gavage once per day for 14 days over a 21-day period. Animals were housed four per cage. Water and feed were available ad libitum.

The rats were observed twice per day and weighed on day 1, 8, and 15, for determination of dosing. Five rats of each sex from each dose group were killed with carbon dioxide within 24 or 72 hours of the last dose. The right kidney was collected and placed in 10% neutral buffered formalin for histologic examination. Paraffin-embedded sections 5-7 µm thick were stained with the Mallory-Heidenhain stain for protein. The amount of positive staining droplets in the tubular epithelial cells was scored as minimal, mild, moderate, or severe. The left kidney was collected for determination of $\alpha_{2\mu}$-globulin with an ELISA test.

An additional two rats of each sex from the 1,200 mg/kg and vehicle control groups were anesthetized with ketamine and xylazine 24 hours after the last dose. The kidney was perfused retrograde via the abdominal aorta with sodium phosphate buffer (pH 7.4) as a flushing solution followed by perfusion with fixative containing 2% paraformaldehyde and 1% glutaraldehyde in sodium phosphate buffer. The animals were exsanguinated and the kidney removed and sliced into 1- to 2-mm sections and placed in fresh fixative for 24 hours at 4° C. Plastic (GMA)-embedded sections of kidney 2 µm thick were stained with labeled antibody to $\alpha_{2\mu}$-globulin for definitive identification of this protein in the protein droplets in the tubular epithelial cells.

III. RESULTS

RATS

SIXTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

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Body Weights and Clinical Signs

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MICE

SIXTEEN-DAY STUDIES

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III. RESULTS: RATS

SIXTEEN-DAY STUDIES

All rats that received 6,600 mg/kg and 5/5 males and 3/5 females that received 3,300 mg/kg *d*-limonene died within the first 2 days (Table 8). The final mean body weight of male rats that received 1,650 mg/kg was 10% lower than that of the vehicle controls. The final mean body weight of female rats that received 3,300 mg/kg was 8% lower than that of the vehicle controls. No compound-related clinical signs were observed in rats that received doses of 1,650 mg/kg or lower. No compound-related lesions were observed.

THIRTEEN-WEEK STUDIES

Five of 10 males and 9/10 females that received 2,400 mg/kg died during week 1 (Table 9). The final mean body weights of male rats that received 600, 1,200, or 2,400 mg/kg were 6%, 12%, or 23% lower than that of the vehicle controls. The final body weight of the female rat that received 2,400 mg/kg and lived to end of the study was 11% lower than the mean of the vehicle controls. Rough hair coats, lethargy, and excessive lacrimation were observed for rats that received 1,200 or 2,400 mg/kg. Nephropathy was identified in all groups of male rats, but there was a dose-related increased severity of the lesion in dosed groups (Table 10). The nephropathy was characterized by degeneration of epithelium in the convoluted tubules, granular casts within tubular lumens, primarily in the outer stripe of the outer medulla, and regeneration of the tubular epithelium. Hyaline droplets (protein reabsorption droplets) were observed in the epithelium of proximal convoluted tubules in all groups of male rats, including vehicle controls. The slides were coded and reevaluated in a "blind" fashion by two pathologists to determine if there was an increased number of these droplets in the dosed male rats. In this review, no

TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SIXTEEN-DAY GAVAGE STUDIES OF *d*-LIMONENE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	115 ± 2	173 ± 3	+58 ± 3	
413	5/5	113 ± 2	171 ± 4	+58 ± 3	99
825	5/5	113 ± 3	173 ± 4	+60 ± 5	100
1,650	5/5	113 ± 3	156 ± 4	+43 ± 3	90
3,300	(d) 0/5	114 ± 2	(e)	(e)	(e)
6,600	(f) 0/5	111 ± 2	(e)	(e)	(e)
FEMALE					
0	5/5	98 ± 1	123 ± 1	+25 ± 1	
413	5/5	101 ± 2	(g) 139 ± 2	+38 ± 3	113
825	5/5	100 ± 2	131 ± 3	+31 ± 4	107
1,650	5/5	100 ± 2	127 ± 1	+27 ± 2	103
3,300	(f) 2/5	100 ± 2	113 ± 8	+10 ± 5	92
6,600	(f) 0/5	100 ± 4	(e)	(e)	(e)

(a) Number surviving/number initially in the 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) Day of death: 1,1,1,1,2
(e) No data are reported due to the 100% mortality in this group.
(f) Day of death: all 1
(g) One final body weight not recorded; weight change based on remaining four animals.

TABLE 9. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF *d*-LIMONENE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	144 ± 2	333 ± 6	+189 ± 5	
150	10/10	145 ± 3	332 ± 4	+187 ± 3	100
300	10/10	149 ± 2	330 ± 3	+181 ± 4	99
600	10/10	148 ± 2	314 ± 5	+166 ± 5	94
1,200	10/10	139 ± 3	292 ± 5	+153 ± 6	88
2,400	(d) 5/10	150 ± 3	255 ± 10	+103 ± 10	77
FEMALE					
0	10/10	118 ± 2	185 ± 2	+67 ± 4	
150	10/10	115 ± 1	186 ± 2	+71 ± 2	101
300	10/10	105 ± 4	181 ± 2	+76 ± 4	98
600	10/10	114 ± 1	184 ± 2	+70 ± 1	99
1,200	10/10	116 ± 2	182 ± 3	+66 ± 3	98
2,400	(d) 1/10	113 ± 1	164	+56	89

(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) Week of death: all 1

TABLE 10. SEVERITY OF KIDNEY LESIONS IN MALE RATS IN THE THIRTEEN-WEEK GAVAGE STUDY OF *d*-LIMONENE (a)

Lesion	Dose (mg/kg) Vehicle Control	150	300	600	1,200	2,400
Regeneration	(b) 0.8	2.4	2.5	2.5	3.7	0.9
Granular casts	0	1.6	2.4	2.7	3.5	0.3

(a) Severity grades: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
(b) Average severity grade for all rats in the group

definite differences in the accumulation of hyaline droplets could be discerned among the slides from different dose groups.

Dose Selection Rationale: Based on the results of the 13-week studies, doses of 75 and 150 mg/kg were selected for male rats for the 2-year studies because of the compound-related kidney lesions observed at 300 mg/kg and higher which were considered potentially life threatening. Doses of 300 and 600 mg/kg were selected for female rats because the large number of deaths at 2,400 mg/kg suggested that long-term exposure at 1,200 mg/kg might result in reduced survival.

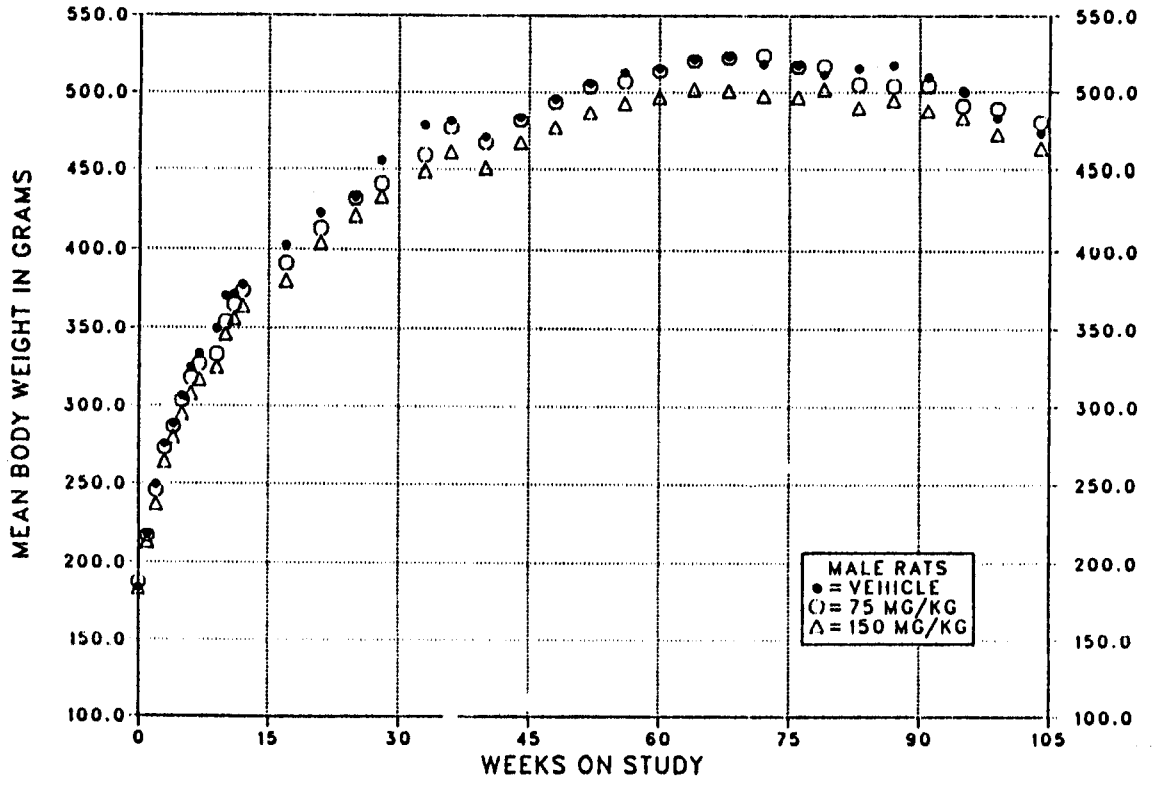
TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male rats were generally 4%-7% lower than those of the vehicle controls from week 2 to the end of the studies (Table 11 and Figure 4). Mean body weights of high dose female rats were generally 4%-7% lower than those of the vehicle controls from week 28 to the end of the studies. No compound-related clinical signs were observed during the 2-year studies.

TABLE 11. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	Low Dose Av. Wt. (grams)	Low Dose Wt. (percent of veh. controls)	Low Dose No. of Survivors	High Dose Av. Wt. (grams)	High Dose Wt. (percent of veh. controls)	High Dose No. of Survivors
MALE				**75 mg/kg**			**150 mg/kg**	
0	184	50	187	102	50	183	99	50
1	218	50	217	100	50	213	98	50
2	250	50	246	98	50	237	95	50
3	276	50	273	99	50	264	96	50
4	289	50	287	99	50	280	97	50
5	307	50	304	99	50	295	96	50
6	325	50	318	98	50	308	95	50
7	334	50	327	98	50	317	95	50
9	350	50	333	95	50	325	93	50
10	371	50	354	95	50	346	93	50
11	372	50	365	98	49	356	96	50
12	378	50	374	99	49	364	96	50
17	403	50	391	97	49	380	94	50
21	423	50	413	98	49	404	96	50
25	433	50	432	100	49	421	97	50
28	456	50	441	97	48	433	95	50
33	479	47	459	96	48	449	94	50
36	482	47	477	99	48	461	96	50
40	471	46	467	99	48	451	96	50
44	484	46	482	100	48	467	96	50
48	496	46	494	100	48	477	96	50
52	506	46	504	100	48	487	96	50
56	513	46	507	99	48	493	96	50
60	516	46	514	100	47	497	96	50
64	522	46	521	100	47	502	96	50
68	524	46	523	100	47	501	96	49
72	519	46	524	101	46	498	96	49
76	518	45	517	100	46	497	96	49
79	512	45	517	101	45	502	98	48
83	516	43	505	98	44	490	95	47
87	518	40	504	97	42	495	95	46
91	510	38	504	99	40	488	96	45
95	501	36	491	98	39	483	94	45
99	483	32	489	101	37	472	98	43
104	473	29	480	101	33	463	98	40
FEMALE				**300 mg/kg**			**600 mg/kg**	
0	132	50	133	101	50	132	100	50
1	146	50	148	101	50	146	100	50
2	158	50	161	102	50	160	101	50
3	166	50	170	102	50	168	101	50
4	175	50	179	102	50	176	101	50
5	184	50	186	101	50	184	100	50
6	189	50	192	102	50	188	99	50
7	192	50	194	101	50	191	99	50
8	198	50	201	102	50	197	99	50
9	198	50	201	102	50	197	99	50
10	202	50	205	101	50	201	100	50
11	206	50	210	102	50	205	100	50
12	210	50	214	102	50	212	101	50
17	213	50	218	102	50	214	100	50
21	222	50	220	99	50	214	96	49
25	226	50	233	103	50	222	98	48
28	237	50	235	99	50	227	96	45
33	246	48	244	99	50	237	96	43
36	247	48	248	100	49	241	98	43
40	250	48	253	101	48	241	96	39
44	254	48	255	100	47	241	95	38
48	261	48	261	100	47	247	95	36
52	269	48	273	101	47	256	95	34
56	281	48	287	102	47	264	94	34
60	289	48	294	102	47	270	93	34
64	294	48	303	103	47	279	95	34
68	305	48	315	103	47	289	95	34
72	311	47	319	103	47	295	95	33
76	320	46	328	103	46	306	96	33
79	322	46	330	102	46	309	96	33
83	327	46	332	102	45	310	95	33
87	326	45	335	103	45	312	96	32
91	322	45	330	102	43	320	99	31
95	333	45	331	99	43	324	97	31
99	340	43	344	101	43	330	97	29
104	339	42	342	101	40	321	95	26



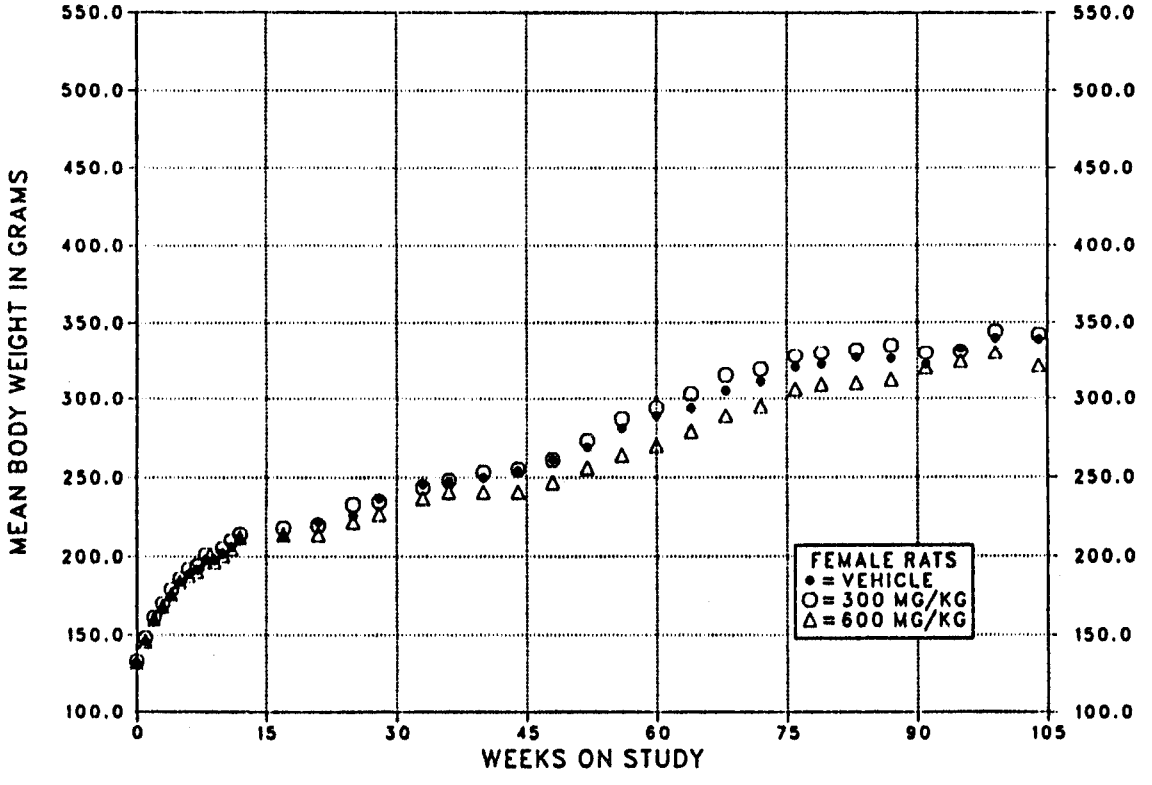


FIGURE 4. GROWTH CURVES FOR RATS ADMINISTERED *d*-LIMONENE IN CORN OIL BY GAVAGE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats administered *d*-limonene at the doses used in these studies and for vehiclecontrols are shown in Table 12 and in the Kaplan and Meier curves in Figure 5. The survival of the high dose male group was significantly greater than that of the vehicle control group after week 81, and survival of the high dose female group was significantly lower than that of the vehicle controls after week 39.

TABLE 12. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
MALE (a)		**75 mg/kg**	**150 mg/kg**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	20	16	5
Accidentally killed	1	1	5
Killed at termination	29	33	40
Survival P values (c)	0.001	0.497	0.001
FEMALE (a)		**300 mg/kg**	**600 mg/kg**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	5	8	16
Accidentally killed	3	2	8
Killed at termination	42	39	24
Died during termination period	0	1	2
Survival P values (c)	0.003	0.571	0.006

(a) Termination period: male--week 104; female--weeks 104-105
(b) Includes animals killed in a moribund condition
(c) 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.

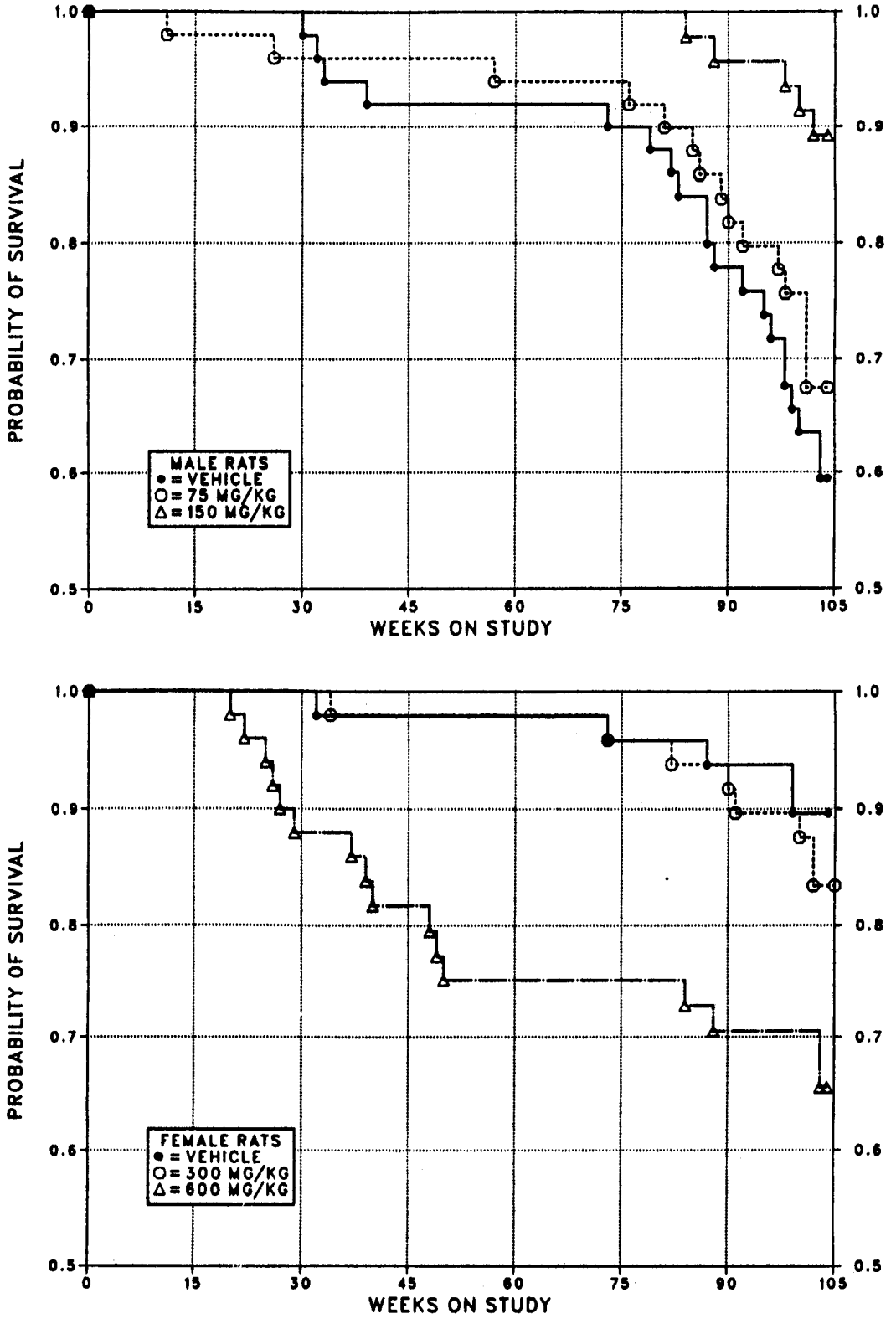


FIGURE 5. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED *d*-LIMONENE IN CORN OIL BY GAVAGE FOR TWO YEARS

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the kidney, uterus, testis, hematopoietic system, skin, subcutaneous tissue, and eye.

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

Kidney: The administration of *d*-limonene to male rats was associated with dose-related increased incidences of mineralization and epithelial hyperplasia (Table 13). These lesions consisted of linear deposits of mineral in the medulla (renal papilla) and focal hyperplasia of the transitional epithelium overlying the papilla. The hyperplasia was often located near the fornices of the renal pelvis and was sometimes bilateral. There was also a dose-related increased severity of spontaneous nephropathy in dosed male rats (Table 14). Nephropathy is an age-related disease characterized by degeneration and atrophy of the tubular epithelium, dilatation of tubules with formation of hyalin and granular casts, regeneration of tubular epithelium, glomerulosclerosis, and interstitial inflammation and fibrosis.

Tubular cell hyperplasia and neoplasia were also observed at increased incidences in dosed male rats (Table 15). Tubular cell adenomas and tubular cell adenomas or adenocarcinomas (combined) in male rats occurred with significant positive trends; the incidences of tubular cell adenomas in high dose male rats and of tubular cell adenomas or adenocarcinomas (combined) in dosed male rats were significantly greater than those in vehicle controls. These rare neoplasms were not observed in vehicle control male rats or in dosed or vehicle control female rats.

Tubular cell hyperplasia, adenomas, and adenocarcinomas were part of a continuous morphologic spectrum. Proliferative lesions diagnosed as hyperplasia generally consisted of one to

TABLE 13. INCIDENCE OF NONNEOPLASTIC RENAL PAPILLA LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

Lesion	Vehicle Control	75 mg/kg	150 mg/kg
Mineralization	7/50	43/50	48/50
Epithelial hyperplasia	0/50	35/50	43/50

TABLE 14. SEVERITY OF NEPHROPATHY IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (a)

	Vehicle Control	75 mg/kg	150 mg/kg
Not present	9	4	1
Minimal	12	12	5
Mild	25	25	28
Moderate	4	7	15
Marked	0	2	1
Mean	1.5	1.8	2.2

(a) Lesions were independently diagnosed and graded by the PWG Chairperson during the PWG review.

TABLE 15. RENAL TUBULAR CELL LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (a)

	Vehicle Control	75 mg/kg	150 mg/kg
Hyperplasia			
Overall Rates	0/50 (0%)	4/50 (8%)	7/50 (14%)
Adenoma			
Overall Rates	0/50 (0%)	4/50 (8%)	8/50 (16%)
Adjusted Rates	0.0%	11.5%	19.3%
Terminal Rates	0/29 (0%)	3/33 (9%)	7/40 (18%)
Week of First Observation		101	96
Life Table Tests	P=0.011	P=0.084	P=0.016
Incidental Tumor Tests	P=0.006	P=0.067	P=0.011
Adenocarcinoma			
Overall Rates	0/50 (0%)	4/50 (8%)	3/50 (6%)
Adjusted Rates	0.0%	11.5%	7.0%
Terminal Rates	0/29 (0%)	3/33 (9%)	1/40 (3%)
Week of First Observation		101	98
Life Table Tests	P=0.202	P=0.084	P=0.179
Incidental Tumor Tests	P=0.111	P=0.067	P=0.081
Adenoma or Adenocarcinoma (b)			
Overall Rates	0/50 (0%)	8/50 (16%)	11/50 (22%)
Adjusted Rates	0.0%	22.6%	25.4%
Terminal Rates	0/29 (0%)	6/33 (18%)	8/40 (20%)
Week of First Observation		101	96
Life Table Tests	P=0.006	P=0.009	P=0.004
Incidental Tumor Tests	P=0.001	P=0.005	P=0.001

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) Historical incidence in NTP studies (mean ± SD): 10/1,943 (0.5% ± 0.9%)

three adjacent normal-sized to slightly enlarged tubules with stratification of the tubular epithelium. In some hyperplasia, the epithelial cells appeared to fill the tubular lumen completely. Tubular cell adenomas varied from greatly enlarged 1-mm diameter tubules containing proliferating epithelial cells up to 1-cm diameter masses. Adenomas consisted of relatively well-differentiated epithelium and exhibited solid, cystic, or papillary patterns of growth. The solid or cystic neoplasms were arranged in solid sheets or small solid nests separated by a delicate vascular stroma and showed little evidence of tubule formation. Papillary neoplasms consisted of layers of epithelium lining a fibrous connective tissue stroma and were arranged in complex branching papillary formations. Adenocarcinomas showed growth patterns similar to those in adenomas but generally were larger (1 cm or more) and exhibited cellular pleomorphism and anaplasia.

In the 21-day studies conducted after the 2-year studies, microscopic examination of paraffin-embedded sections of kidney stained with hematoxylin and eosin showed no visible differences between dosed and vehicle control male and female rats. However, in plastic-embedded sections of kidney stained with Lee's methylene blue basic fuchsin, differences in the distribution, amount, and shape of intracytoplasmic granules in the proximal tubules of dosed and vehicle control males were detected. In vehicle control male rats, focal clusters of proximal convoluted tubules contained varying numbers of round, blue-to-purple granules of varying size. In dosed males, the tubular cells containing the intracytoplasmic granules were more diffusely distributed (although still limited to the convoluted tubules) and increased in number within many of the cells. Furthermore, many granules in the dosed males were rectangular rather than round. These differences were detectable at all

doses for groups receiving necropsies at either 24 or 72 hours after the last dose. The intracytoplasmic granules in vehicle control and males stained positively for $\alpha_{2\mu}$-globulin with the immunohistochemical stain.

In dosed and vehicle control female rats, the plastic-embedded sections of kidney showed occasional tubular epithelial cells with one or several small red granules. However, there were no visible differences in the distribution, amount, or shape of the granules between the dosed and vehicle control groups.

Data were analyzed by the RS/1 multicomparison procedure, using the Wilk-Shapiro test for normality, one-way analysis of variance, and Dunnett's test for parametric multiple comparison or the Kruskal-Wallis test for nonparametric multiple comparison with corresponding vehicle controls (RS/1,1983). The $\alpha_{2\mu}$-globulin in the kidney of dosed male and female rats was quantitated with an ELISA technique and was found to increase significantly in dosed male rats relative to that in vehicle controls (Table 16). No increase was observed in female rats.

Uterus: The incidence of endometrial stromal polyps of the uterus in female rats that received 300 mg/kg was increased compared with that in vehicle controls (Table 17). However, the incidence of this neoplasm in female rats that received 600 mg/kg was not significantly greater than that in vehicle controls and was well below the mean historical incidence of this tumor. In addition, the vehicle control group incidence of this neoplasm is substantially lower than the mean historical incidence in National Toxicology Program (NTP) studies (6% vs. 21%). In the current study, the absence of a dose-related response for endometrial stromal polyps and the low incidence of this tumor in the vehicle controls compared with the historical incidence argue against a compound-related effect on the uterus.

TABLE 16. EFFECTS OF *d*-LIMONENE ON $\alpha_{2\mu}$-GLOBULIN IN THE RAT KIDNEY IN THE TWENTY-ONE-DAY GAVAGE STUDIES (a)

Dose (mg/kg)	Hours Postexposure	$\alpha_{2\mu}$-Globulin	
		Milligrams per Milliliter	Micrograms per Milligram of Total Protein
MALE			
0	24	6.8 ± 0.4	203.9 ± 14.1
75		12.9 ± 0.4 (b) (89%)	408.8 ± 18.5 (c) (101%)
150		15.1 ± 0.8 (b) (121%)	464.5 ± 22.0 (c) (128%)
300		16.0 ± 0.6 (b) (134%)	489.2 ± 13.8 (c) (140%)
600		17.0 ± 1.3 (b) (149%)	504.9 ± 26.6 (c) (148%)
1,200		19.8 ± 0.6 (b) (190%)	560.9 ± 17.5 (c) (175%)
0	72	5.4 ± 0.5	169.4 ± 16.7
1,200		16.4 ± 1.0 (d) (204%)	471.3 ± 15.8 (d) (178%)
FEMALE			
0	24	1.7 ± 0.5	54.2 ± 14.2
75		2.8 ± 0.3	90.1 ± 5.5
150		2.8 ± 0.3	90.6 ± 9.6
300		1.8 ± 0.4	48.3 ± 7.6
600		2.0 ± 0.5	58.1 ± 14.0
1,200		2.4 ± 0.3	70.8 ± 8.8

(a) Male and female F344/N rats were exposed to *d*-limonene by gavage for 14 days at the indicated doses. The left kidney was collected for determination of $\alpha_{2\mu}$-globulin with an ELISA test in kidney homogenates. Total protein was measured in the same aliquots as those for $\alpha_{2\mu}$-globulin by the Lowry method. Results are expressed as mean ± standard error of the mean of five rats per group. The percent increase for significant responses is indicated in parentheses.
(b) Significance at $P<0.01$ vs. vehicle controls by Dunnett's multiple range test (parametric test for normal distribution)
(c) Significance at $P<0.01$ vs. vehicle controls by the Kruskal-Wallis multiple comparison test (nonparametric test for abnormal distribution)
(d) Significance at $P<0.01$ vs. vehicle controls by Student's *t*-test

TABLE 17. UTERINE ENDOMETRIAL STROMAL POLYPS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (a)

	Vehicle Control	300 mg/kg	600 mg/kg
Overall Rates	3/50 (6%)	13/50 (26%)	5/50 (10%)
Adjusted Rates	6.8%	30.7%	17.9%
Terminal Rates	1/42 (2%)	11/40 (28%)	4/26 (15%)
Week of First Observation	99	82	84
Life Table Tests	P=0.094	P=0.007	P=0.155
Incidental Tumor Tests	P=0.176	P=0.011	P=0.305

(a) Historical incidence in NTP studies (mean ± SD): 390/1,934 (20% ± 7%)

Testis: Interstitial cell tumors in male rats occurred with a significant positive trend; the incidences in dosed groups were significantly greater than that in vehicle controls (Table 18). Testicular interstitial cell tumors are commonly occurring neoplasms in aging male F344 rats and are present at a very high incidence in control animals. These neoplasms are generally not life threatening at 24 months, and most male rats (chemically exposed as well as control) will develop this tumor during the latter part of a 2-year study. The marginal difference in the incidence of this neoplasm between chemically exposed and vehicle control male rats is attributed to the low survival of the vehicle control group compared with that of the dosed groups and was not considered to be related to chemical exposure.

Hematopoietic System: Mononuclear cell leukemia in male rats occurred with a positive trend that was significant by the incidental tumor test; the incidences in dosed male rats were not significantly different from that in vehicle controls and were not considered to be related to *d*-limonene administration (Table 19).

Skin: Three squamous cell papillomas or carcinomas occurred in high dose male rats. The incidence of these neoplasms was not significantly different from that in vehicle controls and was within the range of the historical incidences in NTP studies, and thus these tumors were not considered related to *d*-limonene administration.

Subcutaneous Tissue: Fibromas in male rats occurred with a significant negative trend (vehicle control, 8/50; low dose, 2/50; high dose, 3/50; P=0.041); the incidence of fibromas or fibrosarcomas (combined) in dosed male rats was not significantly different from that in vehicle controls (8/50; 4/50; 3/50; P>0.05).

Eye: Cataracts were observed at increased incidences in high dose male and dosed female rats (male: vehicle control, 1/50; low dose, 3/50; high dose, 27/50; female: 0/50; 5/50; 20/50). Retinal degeneration was observed in dosed male and female rats (male: 0/50; 7/50; 37/50; female: 0/50; 21/50; 28/50). These changes are not believed to be related to the administration of *d*-limonene but rather to the proximity of animal cages to the light source in the animal room. These studies were conducted before initiation of routine animal cage rotation, a procedure instituted for the purpose of randomizing animals with respect to light. High dose male and female rats were housed in the top tiers of their respective cage racks. Low dose males and females were housed in intermediate tiers and vehicle control males and females in the bottom tiers.

TABLE 18. TESTICULAR INTERSTITIAL CELL LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	75 mg/kg	150 mg/kg
Hyperplasia			
Overall Rates	11/50 (22%)	2/49 (4%)	6/50 (12%)
Tumor (a)			
Overall Rates	37/50 (74%)	47/49 (96%)	48/50 (96%)
Adjusted Rates	90.2%	100.0%	98.0%
Terminal Rates	25/29 (86%)	33/33 (100%)	39/40 (98%)
Week of First Observation	79	57	78
Life Table Tests	P=0.407N	P=0.216	P=0.481N
Incidental Tumor Tests	P=0.005	P=0.002	P=0.021

(a) Historical incidence in NTP studies (mean ± SD): 1,675/1,944 (86% ± 9%)

TABLE 19. MONONUCLEAR CELL LEUKEMIA IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (a)

	Vehicle Control	75 mg/kg	150 mg/kg
Overall Rates	10/50 (20%)	10/50 (20%)	19/50 (38%)
Adjusted Rates	29.4%	26.5%	45.0%
Terminal Rates	6/29 (21%)	7/33 (21%)	17/40 (43%)
Week of First Observation	87	81	88
Life Table Tests	P=0.147	P=0.491N	P=0.211
Incidental Tumor Tests	P=0.043	P=0.583	P=0.081

(a) Historical incidence of leukemia in NTP studies (mean ± SD): 321/1,949 (16% ± 9%) (range: high, 22/50; low, 1/50)

SIXTEEN-DAY STUDIES

All but one of 20 mice that received 3,300 or 6,600 mg/kg, died within 3 days (Table 20). Vehicle control mice gained little or no weight. No compound-related clinical signs were observed in mice that received 1,650 mg/kg and lived to the end of the studies. No compound-related histopathologic lesions were observed.

THIRTEEN-WEEK STUDIES

One of 10 males and 2/10 females that received 2,000 mg/kg and 1/10 females that received 500 mg/kg died before the end of the studies (Table 21). Several animals in other groups died as a result of gavage error. Clinical signs of rough hair coats and decreased activity were observed at the two highest doses. The final mean body weights of mice that received 1,000 or 2,000 mg/kg were 10% lower than that of the vehicle controls for males and 2% lower for females. An alveolar cell adenoma was observed in the lung of 1/10 females that received 2,000 mg/kg.

Dose Selection Rationale: Because of the deaths in males and females at 2,000 mg/kg and the lower weight gain of males that received 1,000 mg/kg, doses selected for mice for the 2-year studies were 250 and 500 mg/kg *d*-limonene for males and 500 and 1,000 mg/kg *d*-limonene for females, administered in corn oil by gavage, 5 days per week.

TABLE 20. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SIXTEEN-DAY GAVAGE STUDIES OF *d*-LIMONENE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	25.4 ± 0.4	26.0 ± 0.9	+0.6 ± 0.9	
413	5/5	25.2 ± 0.5	23.0 ± 0.9	−2.2 ± 0.8	88.5
825	5/5	24.6 ± 0.2	24.2 ± 0.7	−0.4 ± 0.6	93.1
1,650	(d) 4/5	25.6 ± 0.6	25.3 ± 1.3	−0.5 ± 1.7	97.3
3,300	(e) 1/5	24.8 ± 0.4	19.0	−5.0	73.1
6,600	(f) 0/5	24.6 ± 0.2	(g)	(g)	(g)
FEMALE					
0	5/5	20.2 ± 0.2	21.8 ± 1.1	+1.6 ± 1.1	
413	5/5	21.4 ± 0.5	20.8 ± 0.5	−0.6 ± 0.5	95.4
825	5/5	20.0 ± 0.3	19.8 ± 0.6	−0.2 ± 0.4	90.8
1,650	(h) 4/5	21.2 ± 0.4	22.3 ± 0.6	+1.0 ± 1.0	102.3
3,300	(i) 0/5	20.4 ± 0.4	(g)	(g)	(g)
6,600	(j) 0/5	19.8 ± 0.2	(g)	(g)	(g)

(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) Day of death: 2
(e) Day of death: 2,2,3,3
(f) Day of death: all 1
(g) No data are reported due to the 100% mortality in this group.
(h) Death due to gavage error
(i) Day of death: 1,2,2,2,2
(j) Day of death: 1,1,1,2,2

TABLE 21. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF *d*-LIMONENE

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	26.6 ± 1.0	37.1 ± 1.0	+10.5 ± 1.3	
125	10/10	28.8 ± 0.7	37.9 ± 1.1	+9.1 ± 0.7	102.2
250	(d) 9/10	26.5 ± 0.8	33.9 ± 0.8	+7.6 ± 0.8	91.4
500	(d) 7/10	24.7 ± 0.9	34.4 ± 0.9	+9.7 ± 1.1	92.7
1,000	(d) 9/10	28.2 ± 0.9	33.3 ± 0.8	+5.1 ± 1.1	89.8
2,000	(e) 9/10	27.7 ± 0.7	33.0 ± 0.8	+5.6 ± 0.8	88.9
FEMALE					
0	10/10	21.3 ± 0.2	24.7 ± 0.5	+3.4 ± 0.4	
125	(d) 9/10	20.6 ± 0.3	25.9 ± 0.5	+5.2 ± 0.4	104.9
250	10/10	20.7 ± 0.3	25.4 ± 0.6	+4.7 ± 0.4	102.8
500	(f) 9/10	20.9 ± 0.2	24.9 ± 0.5	+4.1 ± 0.4	100.8
1,000	10/10	20.4 ± 0.2	24.1 ± 0.7	+3.7 ± 0.7	97.6
2,000	(g) 8/10	21.0 ± 0.3	24.1 ± 0.4	+3.4 ± 0.3	97.6

(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) Death due to gavage error
(e) Week of death: 1
(f) Week of death: 5
(g) Week of death: 3,4

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed and vehicle control male mice were similar throughout the studies (Table 22 and Figure 6). Mean body weights of high dose female mice were 5%-15% lower than those of the vehicle controls after week 28. No compound-related clinical signs were observed during the 2-year studies.

TABLE 22. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	Low Dose Av. Wt. (grams)	Low Dose Wt. (percent of veh. controls)	Low Dose No. of Survivors	High Dose Av. Wt. (grams)	High Dose Wt. (percent of veh. controls)	High Dose No. of Survivors
MALE				**250 mg/kg**			**500 mg/kg**	
0	30.2	50	30.3	100	50	30.3	100	50
1	31.1	50	31.4	101	50	31.1	100	50
2	32.0	50	31.6	99	50	31.0	97	50
3	32.0	50	32.3	101	50	32.7	102	50
4	32.7	50	33.4	102	50	32.8	100	50
5	33.5	50	33.9	101	50	33.5	100	50
6	34.8	50	35.7	103	50	35.6	102	50
7	35.0	50	35.8	102	50	35.6	102	50
8	35.5	50	36.2	102	50	36.1	102	50
9	36.2	50	37.2	103	50	37.1	102	50
10	--	--	--	--	--	37.3	--	50
11	37.1	50	37.8	102	50	38.4	104	50
12	38.5	50	38.7	101	47	38.2	99	50
16	41.2	50	41.7	101	47	41.4	100	50
21	43.4	50	43.9	101	47	43.9	101	50
24	--	--	--	--	--	45.7	--	49
25	46.3	50	47.4	102	47	--	--	--
28	46.8	50	47.7	102	46	47.5	101	49
32	47.3	50	48.1	102	46	47.9	101	49
36	48.4	50	47.6	98	46	48.1	99	49
40	48.2	50	48.5	101	45	48.8	101	49
44	47.2	50	46.7	99	45	46.7	99	49
48	48.9	50	48.8	100	45	48.1	98	49
52	47.8	49	47.8	100	45	47.8	100	49
56	48.8	48	48.6	100	44	48.8	100	49
60	48.3	47	47.5	98	41	47.6	99	48
64	49.0	46	48.4	99	40	48.4	99	46
68	47.8	45	48.3	101	39	48.8	102	44
72	48.4	45	48.1	99	39	49.0	101	44
76	50.0	43	48.8	98	38	49.8	100	44
81	48.8	42	49.2	101	36	50.7	104	43
85	48.0	42	49.1	102	34	50.2	105	43
88	49.5	40	49.2	99	32	50.4	102	41
92	46.8	38	46.7	100	32	51.8	111	40
96	45.1	35	45.4	101	31	48.1	107	40
99	44.7	34	45.8	102	28	47.4	106	40
101	45.3	34	46.4	102	27	47.2	104	40
103	44.3	33	44.4	100	24	45.9	104	39
FEMALE				**500 mg/kg**			**1,000 mg/kg**	
0	21.2	50	21.5	101	50	22.0	104	50
1	21.6	50	22.0	102	50	22.1	102	50
2	22.2	50	22.3	100	50	22.3	100	50
3	22.8	50	22.6	99	50	22.8	100	50
4	23.4	50	23.5	100	50	23.4	100	50
5	22.9	50	24.0	105	50	23.6	103	50
6	23.5	50	23.7	101	50	23.6	100	50
7	23.5	50	23.8	101	50	23.9	102	50
8	23.8	50	23.4	98	50	23.8	100	50
9	24.7	50	24.6	100	50	24.3	98	50
11	24.9	50	25.3	102	50	25.2	101	50
12	26.1	50	25.3	97	50	25.3	97	50
16	26.8	50	27.2	101	50	26.2	98	50
21	29.4	50	29.6	101	50	28.9	98	50
24	--	--	--	--	--	29.5	--	50
25	31.3	50	31.2	100	50	--	--	--
28	32.2	50	31.9	99	50	30.3	94	50
32	32.6	50	32.4	99	50	30.9	95	49
36	32.9	50	33.2	101	50	30.6	93	49
40	33.0	50	33.7	102	50	30.9	94	49
44	33.0	50	33.3	101	50	31.3	95	48
48	33.9	50	34.2	101	50	32.3	95	48
52	33.9	50	33.9	100	50	31.9	94	48
56	34.5	50	34.2	99	50	32.1	93	48
60	34.7	50	35.1	101	49	31.7	91	48
64	35.5	50	35.4	100	49	33.6	95	48
68	37.9	50	37.7	99	49	35.4	93	47
72	38.6	50	38.5	100	49	36.0	93	47
76	41.0	50	38.9	95	49	36.7	90	47
81	41.2	48	40.4	98	48	36.9	90	47
85	41.8	48	40.8	98	47	37.6	90	47
88	43.6	46	40.5	93	47	37.1	85	47
92	42.7	45	41.3	97	46	37.8	89	47
96	42.0	45	40.3	96	46	37.4	89	45
99	41.2	43	39.8	97	45	37.0	90	44
101	41.2	43	40.0	97	45	37.6	91	44
103	41.6	43	40.6	98	44	37.3	90	43

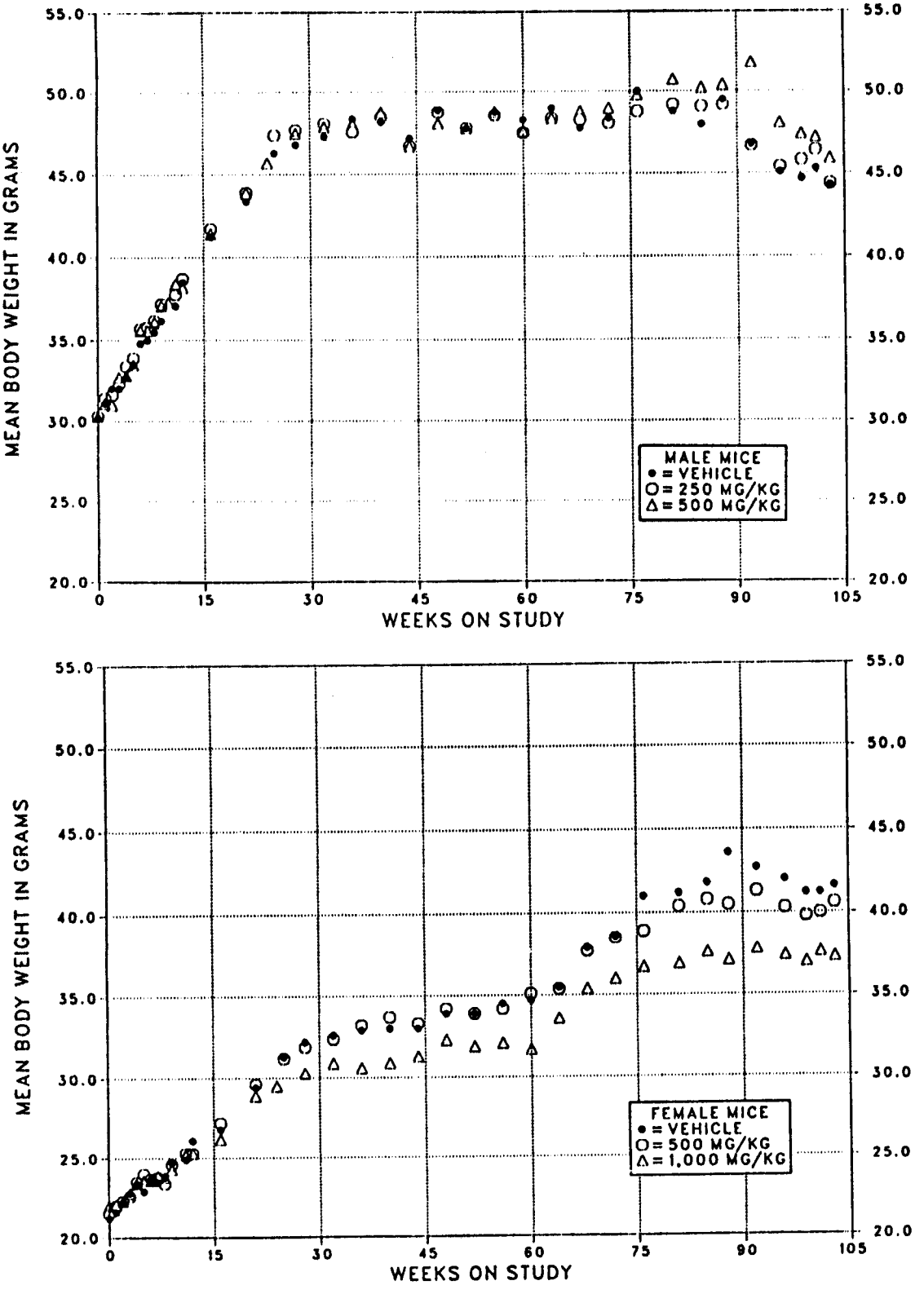


FIGURE 6. GROWTH CURVES FOR MICE ADMINISTERED *d*-LIMONENE IN CORN OIL BY GAVAGE FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female mice administered *d*-limonene at the doses used in these studies and for vehicle controls are shown in Table 23 and in the Kaplan and Meier curves in Figure 7. The survival of the low dose group of male mice was significantly lower than that of the vehicle controls at the end of the studies.

TABLE 23. SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
MALE (a)				
Animals initially in study	50	50	50	
Nonaccidental deaths before termination (b)	14	24	9	
Accidentally killed	2	2	2	
Animals missing	1	0	0	
Killed at termination	33	24	38	
Died during termination period	0	0	1	
Survival P values (c)	0.361	0.048	0.348	
FEMALE (a)				
Animals initially in study	50		50	50
Nonaccidental deaths before termination (b)	7		5	7
Accidentally killed	0		1	0
Killed at termination	42		44	42
Died during termination period	1		0	1
Survival P values (c)	1.000		0.757	0.995

(a) Termination period: week 104
(b) Includes animals killed in a moribund condition
(c) 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.

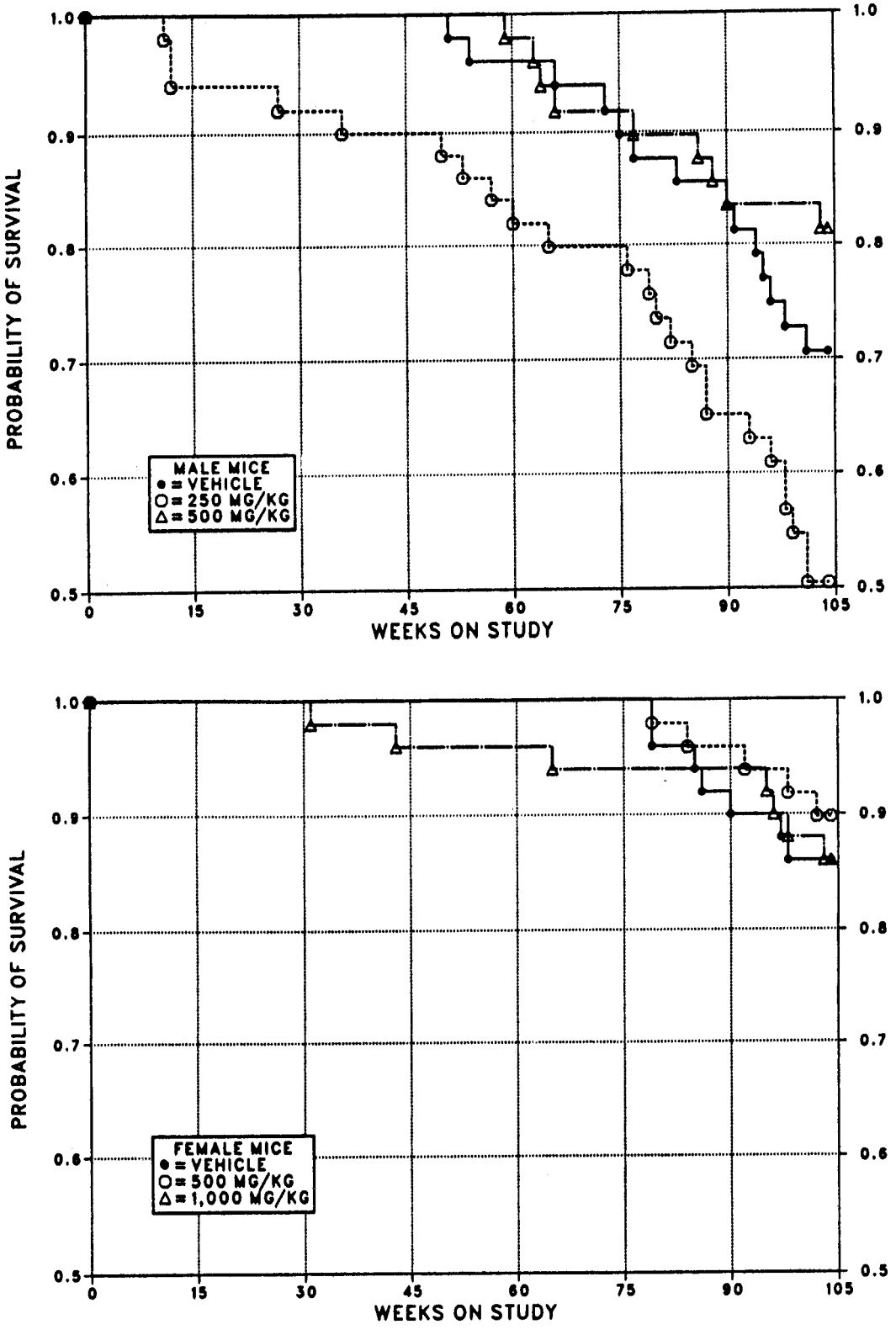


FIGURE 7. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED *d*-LIMONENE IN CORN OIL BY GAVAGE FOR TWO YEARS

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the liver and anterior pituitary gland.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes C and D for male and female mice, respectively.

Liver: Hepatocytes containing three or more nuclei (multinucleated cells) and cytomegaly occurred at increased incidences in high dose male mice (multinucleated cells--male: vehicle control, 8/49 low dose, 4/36; high dose, 32/50; female: none observed; cytomegaly--male: 23/49; 11/36; 38/50; female: none observed). The incidences of hepatocellular adenomas or carcinomas (combined) in dosed mice were not significantly different from those in the vehicle controls (male: 22/49; 14/36; 15/50; female: 4/50; 2/50; 8/49).

Anterior Pituitary Gland: The incidence of adenomas in high dose female mice was significantly lower than in the vehicle controls (Table 24). Focal hyperplasia was observed both in vehicle control and in high dose female mice. Biologically, these represent a continuum of pituitary gland lesions. The combined incidence of hyperplasia, adenomas, or carcinomas (all occurring in different animals) was not significant by the Fisher exact test (vehicle control, 28/49; low dose, 5/8; high dose, 19/48; P = 0.06).

TABLE 24. ANTERIOR PITUITARY GLAND LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (a)

	Vehicle Control	500 mg/kg	1,000 mg/kg
Focal Hyperplasia			
Overall Rates	16/49 (33%)	0/8 (0%)	17/48 (35%)
Adenoma			
Overall Rates	12/49 (24%)	5/8 (63%)	1/48 (2%)
Adjusted Rates	27.9%		2.4%
Terminal Rates	12/43 (28%)		1/41 (2%)
Week of First Observation	104		104
Life Table Test			P=0.002N
Incidental Tumor Test			P=0.002N
Carcinoma			
Overall Rates	0/49 (0%)	0/8 (0%)	1/48 (2%)
Adenoma or Carcinoma (b)			
Overall Rates	12/49 (24%)	5/8 (63%)	2/48 (4%)
Adjusted Rates	27.9%		4.9%
Terminal Rates	12/43 (28%)		2/41 (5%)
Week of First Observation	104		104
Life Table Test			P=0.006N
Incidental Tumor Test			P=0.006N

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table D3 (footnotes).
(b) Historical incidence in NTP studies (mean ± SD): 396/1,798 (22% ± 10%)

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

d-Limonene, a monoterpene found in many volatile oils, especially in citrus oils, is widely used as a flavoring and fragrance additive for foods, cosmetics, soaps, and many kinds of technical goods (Igimi et al., 1974). Other properties of the chemical allow its use as an industrial solvent to substitute for chlorinated hydrocarbon solvents (Merck, 1983). Clinically, it has been used in an attempt to dissolve cholesterol gallstones (Igimi et al., 1976). *d*-Limonene has been tested for mutagenicity in bacteria and cultured mammalian cells but has not been studied in vivo. Neither *d*-limonene nor two presumed intermediary epoxide metabolites were mutagenic in Salmonella (Watabe et al., 1980). *d*-Limonene did not induce either forward gene mutations in mouse lymphoma cells or cytogenetic damage to Chinese hamster ovary cells in culture (Appendix E). Toxicology and carcinogenicity studies of *d*-limonene were conducted because of the high production volume (USEPA, 1977), widespread human exposure in food products and cosmetics, and lack of adequate long-term toxicity data. The instability of *d*-limonene in feed mixtures necessitated gavage administration for these studies.

The short-term toxicity of *d*-limonene was evaluated in rats and mice. In 16-day studies, deaths occurred in rats and mice receiving 3,300 and 6,600 mg/kg and in male mice dosed with 1,650 mg/kg. No compound-related clinical signs or histopathologic lesions were associated with chemical administration. Because of deaths at higher doses, the doses selected for the 13-week studies in rats ranged from 2,400 mg/kg down to 150 mg/kg and in mice, from 2,000 mg/kg down to 125 mg/kg.

In the 13-week studies in rats, compound-related deaths occurred in 5/10 high dose males and in 9/10 high dose females during the first week of the studies. No other deaths occurred during the studies. Body weight gain depression was observed for males only in the three highest dosed groups. No weight gain depression was observed in any of the female dosed groups in which all the animals survived. Histopathologic examinations indicated no compound-related lesions in female rats. The renal lesions that occurred in dosed male rats given *d*-limonene for 13 weeks are remarkably similar to those observed in male rats given, or exposed to, a variety of compounds studied by the National Toxicology Program, including pentachloroethane (NTP, 1983), dimethyl methylphosphonate (NTP, 1987a), and 1,4-dichlorobenzene (NTP, 1987b). Chemicals studied by other investigators, including decalin (Stone et al., 1987a,b; Kanerva et al., 1987a,b,c), unleaded gasoline (Kitchen, 1984), 2,2,4-trimethylpentane (Short et al., 1986, 1987), and other hydrocarbon solvents and fuels (Halder et al., 1984), have shown similar effects. Although the profile of toxicity varied from chemical to chemical with all these compounds, toxic kidney lesions were produced only in male rats and not in female rats or in mice of either sex.

The sex- and species-specific nature of the kidney lesions in male rats exposed to vapors of the light hydrocarbon compounds has been attributed to the accumulation of the low molecular weight protein $\alpha_{2\mu}$-globulin (Stonard et al., 1986). This protein is produced in the liver under the influence of testosterone and is readily filtered through the glomeruli of the kidney. After short-term exposure to decalin, unleaded gasoline, or trimethylpentane, $\alpha_{2\mu}$-globulin accumulates within phagolysozomes of epithelial cells primarily in the P2 segment of the nephron (Charbonneau et al., 1987). The enlarged phagolysozomes containing the protein appear as hyaline droplets by light microscopy. The accumulation of these droplets is accompanied by tubular cell degeneration and granular casts in tubule lumens consisting of necrotic cell debris.

The immediate cause of the kidney tubular cell cytotoxicity is as yet unknown, but reversible binding of a metabolite of trimethylpentane to $\alpha_{2\mu}$-globulin within the kidney has been shown (Lock et al., 1987). Binding of metabolites of volatile hydrocarbons to $\alpha_{2\mu}$-globulin may prevent or decrease the lysozomal catabolism of this protein, resulting in its accumulation within the phagolysozomes of the tubular epithelium. Phagolysozomes containing crystallized proteins, with associated cellular damage, have been reported in human and experimental animal proteinuric conditions that are caused by increased glomerular filtration of the light chain portion of the immunoglobulins (Clyne et al., 1974), lysozyme (Osserman and Azar, 1969), and lysine (Madsen et al., 1976; Males et al., 1984).

An increased rate of cell replication in the P2 segment has been demonstrated by autoradiography in male rats dosed with trimethylpentane, and increased DNA synthesis, as shown by the incorporation of [^{3}H]thymidine into DNA, has been demonstrated in male rats dosed with trimethylpentane or 1,4-dichlorobenzene (Charbonneau et al., 1987, 1989). These findings are consistent with the histologic evidence that regeneration of the tubular epithelium occurs in response to cell necrosis.

At the conclusion of the current 13-week study of *d*-limonene, an accumulation of hyaline droplets in the proximal convoluted tubules of dosed male rats was not demonstrated by light microscopy. Short-term studies (up to 27 days) by other investigators, however, have demonstrated that hyaline droplets consisting of $\alpha_{2\mu}$-globulin do accumulate in the tubular epithelium of male rats given 75, 150, or 300 mg/kg *d*-limonene (Kanerva et al., 1987c). These data indicate that kidney lesions induced in male rats given *d*-limonene for 13 weeks may be related to the accumulation of $\alpha_{2\mu}$-globulin within the kidney. The reason for the difference between findings from these 13-week studies and those from the Kanerva study is unknown but may be related to the interval between the time the chemical was last administered and the time the animals were killed and examined histologically. In the current 13-week studies, 3 days elapsed between the time of the last dose and when the animals were killed. In subsequent 21-day studies, male and female rats were administered *d*-limonene at doses ranging from 75 to 1,200 mg/kg. Microscopic examination of kidney sections from these rats, as well as an ELISA test on kidney homogenates, indicated that *d*-limonene does cause an increase in $\alpha_{2\mu}$-globulin within the proximal convoluted tubular epithelium of male rats relative to vehicle controls but not within that of female rats.

Based on the results of the 13-week studies, doses of 75 and 150 mg/kg were selected for male rats for the 2-year studies because of the compound-related kidney lesions observed at 300 mg/kg and higher which were considered potentially life threatening. Doses of 300 and 600 mg/kg were selected for female rats because the large number of deaths at 2,400 mg/kg suggested that long-term exposure at 1,200 mg/kg might result in reduced survival.

In the 13-week studies in mice, some animals in the 2,000 mg/kg groups died, and an 11% reduction in body weight was observed for males receiving the two highest doses. Doses selected for mice for the 2-year studies were 250 and 500 mg/kg for males and 500 and 1,000 mg/kg for females, based on the number of deaths and decreased body weight gain at higher doses.

In the 2-year studies, mean body weights of rats and mice dosed with *d*-limonene were generally similar to those of the vehicle controls, except for those of high dose female mice which were 5%-15% lower after week 28. Survival of the high dose group of female rats was significantly lower than that of the vehicle control group after week 39 (see Table 13). Survival of the low dose group of male mice was significantly lower than that of the vehicle controls at the end of the study (see Table 23).

Consistent with the results of the 13-week studies, the kidney of male rats was the target organ in the 2-year study. Male rats dosed with *d*-limonene showed a spectrum of compound-related kidney lesions, including exacerbation of the age-related nephropathy, mineralization in the renal medulla, hyperplasia of the transitional epithelium overlying the renal papilla, and proliferative lesions of the renal tubular cell epithelium.

These proliferative lesions consisted of tubular cell hyperplasia, adenomas, and adenocarcinomas. The pathogenesis of renal cortical epithelial neoplasms in rats is thought to involve a progression from tubular cell hyperplasia to tubular cell adenomas and, with increasing size, to adenocarcinomas or carcinomas (Hard, 1986). A similar progression in development was observed with tubular cell neoplasms induced in F344 rats exposed to *N*-(4′-fluoro-4-biphenyl)-acetamide (Dees et al., 1980), tris(2,3-dibromopropyl)phosphate (Reznick et al., 1979), and tetrachloroethylene (NTP, 1986). Studies in which tubular cell neoplasms were induced in Wistar rats exposed to dimethylnitrosamine have shown that once these neoplasms attain macroscopic dimensions (2 cm or larger), they have a high potential for metastasis (Hard, 1984).

IV. DISCUSSION AND CONCLUSIONS

The mechanism of tumor formation in the kidney of male rats given *d*-limonene is uncertain at the present time. The similarity of the nephrotoxicity observed in the 2-year studies of *d*-limonene and 1,4-dichlorobenzene (NTP, 1987b), dimethyl methylphosphonate (NTP, 1987a), unleaded gasoline and JP-5 navy fuel (Halder et al., 1984) and the sex- and species-specific nature of the response provide circumstantial but not definitive evidence that there may be a common mechanism for the induction of both the nonneoplastic lesions and renal neoplasms. Cell degeneration and necrosis in the P2 segment of the proximal convoluted tubules associated with the accumulation of $\alpha_{2\mu}$-globulin have been demonstrated with several of these compounds, and it has been suggested that the increased cell replication rates caused by prolonged degeneration and necrosis may promote spontaneously initiated renal epithelial cells (Charbonneau et al., 1987). It is noteworthy that 1,2-dichlorobenzene, the ortho isomer of 1,4-dichlorobenzene, did not cause the development of these nonneoplastic lesions or tubular cell neoplasms in male rats dosed for 2 years (NTP, 1985), and in one short-term study, this compound also did not induce the accumulation of $\alpha_{2\mu}$-globulin or cause increased cell proliferation in the kidney (Charbonneau et al., 1989).

The issue concerning the mechanism of renal tumor formation in male rats and the possible link to $\alpha_{2\mu}$-globulin is important. In humans, males also develop a greater incidence of kidney neoplasms than do females (Page and Asire, 1985; Pickle et al., 1987). Although humans have not been shown to have $\alpha_{2\mu}$-globulin, they do produce low molecular weight serum proteins that are reabsorbed in the human kidney. If binding of a compound or its metabolite to a protein is necessary for the localization of a material within the cell, it is important to determine if any human serum proteins have a binding site similar to that of $\alpha_{2\mu}$-globulin and to determine if these have the same cytotoxicity as $\alpha_{2\mu}$-globulin.

When compared with the results of the initiation-promotion studies with DMBA and *d*-limonene (Elegbede et al., 1986b), the results of the current study are not inconsistent and show a statistically significant dose-related decrease in the occurrence of mammary gland fibroadenomas, adenomas, cystadenomas, or adenocarcinomas (combined) in female rats (vehicle control, 23/50; low dose, 17/50; high dose, 9/50; $P = 0.044$ for trend). Reduced survival of the high dose female rat group ($P = 0.006$) may be partially responsible for the observations of fewer tumors in that group compared with vehicle controls.

No compound-related neoplasms were observed in mice in the 2-year studies of *d*-limonene. The incidence of adenomas of the anterior pituitary gland in high dose female mice was significantly lower than those in the vehicle controls (see Table 24) and may be attributed to the administration of *d*-limonene. A toxic response in the liver of high dose male mice was demonstrated by the presence of cells with an abnormal number of nuclei and cytomegaly.

The experimental and tabulated data for the NTP Technical Report on *d*-limonene were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix H, 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.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of *d*-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was *no evidence of carcinogenic activity* of *d*-limonene for female F344/N rats that received 300 or 600 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for male B6C3F$_1$ mice that received 250 or 500 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for female B6C3F$_1$ mice that received 500 or 1,000 mg/kg.

An increased severity of spontaneous nephropathy, increased incidences of linear mineralization of the renal medulla and papilla, and hyperplasia of the transitional epithelium of the renal papilla were present in dosed male rats.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 9-10.

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

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

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)
Squamous cell carcinoma			2 (4%)
Basal cell tumor			1 (2%)
Trichoepithelioma	1 (2%)		
Keratoacanthoma	2 (4%)	1 (2%)	2 (4%)
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS	1 (2%)		
Fibroma	8 (16%)	2 (4%)	3 (6%)
Fibrosarcoma		2 (4%)	
Lipoma	2 (4%)	1 (2%)	
Chordoma		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(50)	(16)	(50)
Squamous cell carcinoma		1 (6%)	
Alveolar/bronchiolar adenoma	2 (4%)	1 (6%)	
Alveolar/bronchiolar carcinoma	1 (2%)	1 (6%)	1 (2%)
Osteosarcoma, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	10 (20%)	10 (20%)	19 (38%)
#Spleen	(50)	(50)	(50)
Fibroma		1 (2%)	
CIRCULATORY SYSTEM			
*Abdominal wall	(50)	(50)	(50)
Hemangioma			1 (2%)
#Heart	(50)	(12)	(50)
Osteosarcoma, metastatic	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Neoplastic nodule	2 (4%)	2 (4%)	1 (2%)
Hepatocellular carcinoma			1 (2%)
#Pancreas	(45)	(12)	(49)
Acinar cell adenoma	2 (4%)	1 (8%)	1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Tubular cell adenoma		4 (8%)	8 (16%)
Tubular cell adenocarcinoma		4 (8%)	3 (6%)
#Urinary bladder	(48)	(11)	(47)
Transitional cell papilloma	1 (2%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(50)	(17)	(50)
Adenoma, NOS	16 (32%)	6 (35%)	11 (22%)

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

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal	(50)	(50)	(50)
Pheochromocytoma	15 (30%)	18 (36%)	25 (50%)
Pheochromocytoma, malignant	3 (6%)	4 (8%)	2 (4%)
#Thyroid	(48)	(10)	(50)
Follicular cell adenoma			1 (2%)
Follicular cell carcinoma	1 (2%)		
C-cell adenoma	1 (2%)		2 (4%)
C-cell carcinoma	1 (2%)		2 (4%)
#Pancreatic islets	(45)	(12)	(49)
Islet cell adenoma	4 (9%)	1 (8%)	6 (12%)
Islet cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenoma, NOS		1 (2%)	
Adenocarcinoma, NOS		1 (2%)	
Fibroadenoma	3 (6%)	2 (4%)	3 (6%)
*Preputial gland	(50)	(50)	(50)
Adenoma, NOS	2 (4%)	4 (8%)	4 (8%)
#Testis	(50)	(49)	(50)
Interstitial cell tumor	37 (74%)	47 (96%)	48 (96%)
Mesothelioma, malignant	2 (4%)	6 (12%)	4 (8%)
NERVOUS SYSTEM			
#Brain	(50)	(11)	(50)
Granular cell tumor, NOS			1 (2%)
Astrocytoma			1 (2%)
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS		1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
*Bone	(50)	(50)	(50)
Chondrosarcoma	1 (2%)		
BODY CAVITIES			
*Thoracic cavity	(50)	(50)	(50)
Mesothelioma, malignant			1 (2%)
*Pelvis	(50)	(50)	(50)
Osteosarcoma	2 (4%)		
*Mesentery	(50)	(50)	(50)
Mesothelioma, metastatic			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mesothelioma, metastatic	1 (2%)	3 (6%)	2 (4%)
Head			
Carcinoma, NOS	1		

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

	Vehicle Control	Low Dose	High Dose
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	18	12	4
Moribund sacrifice	2	4	1
Terminal sacrifice	29	33	40
Dosing accident	1	1	5
TUMOR SUMMARY			
Total animals with primary tumors**	46	47	48
Total primary tumors	122	123	157
Total animals with benign tumors	44	47	48
Total benign tumors	96	90	117
Total animals with malignant tumors	19	28	29
Total malignant tumors	24	31	38
Total animals with secondary tumors##	2	3	3
Total secondary tumors	3	3	3
Total animals with tumors-- uncertain benign or malignant	2	2	2
Total uncertain tumors	2	2	2

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

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

ANIMAL NUMBER	C 45	C 12	C 42	C 02	C 17	C 08	C 50	C 47	C 07	C 37	C 46	C 18	C 39	C 35	C 23	C 11	C 31	C 32	C 33	C 26	C 27	C 01	C 03	C 04	C 05
WEEKS ON STUDY	030	032	033	039	073	079	082	083	086	087	087	088	092	095	096	098	098	099	100	103	103	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
Trichoepithelioma														X											
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
Sarcoma, NOS								X																	
Fibroma										X		X						X							
Lipoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Osteosarcoma, metastatic															X										
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	−	−	−	+	+	−	+	+	+	+	−	+	−	−	+	+	−	+	+	+	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic															X										
DIGESTIVE SYSTEM																									
Salivary gland	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	+
Acinar cell adenoma																							X		
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	+	+	−	−	+	+	+	+
Large intestine	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Transitional cell papilloma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS								X				X					X						X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma													X	X				X	X	X		X		X	X
Pheochromocytoma, malignant																	X								
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma																	X								
C-cell carcinoma																									
Parathyroid	+	+	−	+	+	+	+	+	−	+	−	+	−	−	+	−	−	+	−	+	+	−	+	+	+
Pancreatic islets	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	+
Islet cell adenoma																									
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	N	N	+	+	N	N	N	N	N	+	+	+	N	+	+	+
Fibroadenoma																									X
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor						X			X	X	X		X	X	X	X		X	X	X	X	X	X	X	X
Mesothelioma, malignant																							X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS									X													X			
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Chondrosarcoma							X																		
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Osteosarcoma						X									X										
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, metastatic																									
Leukemia, mononuclear cell											X					X		X	X				X		
Head, NOS																									
Carcinoma, NOS					X																				

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: *Necropsy, no histology* due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	C 0 0 6	C 0 0 9	C 0 1 0	C 0 1 3	C 0 1 4	C 0 1 5	C 0 1 6	C 0 1 9	C 0 2 0	C 0 2 1	C 0 2 2	C 0 2 4	C 0 2 5	C 0 2 8	C 0 2 9	C 0 3 0	C 0 3 4	C 0 3 6	C 0 3 8	C 0 4 0	C 0 4 1	C 0 4 3	C 0 4 4	C 0 4 8	C 0 4 9	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Trichoepithelioma																										1
Keratoacanthoma	X																		X							2
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
Fibroma			X											X							X	X	X			8
Lipoma										X														X		2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																					X			2
Alveolar/bronchiolar carcinoma																							X			1
Osteosarcoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	−	−	+	−	+	+	+	−	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	35
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic																										1
DIGESTIVE SYSTEM																										
Salivary gland	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule									X				X													2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Acinar cell adenoma											X															2
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	48
Transitional cell papilloma							X																			1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS	X	X	X		X					X	X				X			X	X			X	X			16
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma			X			X			X			X	X							X	X					15
Pheochromocytoma, malignant	X					X																				3
Thyroid	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell carcinoma																								X		1
C-cell adenoma																										1
C-cell carcinoma																									X	1
Parathyroid	+	−	+	−	−	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	−	−	+	−	33
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Islet cell adenoma		X						X			X									X						4
Islet cell carcinoma																						X				1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	*50
Fibroadenoma												X				X										3
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X		X	X	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X		X	X	37
Mesothelioma, malignant						X																				2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	49
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																										2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Chondrosarcoma																										1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Osteosarcoma																										2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, metastatic						X																				1
Leukemia, mononuclear cell											X									X		X	X		X	10
Head, NOS																										
Carcinoma, NOS																										1

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: LOW DOSE

ANIMAL NUMBER	C28	C26	C01	C37	C32	C10	C36	C21	C33	C27	C20	C24	C34	C22	C25	C29	C46	C02	C03	C04	C05	C06	C07	C08	C09
WEEKS ON STUDY	011	026	057	069	076	081	085	086	089	090	092	097	098	101	101	101	101	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma																				X					
Fibrosarcoma							X																		
Lipoma																							X		
Chordoma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	−	+	−	−	+	−	−	−	+	−
Squamous cell carcinoma																								X	
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																				X					
Trachea	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Lymph nodes	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Thymus	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Small intestine	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Large intestine	+	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma															X			X							
Tubular cell adenocarcinoma														X							X				
Urinary bladder	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	+	−	−	−
Adenoma, NOS										X										X		X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma									X					X				X	X				X	X	
Pheochromocytoma, malignant							X								X										
Thyroid	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Parathyroid	+	+	−	+	+	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pancreatic islets	+	+	+	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	N	N	+	+	+	+	+	N	N	N	N	N	N	N	N	N	+	N	N	N	N
Adenoma, NOS																									
Adenocarcinoma, NOS																									
Fibroadenoma									X												X				
Testis	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor			X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mesothelioma, malignant											X		X			X	X			X					
Prostate	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, metastatic												X					X	X							
Leukemia, mononuclear cell						X		X	X										X						

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	C 1 1 1	C 1 1 2	C 1 1 3	C 1 1 4	C 1 1 5	C 1 1 6	C 1 1 7	C 1 1 8	C 1 1 9	C 1 2 3	C 1 3 0	C 1 3 1	C 1 3 5	C 1 3 8	C 1 3 9	C 1 4 0	C 1 4 1	C 1 4 2	C 1 4 3	C 1 4 4	C 1 4 5	C 1 4 7	C 1 4 8	C 1 4 9	C 1 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	N	N	+	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Keratoacanthoma			X																							1
Subcutaneous tissue	N	N	+	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibroma								X																		2
Fibrosarcoma																								X		2
Lipoma																										1
Chordoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	16
Squamous cell carcinoma																										1
Alveolar/bronchiolar adenoma												X														1
Alveolar/bronchiolar carcinoma																										1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma					X																					1
Lymph nodes	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	13
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	12
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	11
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule					X								X													2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	12
Acinar cell adenoma																		X								1
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma														X								X				4
Tubular cell adenocarcinoma												X			X											4
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
ENDOCRINE SYSTEM																										
Pituitary	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+	–	–	–	–	+	–	17
Adenoma, NOS											X							X	X							6
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma				X	X		X			X	X		X			X		X		X	X	X			X	18
Pheochromocytoma, malignant															X	X										4
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Pancreatic islets	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	12
Islet cell adenoma																		X								1
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	+	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	*50
Adenoma, NOS											X															1
Adenocarcinoma, NOS																						X				1
Fibroadenoma																										2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	47
Mesothelioma, malignant				X																						6
Prostate	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	12
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS					X														X		X				X	4
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS														X												1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, metastatic																										3
Leukemia, mononuclear cell			X						X	X			X				X				X					10

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: HIGH DOSE

ANIMAL NUMBER	C 1 6	C 2 9	C 0 2	C 3 1	C 3 2	C 3 5	C 0 3	C 0 7	C 1 7	C 2 5	C 0 1	C 0 4	C 0 5	C 0 6	C 0 8	C 0 9	C 1 0	C 1 1	C 1 2	C 1 3	C 1 4	C 1 5	C 1 8	C 1 9	C 2 0
WEEKS ON STUDY	0 6 6	0 7 8	0 8 1	0 8 4	0 8 8	0 9 6	0 9 8	1 0 0	1 0 2	1 0 3	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Skin	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell carcinoma								X																	
Basal cell tumor																									
Keratoacanthoma												X													
Subcutaneous tissue	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	−	+	+	+	+	−	−	+	+	−	+	−	+	+	+	−	−	+	+	+	+	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule					X																				
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Acinar cell adenoma										X															
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma						X					X									X					X
Tubular cell adenocarcinoma							X		X																
Urinary bladder	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																X		X					X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma				X	X			X			X	X					X	X	X	X	X				
Pheochromocytoma, malignant				X																					
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma											X														
C-cell adenoma																									
C-cell carcinoma																									
Parathyroid	−	+	+	−	+	+	+	+	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Islet cell adenoma												X			X							X	X		
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																			X						
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Mesothelioma, malignant									X																
Prostate	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS			X				X																		
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor, NOS																									
Astrocytoma																		X							
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS								X																	
BODY CAVITIES																									
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, malignant																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hemangioma																									
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, metastatic																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, metastatic									X																
Leukemia, mononuclear cell					X		X				X	X			X	X			X			X		X	

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	C 2 1	C 2 2	C 2 3	C 2 4	C 2 6	C 2 7	C 2 8	C 3 0	C 3 3	C 3 4	C 3 6	C 3 7	C 3 8	C 3 9	C 4 0	C 4 1	C 4 2	C 4 3	C 4 4	C 4 5	C 4 6	C 4 7	C 4 8	C 4 9	C 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma		X																								1
Squamous cell carcinoma									X																	2
Basal cell tumor								X																		1
Keratoacanthoma					X																					2
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma										X													X			3
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma					X																					1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	−	+	−	+	−	+	+	+	+	−	+	−	+	−	−	+	+	+	+	−	−	+	−	−	31
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																										1
Hepatocellular carcinoma									X																	1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinar cell adenoma																										1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma										X											X	X			X	8
Tubular cell adenocarcinoma															X											3
Urinary bladder	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS					X		X		X				X		X			X			X				X	11
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X	X		X			X	X	X		X		X		X			X	X	X		X	X		X	25
Pheochromocytoma, malignant																						X				2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
C-cell adenoma						X	X																			2
C cell carcinoma														X			X									2
Parathyroid	+	+	−	+	−	+	+	−	+	+	−	−	+	+	+	+	+	−	−	+	+	+	+	+	+	38
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma					X	X																				6
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	N	+	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	+	N	*50
Fibroadenoma		X																X								3
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	48
Mesothelioma, malignant	X										X			X												4
Prostate	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS		X																						X		4
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor, NOS														X												1
Astrocytoma																										1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	*50
Carcinoma, NOS																				X						2
BODY CAVITIES																										
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, malignant																X										1
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hemangioma												X														1
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, metastatic											X															1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, metastatic														X												2
Leukemia, mononuclear cell		X				X			X	X		X				X	X				X			X		19

* Animals necropsied

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

	Vehicle Control	75 mg/kg	150 mg/kg
Skin: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	0.0%	0.0%	7.2%
Terminal Rates (c)	0/29 (0%)	0/33 (0%)	2/40 (5%)
Week of First Observation			100
Life Table Tests (d)	P=0.060	(e)	P=0.184
Incidental Tumor Tests (d)	P=0.041	(e)	P=0.129
Cochran-Armitage Trend Test (d)	P=0.037		
Fisher Exact Test (d)		(e)	P=0.121
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	8/50 (16%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	23.7%	6.1%	7.5%
Terminal Rates (c)	5/29 (17%)	2/33 (6%)	3/40 (7%)
Week of First Observation	87	104	104
Life Table Tests (d)	P=0.021N	P=0.034N	P=0.039N
Incidental Tumor Tests (d)	P=0.041N	P=0.048N	P=0.088N
Cochran-Armitage Trend Test (d)	P=0.055N		
Fisher Exact Test (d)		P=0.046N	P=0.100N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	8/50 (16%)	4/50 (8%)	3/50 (6%)
Adjusted Rates (b)	23.7%	11.2%	7.5%
Terminal Rates (c)	5/29 (17%)	3/33 (9%)	3/40 (7%)
Week of First Observation	87	85	104
Life Table Tests (d)	P=0.026N	P=0.136N	P=0.039N
Incidental Tumor Tests (d)	P=0.058N	P=0.182N	P=0.088N
Cochran-Armitage Trend Test (d)	P=0.067N		
Fisher Exact Test (d)		P=0.178N	P=0.100N
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	9/50 (18%)	4/50 (8%)	3/50 (6%)
Adjusted Rates (b)	25.5%	11.2%	7.5%
Terminal Rates (c)	5/29 (17%)	3/33 (9%)	3/40 (7%)
Week of First Observation	83	85	104
Life Table Tests (d)	P=0.014N	P=0.090N	P=0.023N
Incidental Tumor Tests (d)	P=0.038N	P=0.126N	P=0.068N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.117N	P=0.061N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	10/50 (20%)	10/50 (20%)	19/50 (38%)
Adjusted Rates (b)	29.4%	26.5%	45.0%
Terminal Rates (c)	6/29 (21%)	7/33 (21%)	17/40 (43%)
Week of First Observation	87	81	88
Life Table Tests (d)	P=0.147	P=0.491N	P=0.211
Incidental Tumor Tests (d)	P=0.043	P=0.583	P=0.081
Cochran-Armitage Trend Test (d)	P=0.026		
Fisher Exact Test (d)		P=0.598	P=0.038
Kidney: Tubular Cell Adenoma			
Overall Rates (a)	0/50 (0%)	4/50 (8%)	8/50 (16%)
Adjusted Rates (b)	0.0%	11.5%	19.3%
Terminal Rates (c)	0/29 (0%)	3/33 (9%)	7/40 (18%)
Week of First Observation		101	96
Life Table Tests (d)	P=0.011	P=0.084	P=0.016
Incidental Tumor Tests (d)	P=0.006	P=0.067	P=0.011
Cochran-Armitage Trend Test (d)	P=0.003		
Fisher Exact Test (d)		P=0.059	P=0.003

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

	Vehicle Control	75 mg/kg	150 mg/kg
Kidney: Tubular Cell Adenocarcinoma			
Overall Rates (a)	0/50 (0%)	4/50 (8%)	3/50 (6%)
Adjusted Rates (b)	0.0%	11.5%	7.0%
Terminal Rates (c)	0/29 (0%)	3/33 (9%)	1/40 (3%)
Week of First Observation		101	98
Life Table Tests (d)	P=0.202	P=0.084	P=0.179
Incidental Tumor Tests (d)	P=0.111	P=0.067	P=0.081
Cochran-Armitage Trend Test (d)	P=0.118		
Fisher Exact Test (d)		P=0.059	P=0.121
Kidney: Tubular Cell Adenoma or Adenocarcinoma			
Overall Rates (a)	0/50 (0%)	8/50 (16%)	11/50 (22%)
Adjusted Rates (b)	0.0%	22.6%	25.4%
Terminal Rates (c)	0/29 (0%)	6/33 (18%)	8/40 (20%)
Week of First Observation		101	96
Life Table Tests (d)	P=0.006	P=0.009	P=0.004
Incidental Tumor Tests (d)	P=0.001	P=0.005	P=0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.003	P<0.001
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	16/50 (32%)	(f) 6/17 (35%)	11/50 (22%)
Adjusted Rates (b)	47.6%		27.5%
Terminal Rates (c)	12/29 (41%)		11/40 (28%)
Week of First Observation	83		104
Life Table Test (d)			P=0.033N
Incidental Tumor Test (d)			P=0.071N
Fisher Exact Test (d)			P=0.184N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	15/50 (30%)	18/50 (36%)	25/50 (50%)
Adjusted Rates (b)	43.6%	51.1%	57.9%
Terminal Rates (c)	10/29 (34%)	16/33 (48%)	22/40 (55%)
Week of First Observation	92	89	84
Life Table Tests (d)	P=0.232	P=0.502	P=0.273
Incidental Tumor Tests (d)	P=0.083	P=0.385	P=0.086
Cochran-Armitage Trend Test (d)	P=0.026		
Fisher Exact Test (d)		P=0.335	P=0.033
Adrenal Gland: Malignant Pheochromocytoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	9.6%	10.7%	4.6%
Terminal Rates (c)	2/29 (7%)	2/33 (6%)	1/40 (3%)
Week of First Observation	98	85	84
Life Table Tests (d)	P=0.298N	P=0.563	P=0.384N
Incidental Tumor Tests (d)	P=0.477N	P=0.473	P=0.539N
Cochran-Armitage Trend Test (d)	P=0.417N		
Fisher Exact Test (d)		P=0.500	P=0.500N
Adrenal Gland: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	17/50 (34%)	21/50 (42%)	25/50 (50%)
Adjusted Rates (b)	48.1%	56.2%	57.9%
Terminal Rates (c)	11/29 (38%)	17/33 (52%)	22/40 (55%)
Week of First Observation	92	85	84
Life Table Tests (d)	P=0.410	P=0.446	P=0.442
Incidental Tumor Tests (d)	P=0.153	P=0.287	P=0.171
Cochran-Armitage Trend Test (d)	P=0.064		
Fisher Exact Test (d)		P=0.268	P=0.078

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

	Vehicle Control	75 mg/kg	150 mg/kg
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	2/48 (4%)	(f) 0/10 (0%)	4/50 (8%)
Adjusted Rates (b)	6.3%		10.0%
Terminal Rates (c)	1/28 (4%)		4/40 (10%)
Week of First Observation	98		104
Life Table Test (d)			P=0.499
Incidental Tumor Test (d)			P=0.441
Fisher Exact Test (d)			P=0.358
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	4/45 (9%)	(f) 1/12 (8%)	6/49 (12%)
Adjusted Rates (b)	13.8%		15.4%
Terminal Rates (c)	4/29 (14%)		6/39 (15%)
Week of First Observation	104		104
Life Table Test (d)			P=0.564
Incidental Tumor Test (d)			P=0.564
Fisher Exact Test (d)			P=0.426
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	5/45 (11%)	(f) 1/12 (8%)	6/49 (12%)
Adjusted Rates (b)	17.2%		15.4%
Terminal Rates (c)	5/29 (17%)		6/39 (15%)
Week of First Observation	104		104
Life Table Test (d)			P=0.550N
Incidental Tumor Test (d)			P=0.550N
Fisher Exact Test (d)			P=0.561
Mammary Gland: Fibroadenoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	10.3%	5.3%	7.5%
Terminal Rates (c)	3/29 (10%)	1/33 (3%)	3/40 (7%)
Week of First Observation	104	89	104
Life Table Tests (d)	P=0.445N	P=0.444N	P=0.507N
Incidental Tumor Tests (d)	P=0.507N	P=0.479N	P=0.507N
Cochran-Armitage Trend Test (d)	P=0.588		
Fisher Exact Test (d)		P=0.500N	P=0.661
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	10.3%	8.3%	7.5%
Terminal Rates (c)	3/29 (10%)	2/33 (6%)	3/40 (7%)
Week of First Observation	104	89	104
Life Table Tests (d)	P=0.430N	P=0.604N	P=0.507N
Incidental Tumor Tests (d)	P=0.488N	P=0.635N	P=0.507N
Cochran-Armitage Trend Test (d)	P=0.583		
Fisher Exact Test (d)		P=0.661	P=0.661
Preputial Gland: Adenoma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	5.7%	12.1%	9.1%
Terminal Rates (c)	1/29 (3%)	4/33 (12%)	2/40 (5%)
Week of First Observation	86	104	81
Life Table Tests (d)	P=0.405	P=0.390	P=0.447
Incidental Tumor Tests (d)	P=0.273	P=0.365	P=0.233
Cochran-Armitage Trend Test (d)	P=0.274		
Fisher Exact Test (d)		P=0.339	P=0.339

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

	Vehicle Control	75 mg/kg	150 mg/kg
Testis: Interstitial Cell Tumor			
Overall Rates (a)	37/50 (74%)	47/49 (96%)	48/50 (96%)
Adjusted Rates (b)	90.2%	100.0%	98.0%
Terminal Rates (c)	25/29 (86%)	33/33 (100%)	39/40 (98%)
Week of First Observation	79	57	78
Life Table Tests (d)	P=0.407N	P=0.216	P=0.481N
Incidental Tumor Tests (d)	P=0.005	P=0.002	P=0.021
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.002	P=0.002
All Sites: Malignant Mesothelioma			
Overall Rates (a)	2/50 (4%)	6/50 (12%)	5/50 (10%)
Adjusted Rates (b)	6.9%	15.6%	12.1%
Terminal Rates (c)	2/29 (7%)	2/33 (6%)	4/40 (10%)
Week of First Observation	104	92	102
Life Table Tests (d)	P=0.338	P=0.185	P=0.362
Incidental Tumor Tests (d)	P=0.178	P=0.109	P=0.307
Cochran-Armitage Trend Test (d)	P=0.187		
Fisher Exact Test (d)		P=0.134	P=0.218
All Sites: Benign Tumors			
Overall Rates (a)	44/50 (88%)	47/50 (94%)	48/50 (96%)
Adjusted Rates (b)	100.0%	100.0%	98.0%
Terminal Rates (c)	29/29 (100%)	33/33 (100%)	39/40 (98%)
Week of First Observation	79	57	78
Life Table Tests (d)	P=0.042N	P=0.419N	P=0.050N
Incidental Tumor Tests (d)	P=0.551	P=0.271	P=0.650
Cochran-Armitage Trend Test (d)	P=0.090		
Fisher Exact Test (d)		P=0.243	P=0.134
All Sites: Malignant Tumors			
Overall Rates (a)	19/50 (38%)	28/50 (56%)	29/50 (58%)
Adjusted Rates (b)	47.1%	63.6%	64.3%
Terminal Rates (c)	9/29 (31%)	17/33 (52%)	24/40 (60%)
Week of First Observation	73	81	84
Life Table Tests (d)	P=0.339	P=0.181	P=0.337
Incidental Tumor Tests (d)	P=0.024	P=0.032	P=0.050
Cochran-Armitage Trend Test (d)	P=0.029		
Fisher Exact Test (d)		P=0.054	P=0.036
All Sites: All Tumors			
Overall Rates (a)	46/50 (92%)	47/50 (94%)	48/50 (96%)
Adjusted Rates (b)	100.0%	100.0%	98.0%
Terminal Rates (c)	29/29 (100%)	33/33 (100%)	39/40 (98%)
Week of First Observation	73	57	78
Life Table Tests (d)	P=0.019N	P=0.288N	P=0.024N
Incidental Tumor Tests (d)	P=0.187N	P=0.718N	P=0.355N
Cochran-Armitage Trend Test (d)	P=0.264		
Fisher Exact Test (d)		P=0.500	P=0.339

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 incidental tumor 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 is indicated by (N).
(e) No P value is reported because no tumors were observed in the 75 mg/kg and vehicle control groups.
(f) Incomplete sampling of tissues

TABLE A4a. HISTORICAL INCIDENCE OF KIDNEY TUBULAR CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence of Adenomas or Adenocarcinomas in Vehicle Controls
Historical Incidence at Microbiological Associates	
d-Limonene	0/50
Benzyl alcohol	0/50
TOTAL	0/100 (0.0%)
Overall Historical Incidence	
TOTAL	(b) 10/1,943 (0.5%)
SD (c)	0.89%
Range (d)	
High	1/48
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes three tubular cell adenomas, two adenocarcinomas, NOS, and five tubular cell adenocarcinomas; an adenoma, NOS, was also observed in an animal with a tubular cell adenoma.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4b. HISTORICAL INCIDENCE OF TESTICULAR INTERSTITIAL CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence in Vehicle Controls
Historical Incidence at Microbiological Associates	
d-Limonene	37/50
Benzyl alcohol	39/49
TOTAL	76/99 (76.8%)
Overall Historical Incidence	
TOTAL	(b) 1,675/1,944 (86.2%)
SD (c)	9.47%
Range (d)	
High	48/50
Low	31/49

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes two malignant interstitial cell tumors
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4c. HISTORICAL INCIDENCE OF LEUKEMIA IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence in Vehicle Controls
Historical Incidence at Microbiological Associates	
d-Limonene	10/50
Benzyl alcohol	15/50
TOTAL	25/100 (25.0%)
Overall Historical Incidence	
TOTAL	321/1,949 (16.5%)
SD (b)	8.95%
Range (c)	
High	(d) 22/50
Low	1/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Second highest: 15/50

TABLE A4d. HISTORICAL INCIDENCE OF SKIN SQUAMOUS CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence in Vehicle Controls		
	Papilloma	Carcinoma	Papilloma or Carcinoma
Historical Incidence at Microbiological Associates			
d-Limonene	0/50	0/50	0/50
Benzyl alcohol	(b) 2/50	0/50	2/50
TOTAL	2/100 (2.0%)	0/100 (0.0%)	2/100 (2.0%)
Overall Historical Incidence			
TOTAL	(c) 39/1,949 (2.3%)	12/1,949 (0.6%)	(c) 51/1,949 (2.6%)
SD (d)	2.47%	1.23%	2.83%
Range (e)			
High	4/50	3/50	5/50
Low	0/50	0/50	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Papillomas, NOS
(c) Includes 10 papillomas, NOS
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Hematoma, NOS			1 (2%)
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Hemorrhage	2 (4%)		1 (2%)
Inflammation, suppurative	9 (18%)	2 (4%)	9 (18%)
Inflammation, chronic	1 (2%)		1 (2%)
Foreign material, NOS		1 (2%)	
Hyperkeratosis			2 (4%)
*Nose/respiratory region	(50)	(50)	(50)
Hyperplasia, epithelial			1 (2%)
Metaplasia, squamous			1 (2%)
Regeneration, NOS			1 (2%)
*Nose/olfactory region	(50)	(50)	(50)
Regeneration, NOS			1 (2%)
*Larynx	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, suppurative	1 (2%)		
#Lung	(50)	(16)	(50)
Congestion, NOS	4 (8%)	5 (31%)	2 (4%)
Edema, NOS	2 (4%)	3 (19%)	
Hemorrhage	6 (12%)		1 (2%)
Bronchopneumonia, NOS			1 (2%)
Inflammation, interstitial	4 (8%)		5 (10%)
Inflammation, acute			1 (2%)
Inflammation, granulomatous focal			2 (4%)
Infarct, NOS	1 (2%)		
Foreign material, NOS			1 (2%)
Hemosiderosis	1 (2%)		
Epithelialization	3 (6%)	1 (6%)	4 (8%)
#Lung/alveoli	(50)	(16)	(50)
Histiocytosis	22 (44%)		22 (44%)
HEMATOPOIETIC SYSTEM			
#Spleen	(50)	(50)	(50)
Fibrosis, focal			1 (2%)
Fibrosis, diffuse			3 (6%)
Hemosiderosis			1 (2%)
Angiectasis			1 (2%)
Hyperplasia, lymphoid		1 (2%)	1 (2%)
#Splenic follicles	(50)	(50)	(50)
Atrophy, NOS		1 (2%)	
#Lymph node	(49)	(13)	(50)
Degeneration, cystic		1 (8%)	
#Mandibular lymph node	(49)	(13)	(50)
Degeneration, cystic	10 (20%)		5 (10%)
Hyperplasia, lymphoid		1 (8%)	
#Mediastinal lymph node	(49)	(13)	(50)
Hemorrhage	12 (24%)	3 (23%)	13 (26%)
Degeneration, cystic	4 (8%)		7 (14%)
Hemosiderosis	1 (2%)	1 (8%)	2 (4%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mesenteric lymph node	(49)	(13)	(50)
Hemorrhage	1 (2%)		2 (4%)
Degeneration, cystic	1 (2%)		2 (4%)
Hemosiderosis	1 (2%)		
#Renal lymph node	(49)	(13)	(50)
Hemorrhage	1 (2%)		
Degeneration, cystic	1 (2%)		
#Thymus	(35)	(11)	(31)
Cyst, NOS		1 (9%)	
Hemorrhage	2 (6%)	2 (18%)	
Depletion, lymphoid		1 (9%)	
Hyperplasia, epithelial	18 (51%)	3 (27%)	17 (55%)
Lymphocytosis		1 (9%)	
CIRCULATORY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Embolus, septic	1 (2%)		
#Spleen	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
#Heart	(50)	(12)	(50)
Inflammation, suppurative	1 (2%)		
#Heart/atrium	(50)	(12)	(50)
Thrombosis, NOS	2 (4%)		4 (8%)
#Myocardium	(50)	(12)	(50)
Hemorrhage		1 (8%)	
Inflammation, chronic	4 (8%)	1 (8%)	
Fibrosis	30 (60%)	4 (33%)	33 (66%)
*Pulmonary artery	(50)	(50)	(50)
Mineralization	15 (30%)	2 (4%)	18 (36%)
#Liver	(50)	(50)	(50)
Thrombosis, NOS	1 (2%)		
#Pancreas	(45)	(12)	(49)
Polyangiitis			1 (2%)
#Adrenal	(50)	(50)	(50)
Thrombosis, NOS	1 (2%)		
DIGESTIVE SYSTEM			
*Mouth	(50)	(50)	(50)
Hemorrhage	1 (2%)		
#Salivary gland	(48)	(11)	(49)
Inflammation, chronic			1 (2%)
Metaplasia, squamous			4 (8%)
#Liver	(50)	(50)	(50)
Hernia, NOS	2 (4%)		
Congestion, NOS	1 (2%)		
Hemorrhagic cyst			1 (2%)
Inflammation, multifocal	1 (2%)	1 (2%)	
Inflammation, chronic	2 (4%)		
Cholangiofibrosis	2 (4%)	3 (6%)	4 (8%)
Necrosis, NOS	1 (2%)		
Necrosis, coagulative			1 (2%)
Metamorphosis, fatty	6 (12%)		1 (2%)
Cytoplasmic change, NOS	1 (2%)	1 (2%)	3 (6%)
Basophilic cyto change	14 (28%)	14 (28%)	17 (34%)
Ground glass cyto change		1 (2%)	
Eosinophilic cyto change	1 (2%)		
Hyperplasia, focal			1 (2%)
Angiectasis	7 (14%)	1 (2%)	4 (8%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Liver/centrilobular	(50)	(50)	(50)
Necrosis, NOS	4 (8%)	4 (8%)	
Metamorphosis, fatty	1 (2%)	2 (4%)	
Atrophy, NOS			1 (2%)
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS	37 (74%)	43 (86%)	48 (96%)
#Pancreas	(45)	(12)	(49)
Hemorrhage	1 (2%)		
Inflammation, NOS		1 (8%)	
#Pancreatic acinus	(45)	(12)	(49)
Atrophy, NOS	7 (16%)		7 (14%)
Hyperplasia, NOS	5 (11%)		5 (10%)
#Esophagus	(50)	(10)	(50)
Foreign body, NOS	1 (2%)		
Inflammation, acute		1 (10%)	
Inflammation, granulomatous focal	1 (2%)		
#Glandular stomach	(48)	(12)	(50)
Erosion		1 (8%)	
Fibrosis		1 (8%)	1 (2%)
#Forestomach	(48)	(12)	(50)
Ulcer, NOS	1 (2%)		2 (4%)
Inflammation, chronic	2 (4%)		3 (6%)
Hyperplasia, NOS			2 (4%)
Hyperplasia, epithelial			2 (4%)
#Gastric fundus	(48)	(12)	(50)
Mineralization			1 (2%)
Inflammation, acute			1 (2%)
Erosion			1 (2%)
Degeneration, cystic	25 (52%)	4 (33%)	30 (60%)
#Colon	(48)	(10)	(50)
Inflammation, acute			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Hydronephrosis	1 (2%)		
Cyst, NOS		2 (4%)	
Hemorrhage	3 (6%)	1 (2%)	1 (2%)
Pyelonephritis, NOS	2 (4%)	1 (2%)	8 (16%)
Inflammation, suppurative	1 (2%)	1 (2%)	
Nephropathy	43 (86%)	45 (90%)	50 (100%)
Necrosis, NOS		1 (2%)	
Pigmentation, NOS	1 (2%)	5 (10%)	1 (2%)
Hyperplasia, tubular cell		4 (8%)	7 (14%)
#Renal papilla	(50)	(50)	(50)
Mineralization	7 (14%)	43 (86%)	48 (96%)
Hyperplasia, epithelial		35 (70%)	43 (86%)
#Urinary bladder	(48)	(11)	(47)
Inflammation, suppurative	1 (2%)		
Inflammation, chronic			3 (6%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(50)	(17)	(50)
Cyst, NOS	3 (6%)	2 (12%)	5 (10%)
Hemorrhage	3 (6%)		1 (2%)
Hyperplasia, focal	5 (10%)		3 (6%)
Angiectasis	1 (2%)	1 (6%)	3 (6%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal	(50)	(50)	(50)
Cyst, NOS	1 (2%)		
Necrosis, NOS		1 (2%)	
Lipoidosis	7 (14%)	1 (2%)	2 (4%)
Angiectasis		2 (4%)	2 (4%)
#Adrenal cortex	(50)	(50)	(50)
Hypertrophy, focal	1 (2%)		
Hyperplasia, focal	1 (2%)	2 (4%)	
#Adrenal medulla	(50)	(50)	(50)
Hyperplasia, focal	15 (30%)	12 (24%)	16 (32%)
#Thyroid	(48)	(10)	(50)
Ultimobranchial cyst			1 (2%)
Inflammation, acute		1 (10%)	
Hyperplasia, C-cell	6 (13%)		6 (12%)
#Thyroid follicle	(48)	(10)	(50)
Hyperplasia, cystic			1 (2%)
#Parathyroid	(33)	(8)	(38)
Hypertrophy, focal			1 (3%)
Hyperplasia, NOS			2 (5%)
#Pancreatic islets	(45)	(12)	(49)
Hyperplasia, NOS	1 (2%)	1 (8%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation/ducts	2 (4%)		
Galactocele		1 (2%)	
Inflammation, granulomatous			1 (2%)
*Mammary duct	(50)	(50)	(50)
Polyp, inflammatory	1 (2%)		
*Preputial gland	(50)	(50)	(50)
Dilatation/ducts	1 (2%)	1 (2%)	1 (2%)
Abscess, NOS	2 (4%)		
Inflammation, chronic	7 (14%)		16 (32%)
Hyperplasia, NOS			1 (2%)
#Prostate	(49)	(12)	(47)
Inflammation, suppurative	3 (6%)		1 (2%)
Abscess, NOS	1 (2%)	1 (8%)	
Inflammation, chronic	5 (10%)		12 (26%)
Hyperplasia, epithelial	5 (10%)	1 (8%)	8 (17%)
*Seminal vesicle	(50)	(50)	(50)
Abscess, NOS	1 (2%)		
Inflammation, chronic	4 (8%)		
Atrophy, NOS	2 (4%)	1 (2%)	1 (2%)
#Testis	(50)	(49)	(50)
Mineralization		1 (2%)	
Hemosiderosis		1 (2%)	
Atrophy, NOS	1 (2%)		
Hypospermatogenesis	4 (8%)	3 (6%)	4 (8%)
Hyperplasia, interstitial cell	11 (22%)	2 (4%)	6 (12%)
NERVOUS SYSTEM			
#Brain/meninges	(50)	(11)	(50)
Hemorrhage		1 (9%)	
#Brain	(50)	(11)	(50)
Hemorrhage		1 (9%)	2 (4%)
Gliosis			1 (2%)
Status spongiosus	1 (2%)		
#Brain stem	(50)	(11)	(50)
Displacement, NOS	4 (8%)		2 (4%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
NERVOUS SYSTEM (Continued)			
*Spinal cord	(50)	(50)	(50)
Hemorrhage			1 (2%)
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Hemorrhage	1 (2%)		6 (12%)
Inflammation, suppurative	1 (2%)		
Cataract	1 (2%)	3 (6%)	27 (54%)
Phthisis bulbi	1 (2%)		
*Eye/sclera	(50)	(50)	(50)
Mineralization	35 (70%)	21 (42%)	30 (60%)
*Eye/retina	(50)	(50)	(50)
Degeneration, NOS		7 (14%)	37 (74%)
*Middle ear	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Fibrous osteodystrophy			1 (2%)
Osteosclerosis	1 (2%)		
*Skeletal muscle	(50)	(50)	(50)
Hemorrhage		1 (2%)	
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Inflammation, chronic			1 (2%)
*Abdominal cavity	(50)	(50)	(50)
Necrosis, fat	5 (10%)	5 (10%)	2 (4%)
*Mesentery	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
Fibrosis			1 (2%)
*Tunica vaginalis	(50)	(50)	(50)
Hyperplasia, mesothelial		1 (2%)	
ALL OTHER SYSTEMS			
Adipose tissue			
Necrosis, fat	1		
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

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

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Basal cell tumor	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma			1 (2%)
Myxosarcoma		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(50)
Squamous cell carcinoma, unclear primary/meta		1 (2%)	
Alveolar/bronchiolar adenoma	1 (2%)	2 (4%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	10 (20%)	16 (32%)	10 (20%)
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	
#Lung	(50)	(50)	(50)
Hemangiosarcoma, metastatic		1 (2%)	
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Neoplastic nodule	2 (4%)		
#Pancreas	(50)	(49)	(49)
Acinar cell adenoma			1 (2%)
#Forestomach	(50)	(49)	(50)
Squamous cell carcinoma			1 (2%)
URINARY SYSTEM			
#Urinary bladder	(50)	(48)	(48)
Transitional cell papilloma		1 (2%)	
ENDOCRINE SYSTEM			
#Anterior pituitary	(50)	(49)	(49)
Carcinoma, NOS		1 (2%)	
Adenoma, NOS	17 (34%)	20 (41%)	12 (24%)
#Adrenal	(50)	(50)	(50)
Pheochromocytoma	3 (6%)	6 (12%)	
#Thyroid	(50)	(50)	(50)
Follicular cell adenoma	1 (2%)	2 (4%)	
Follicular cell carcinoma			1 (2%)
C-cell adenoma	4 (8%)	2 (4%)	
C-cell carcinoma		2 (4%)	

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

	Vehicle Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenoma, NOS	1 (2%)	1 (2%)	
Adenocarcinoma, NOS	1 (2%)	1 (2%)	
Cystadenoma, NOS	1 (2%)	2 (4%)	
Fibroadenoma	21 (42%)	14 (28%)	9 (18%)
*Clitoral gland	(50)	(50)	(50)
Carcinoma, NOS		1 (2%)	
Adenoma, NOS	4 (8%)	1 (2%)	1 (2%)
#Uterus	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
Endometrial stromal polyp	3 (6%)	13 (26%)	5 (10%)
#Ovary	(50)	(50)	(50)
Granulosa cell tumor	1 (2%)		
NERVOUS SYSTEM			
#Brain	(50)	(50)	(50)
Astrocytoma			1 (2%)
#Brain stem	(50)	(50)	(50)
Carcinoma, NOS, invasive		1 (2%)	
#Cerebellum	(50)	(50)	(50)
Granular cell tumor, NOS			1 (2%)
SPECIAL SENSE ORGANS			
None			
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
Head			
Carcinoma, NOS			1
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	5	7	17
Moribund sacrifice		2	1
Terminal sacrifice	42	39	24
Dosing accident	3	2	8

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

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	37	44	26
Total primary tumors	71	88	45
Total animals with benign tumors	32	39	21
Total benign tumors	57	64	29
Total animals with malignant tumors	11	22	13
Total malignant tumors	11	23	15
Total animals with secondary tumors##		2	
Total secondary tumors		2	
Total animals with tumors-- uncertain benign or malignant	3		1
Total uncertain tumors	3		1
Total animals with tumors-- uncertain primary or metastatic		1	
Total uncertain tumors		1	

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

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

ANIMAL NUMBER	C 4 9	C 4 7	C 2 0	C 2 2	C 4 1	C 4 0	C 4 3	C 3 0	C 0 1	C 0 2	C 0 3	C 0 4	C 0 5	C 0 6	C 0 7	C 0 8	C 0 9	C 1 0	C 1 1	C 1 2	C 1 3	C 1 4	C 1 5	C 1 6	C 1 7
WEEKS ON STUDY	0 3 1	0 3 2	0 7 1	0 7 3	0 8 7	0 9 9	0 9 9	1 0 0	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell tumor			X																						
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule															X										
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS							X		X	X			X				X						X	X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									X
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
C cell adenoma																			X	X					
Parathyroid	−	+	+	+	+	+	−	−	−	+	+	+	−	+	+	+	−	−	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																						X			
Adenocarcinoma, NOS																									X
Cystadenoma, NOS									X																
Fibroadenoma								X				X	X					X	X	X			X	X	X
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS							X															X			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp						X		X																	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																						X			
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell					X			X						X			X				X		X	

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	C 1 1 8	C 1 1 9	C 2 1 1	C 2 2 3	C 2 2 4	C 2 2 5	C 2 2 6	C 2 2 7	C 2 2 8	C 2 2 9	C 3 1 1	C 3 2 2	C 3 3 3	C 3 3 4	C 3 3 5	C 3 3 6	C 3 3 7	C 3 3 8	C 3 3 9	C 4 2 2	C 4 4 4	C 4 4 5	C 4 4 6	C 4 4 8	C 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell tumor																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma															X											1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	44
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule									X																	2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	50
Adenoma, NOS				X	X						X	X	X		X	X	X						X			17
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X															X										3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma										X																1
C-cell adenoma											X										X					4
Parathyroid	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	37
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenoma, NOS																										1
Adenocarcinoma, NOS																										1
Cystadenoma, NOS																										1
Fibroadenoma	X				X		X		X	X	X	X	X	X	X		X								X	21
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS							X														X					4
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp									X																	3
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell										X		X							X		X					10

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: LOW DOSE

ANIMAL NUMBER	C 2 8	C 3 7	C 3 0	C 3 5	C 0 9	C 1 9	C 4 2	C 3 4	C 1 4	C 5 0	C 0 1	C 0 2	C 0 3	C 0 4	C 0 5	C 0 6	C 0 7	C 0 8	C 1 0	C 4 9	C 1 1	C 1 2	C 1 3	C 1 5	C 1 6
WEEKS ON STUDY	0 3 4	0 4 0	0 4 3	0 7 3	0 8 2	0 9 0	0 9 1	1 0 0	1 0 2	1 0 2	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myxosarcoma													X												
Hemangiosarcoma	X																								
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma unclear prim/metastatic																									
Alveolar/bronchiolar adenoma																									
Hemangiosarcoma, metastatic	X																								
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell papilloma																				X					
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS				X		X			X		X			X	X		X					X	X	X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma					X												X				X				
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																X									
C cell adenoma																							X		
C cell carcinoma																	X								
Parathyroid	−	+	+	+	+	−	+	−	+	−	+	+	+	−	+	+	+	+	+	−	+	−	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adenocarcinoma, NOS																									
Cystadenoma, NOS																					X			X	
Fibroadenoma										X				X	X							X			
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp					X	X													X		X		X	X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell					X		X	X		X	X									X					

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	C 1 7	C 1 8	C 2 0	C 2 1	C 2 2	C 2 3	C 2 4	C 2 5	C 2 6	C 2 7	C 2 9	C 3 1	C 3 2	C 3 3	C 3 6	C 3 8	C 3 9	C 4 0	C 4 1	C 4 3	C 4 4	C 4 5	C 4 6	C 4 7	C 4 8	TOTAL TISSUES TUMORS
WEEKS ON STUDY	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	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	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Myxosarcoma																										1
Hemangiosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	50
Squamous cell carcinoma unclear prime																			X							1
Alveolar/bronchiolar adenoma												X			X											2
Hemangiosarcoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	44
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	48
Transitional cell papilloma																										1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																							X			1
Adenoma, NOS	X	X							X		X	X	X						X		X			X		20
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																			X			X		X		6
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma				X																						2
C cell adenoma																				X						2
C cell carcinoma				X																						2
Parathyroid	+	–	+	+	+	+	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	38
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenoma, NOS		X																								1
Adenocarcinoma, NOS																			X							1
Cystadenoma, NOS																										2
Fibroadenoma					X	X		X	X		X			X			X		X			X	X			14
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS								X																		1
Adenoma, NOS			X																							1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp				X									X	X		X	X		X						X	13
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, invasive																							X			1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell		X				X		X		X	X	X	X				X				X	X				16

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: HIGH DOSE

ANIMAL NUMBER	C 2 9	C 1 9	C 3 7	C 2 4	C 4 8	C 4 6	C 2 1	C 0 2	C 4 7	C 1 2	C 1 8	C 1 7	C 1 3	C 1 6	C 4 2	C 3 2	C 3 1	C 0 7	C 0 4	C 0 6	C 0 5	C 2 3	C 2 6	C 4 9	C 0 1
WEEKS ON STUDY	0 2 0	0 2 2	0 2 5	0 2 6	0 2 7	0 2 9	0 3 2	0 3 7	0 3 7	0 3 9	0 3 9	0 4 0	0 4 4	0 4 8	0 4 9	0 5 0	0 7 1	0 8 4	0 8 8	0 9 6	0 9 7	1 0 0	1 0 3	1 0 3	1 0 4
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																			X					X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
Parathyroid	+	+	−	−	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	+	+	N	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																							X		
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Endometrial stromal polyp																		X							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor, NOS																									
Astrocytoma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell																			X						
Head, NOS																									
Carcinoma, NOS,																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	C 0 3	C 0 8	C 0 9	C 1 0	C 1 1	C 1 4	C 1 5	C 2 0	C 2 2	C 2 5	C 2 7	C 2 8	C 3 0	C 3 3	C 3 4	C 3 5	C 3 6	C 3 8	C 3 9	C 4 0	C 4 1	C 4 3	C 4 4	C 4 5	C 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES / TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma													X													1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	40
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinar cell adenoma															X											1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																				X						1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X		X	X	X		X			X	X			X	X								X			12
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma				X																						1
Parathyroid	+	−	+	−	+	−	+	+	−	−	+	−	−	+	−	+	+	−	+	−	+	+	+	+	+	35
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	*50
Fibroadenoma	X									X	X				X				X		X	X			X	9
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS												X														1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma				X																						1
Endometrial stromal polyp				X								X					X					X			5
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor, NOS																			X							1
Astrocytoma					X																					1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell	X					X	X	X				X				X				X		X	X			10
Head, NOS																										
Carcinoma, NOS									X																	1

* Animals necropsied

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

	Vehicle Control	300 mg/kg	600 mg/kg
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	10/50 (20%)	16/50 (32%)	10/50 (20%)
Adjusted Rates (b)	22.6%	36.2%	36.7%
Terminal Rates (c)	8/42 (19%)	12/40 (30%)	9/26 (35%)
Week of First Observation	87	82	88
Life Table Tests (d)	P=0.134	P=0.116	P=0.181
Incidental Tumor Tests (d)	P=0.255	P=0.173	P=0.289
Cochran-Armitage Trend Test (d)	P=0.547		
Fisher Exact Test (d)		P=0.127	P=0.598
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	17/50 (34%)	20/49 (41%)	12/49 (24%)
Adjusted Rates (b)	39.5%	47.3%	42.5%
Terminal Rates (c)	16/42 (38%)	17/39 (44%)	10/26 (38%)
Week of First Observation	99	73	88
Life Table Tests (d)	P=0.355	P=0.253	P=0.435
Incidental Tumor Tests (d)	P=0.472	P=0.262	P=0.567
Cochran-Armitage Trend Test (d)	P=0.187N		
Fisher Exact Test (d)		P=0.311	P=0.207N
Anterior Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	17/50 (34%)	21/49 (43%)	12/49 (24%)
Adjusted Rates (b)	39.5%	49.7%	42.5%
Terminal Rates (c)	16/42 (38%)	18/39 (46%)	10/26 (38%)
Week of First Observation	99	73	88
Life Table Tests (d)	P=0.343	P=0.191	P=0.435
Incidental Tumor Tests (d)	P=0.459	P=0.198	P=0.567
Cochran-Armitage Trend Test (d)	P=0.188N		
Fisher Exact Test (d)		P=0.242	P=0.207N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	3/50 (6%)	6/50 (12%)	0/50 (0%)
Adjusted Rates (b)	7.1%	14.4%	0.0%
Terminal Rates (c)	3/42 (7%)	5/40 (13%)	0/26 (0%)
Week of First Observation	104	82	
Life Table Tests (d)	P=0.297N	P=0.223	P=0.218N
Incidental Tumor Tests (d)	P=0.254N	P=0.275	P=0.218N
Cochran-Armitage Trend Test (d)	P=0.146N		
Fisher Exact Test (d)		P=0.243	P=0.121N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	9.5%	5.0%	0.0%
Terminal Rates (c)	4/42 (10%)	2/40 (5%)	0/26 (0%)
Week of First Observation	104	104	
Life Table Tests (d)	P=0.081N	P=0.359N	P=0.139N
Incidental Tumor Tests (d)	P=0.081N	P=0.359N	P=0.139N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.339N	P=0.059N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	4/50 (8%)	0/50 (0%)
Adjusted Rates (b)	9.5%	10.0%	0.0%
Terminal Rates (c)	4/42 (10%)	4/40 (10%)	0/26 (0%)
Week of First Observation	104	104	
Life Table Tests (d)	P=0.139N	P=0.617	P=0.139N
Incidental Tumor Tests (d)	P=0.139N	P=0.617	P=0.139N
Cochran-Armitage Trend Test (d)	P=0.060N		
Fisher Exact Test (d)		P=0.643	P=0.059N

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

	Vehicle Control	300 mg/kg	600 mg/kg
Mammary Gland: Fibroadenoma			
Overall Rates (a)	21/50 (42%)	14/50 (28%)	9/50 (18%)
Adjusted Rates (b)	48.8%	34.1%	33.2%
Terminal Rates (c)	20/42 (48%)	13/40 (33%)	8/26 (31%)
Week of First Observation	100	102	103
Life Table Tests (d)	P=0.113N	P=0.134N	P=0.170N
Incidental Tumor Tests (d)	P=0.075N	P=0.132N	P=0.119N
Cochran-Armitage Trend Test (d)	P=0.006N		
Fisher Exact Test (d)		P=0.104N	P=0.008N
Mammary Gland: Adenoma or Cystadenoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	4.8%	7.5%	0.0%
Terminal Rates (c)	2/42 (5%)	3/40 (7%)	0/26 (0%)
Week of First Observation	104	104	
Life Table Tests (d)	P=0.329N	P=0.478	P=0.349N
Incidental Tumor Tests (d)	P=0.329N	P=0.478	P=0.349N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.500	P=0.247N
Mammary Gland: Adenoma, Cystadenoma, or Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	0/50 (0%)
Adjusted Rates (b)	7.1%	10.0%	0.0%
Terminal Rates (c)	3/42 (7%)	4/40 (10%)	0/26 (0%)
Week of First Observation	104	104	
Life Table Tests (d)	P=0.233N	P=0.473	P=0.218N
Incidental Tumor Tests (d)	P=0.233N	P=0.473	P=0.218N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Test (d)		P=0.500	P=0.121N
Mammary Gland: Adenoma, Cystadenoma, or Fibroadenoma			
Overall Rates (a)	(e) 23/50 (46%)	(e) 17/50 (34%)	9/50 (18%)
Adjusted Rates (b)	53.5%	41.4%	33.2%
Terminal Rates (c)	22/42 (52%)	16/40 (40%)	8/26 (31%)
Week of First Observation	100	102	103
Life Table Tests (d)	P=0.069N	P=0.196N	P=0.096N
Incidental Tumor Tests (d)	P=0.044N	P=0.194N	P=0.063N
Cochran-Armitage Trend Test (d)	P=0.002N		
Fisher Exact Test (d)		P=0.154N	P=0.002N
Clitoral Gland: Adenoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	9.2%	2.5%	3.8%
Terminal Rates (c)	3/42 (7%)	1/40 (3%)	1/26 (4%)
Week of First Observation	99	104	104
Life Table Tests (d)	P=0.194N	P=0.197N	P=0.347N
Incidental Tumor Tests (d)	P=0.154N	P=0.194N	P=0.270N
Cochran-Armitage Trend Test (d)	P=0.101N		
Fisher Exact Test (d)		P=0.181N	P=0.181N
Clitoral Gland: Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	9.2%	5.0%	3.8%
Terminal Rates (c)	3/42 (7%)	2/40 (5%)	1/26 (4%)
Week of First Observation	99	104	104
Life Table Tests (d)	P=0.233N	P=0.361N	P=0.347N
Incidental Tumor Tests (d)	P=0.190N	P=0.357N	P=0.270N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Test (d)		P=0.339N	P=0.181N

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

	Vehicle Control	300 mg/kg	600 mg/kg
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	3/50 (6%)	13/50 (26%)	5/50 (10%)
Adjusted Rates (b)	6.8%	30.7%	17.9%
Terminal Rates (c)	1/42 (2%)	11/40 (28%)	4/26 (15%)
Week of First Observation	99	82	84
Life Table Tests (d)	P=0.094	P=0.007	P=0.155
Incidental Tumor Tests (d)	P=0.176	P=0.011	P=0.305
Cochran-Armitage Trend Test (d)	P=0.333		
Fisher Exact Test (d)		P=0.006	P=0.357
All Sites: Benign Tumors			
Overall Rates (a)	32/50 (64%)	39/50 (78%)	21/50 (42%)
Adjusted Rates (b)	69.5%	86.6%	69.8%
Terminal Rates (c)	28/42 (67%)	34/40 (85%)	17/26 (65%)
Week of First Observation	71	73	84
Life Table Tests (d)	P=0.351	P=0.062	P=0.490
Incidental Tumor Tests (d)	P=0.477N	P=0.046	P=0.408N
Cochran-Armitage Trend Test (d)	P=0.016N		
Fisher Exact Test (d)		P=0.093	P=0.023N
All Sites: Malignant Tumors			
Overall Rates (a)	11/50 (22%)	22/50 (44%)	13/50 (26%)
Adjusted Rates (b)	24.9%	48.6%	47.8%
Terminal Rates (c)	9/42 (21%)	17/40 (43%)	12/26 (46%)
Week of First Observation	87	34	88
Life Table Tests (d)	P=0.037	P=0.017	P=0.057
Incidental Tumor Tests (d)	P=0.108	P=0.027	P=0.103
Cochran-Armitage Trend Test (d)	P=0.372		
Fisher Exact Test (d)		P=0.016	P=0.407
All Sites: All Tumors			
Overall Rates (a)	37/50 (74%)	44/50 (88%)	26/50 (52%)
Adjusted Rates (b)	78.7%	91.7%	86.6%
Terminal Rates (c)	32/42 (76%)	36/40 (90%)	22/26 (85%)
Week of First Observation	71	34	84
Life Table Tests (d)	P=0.198	P=0.060	P=0.291
Incidental Tumor Tests (d)	P=0.524N	P=0.048	P=0.585N
Cochran-Armitage Trend Test (d)	P=0.010N		
Fisher Exact Test (d)		P=0.062	P=0.019N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 incidental tumor 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 is indicated by (N).
(e) An adenocarcinoma was observed in an animal with a fibroadenoma.

TABLE B4. HISTORICAL INCIDENCE OF UTERINE ENDOMETRIAL STROMAL POLYPS IN FEMALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence in Vehicle Controls
Historical Incidence at Microbiological Associates	
d-Limonene	3/50
Benzyl alcohol	12/49
TOTAL	15/99 (15.2%)
Overall Historical Incidence	
TOTAL	390/1,934 (20.2%)
SD (b)	6.53%
Range (c)	
High	17/50
Low	3/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Ulcer, NOS		2 (4%)	
Inflammation, acute			1 (2%)
Inflammation, chronic		1 (2%)	
Erosion			1 (2%)
Exfoliative dermatitis			1 (2%)
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)
Inflammation, serous		1 (2%)	
Inflammation, suppurative		1 (2%)	2 (4%)
Inflammation, chronic		1 (2%)	
*Larynx	(50)	(50)	(50)
Hemorrhage			3 (6%)
Inflammation, suppurative			1 (2%)
#Trachea	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate			1 (2%)
#Lung	(50)	(50)	(50)
Foreign body, NOS	1 (2%)		
Bronchiectasis			1 (2%)
Congestion, NOS	4 (8%)	2 (4%)	10 (20%)
Edema, NOS	2 (4%)		4 (8%)
Hemorrhage		3 (6%)	7 (14%)
Bronchopneumonia, NOS	1 (2%)		1 (2%)
Lymphocytic inflammatory infiltrate		1 (2%)	2 (4%)
Inflammation, interstitial	16 (32%)	12 (24%)	4 (8%)
Inflammation, acute	1 (2%)		1 (2%)
Inflammation, granulomatous	2 (4%)	9 (18%)	1 (2%)
Perivascular cuffing			3 (6%)
Foreign material, NOS	1 (2%)		
Hemosiderosis	5 (10%)	1 (2%)	1 (2%)
Epithelialization	1 (2%)	1 (2%)	2 (4%)
#Lung/alveoli	(50)	(50)	(50)
Histiocytosis	33 (66%)	36 (72%)	25 (50%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(50)	(50)
Atrophy, NOS			1 (2%)
#Spleen	(49)	(48)	(50)
Hemosiderosis			1 (2%)
Hyperplastic nodule	1 (2%)		
Hyperplasia, lymphoid	1 (2%)		
Hematopoiesis	1 (2%)		
#Lymph node	(50)	(49)	(50)
Plasmacytosis		1 (2%)	
Hyperplasia, lymphoid		1 (2%)	
#Mandibular lymph node	(50)	(49)	(50)
Hemorrhage	1 (2%)		
Degeneration, cystic	4 (8%)	5 (10%)	4 (8%)
Necrosis, NOS		1 (2%)	
Plasmacytosis		1 (2%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Mediastinal lymph node	(50)	(49)	(50)
Hemorrhage	16 (32%)	6 (12%)	4 (8%)
Hemosiderosis	1 (2%)	1 (2%)	
#Pancreatic lymph node	(50)	(49)	(50)
Degeneration, cystic	1 (2%)		1 (2%)
Hemosiderosis	1 (2%)		
#Mesenteric lymph node	(50)	(49)	(50)
Hemorrhage	4 (8%)		
Degeneration, cystic	2 (4%)		2 (4%)
*Femur	(50)	(50)	(50)
Myelofibrosis		2 (4%)	1 (2%)
#Lung	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)
#Thymus	(44)	(44)	(40)
Ectopia		2 (5%)	1 (3%)
Cyst, NOS	1 (2%)		
Congestion, NOS			1 (3%)
Hemorrhage	1 (2%)	1 (2%)	1 (3%)
Fibrosis	1 (2%)		
Depletion, lymphoid			1 (3%)
Hyperplasia, epithelial	29 (66%)	32 (73%)	19 (48%)
CIRCULATORY SYSTEM			
#Heart	(50)	(50)	(50)
Embolus, septic		1 (2%)	
Arteriosclerosis, NOS	1 (2%)		
Fibroelastosis, NOS		1 (2%)	
#Heart/atrium	(50)	(50)	(50)
Thrombosis, NOS		2 (4%)	
#Myocardium	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate			1 (2%)
Inflammation, chronic			4 (8%)
Fibrosis	29 (58%)	15 (30%)	14 (28%)
Degeneration, NOS	1 (2%)		
*Pulmonary artery	(50)	(50)	(50)
Mineralization	3 (6%)	2 (4%)	4 (8%)
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(48)	(50)
Lymphocytic inflammatory infiltrate		1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)	3 (6%)
Metaplasia, squamous	2 (4%)		1 (2%)
#Liver	(50)	(50)	(50)
Ectopia		2 (4%)	
Hernia, NOS		1 (2%)	
Inflammation, multifocal	19 (38%)	12 (24%)	8 (16%)
Inflammation, granulomatous focal		1 (2%)	
Cholangiofibrosis			2 (4%)
Necrosis, NOS		1 (2%)	
Necrosis, coagulative		2 (4%)	1 (2%)
Metamorphosis, fatty	2 (4%)	3 (6%)	
Cytoplasmic change, NOS	5 (10%)		2 (4%)
Basophilic cyto change	34 (68%)	28 (56%)	15 (30%)
Ground glass cyto change	3 (6%)	1 (2%)	
Focal cellular change	1 (2%)		
Hyperplasia, focal	2 (4%)		
Angiectasis	3 (6%)		6 (12%)
#Liver/centrilobular	(50)	(50)	(50)
Necrosis, NOS			2 (4%)
Metamorphosis, fatty		1 (2%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Liver/periportal	(50)	(50)	(50)
Metamorphosis, fatty	1 (2%)	1 (2%)	
#Bile duct	(50)	(50)	(50)
Cyst, NOS		1 (2%)	
Hyperplasia, NOS	31 (62%)	35 (70%)	20 (40%)
#Pancreas	(50)	(49)	(49)
Metaplasia, NOS		2 (4%)	1 (2%)
#Pancreatic acinus	(50)	(49)	(49)
Atrophy, NOS	8 (16%)	8 (16%)	5 (10%)
Hyperplasia, NOS			1 (2%)
#Esophagus	(50)	(50)	(50)
Hemorrhage		1 (2%)	
#Glandular stomach	(50)	(49)	(50)
Fibrosis	1 (2%)		
#Forestomach	(50)	(49)	(50)
Ulcer, NOS		1 (2%)	
Inflammation, chronic	1 (2%)		2 (4%)
Hyperplasia, epithelial			3 (6%)
#Gastric fundus	(50)	(49)	(50)
Degeneration, cystic	36 (72%)	33 (67%)	25 (50%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Hydronephrosis			1 (2%)
Cyst, NOS			1 (2%)
Hemorrhage	4 (8%)	3 (6%)	
Pyelonephritis, NOS			1 (2%)
Inflammation, interstitial	1 (2%)		
Nephropathy	30 (60%)	33 (66%)	29 (58%)
Metamorphosis, fatty		2 (4%)	
Pigmentation, NOS		1 (2%)	
#Renal papilla	(50)	(50)	(50)
Mineralization	1 (2%)	1 (2%)	3 (6%)
*Ureter	(50)	(50)	(50)
Dilatation, NOS			1 (2%)
#Urinary bladder	(50)	(48)	(48)
Inflammation, acute			1 (2%)
Fibrosis, focal		1 (2%)	
ENDOCRINE SYSTEM			
#Anterior pituitary	(50)	(49)	(49)
Cyst, NOS	16 (32%)	14 (29%)	16 (33%)
Hemorrhage	3 (6%)		
Cholesterol deposit	1 (2%)		
Hyperplasia, focal		4 (8%)	1 (2%)
Angiectasis	2 (4%)	6 (12%)	2 (4%)
#Adrenal	(50)	(50)	(50)
Degeneration, lipoid		1 (2%)	
Lipoidosis	6 (12%)	5 (10%)	5 (10%)
Angiectasis	3 (6%)	3 (6%)	
#Adrenal cortex	(50)	(50)	(50)
Ectopia	1 (2%)		
Necrosis, focal			1 (2%)
Hemosiderosis		1 (2%)	
Hyperplasia, focal	1 (2%)	4 (8%)	3 (6%)
#Adrenal medulla	(50)	(50)	(50)
Hyperplasia, focal	6 (12%)	9 (18%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(50)	(50)
Ultimobranchial cyst	2 (4%)	3 (6%)	1 (2%)
Cystic follicles	1 (2%)	1 (2%)	2 (4%)
Lymphocytic inflammatory infiltrate	1 (2%)		
Hyperplasia, C-cell	7 (14%)	4 (8%)	2 (4%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation/ducts	2 (4%)	4 (8%)	
Galactocele	1 (2%)		3 (6%)
Inflammation, granulomatous	1 (2%)		1 (2%)
Hyperplasia, nodular	1 (2%)		
*Clitoral gland	(50)	(50)	(50)
Dilatation/ducts	3 (6%)	2 (4%)	1 (2%)
Inflammation, suppurative	1 (2%)		2 (4%)
Abscess, NOS	1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)		
Hyperplasia, epithelial	1 (2%)		2 (4%)
*Vagina	(50)	(50)	(50)
Epidermal inclusion cyst			2 (4%)
Inflammation, suppurative			1 (2%)
Hyperkeratosis			1 (2%)
#Uterus	(50)	(50)	(50)
Hydrometra	1 (2%)		
Hematoma, NOS	1 (2%)		1 (2%)
Inflammation, suppurative			2 (4%)
#Uterus/endometrium	(50)	(50)	(50)
Cyst, NOS	4 (8%)	6 (12%)	
Hyperplasia, cystic		1 (2%)	2 (4%)
#Ovary	(50)	(50)	(50)
Parovarian cyst	1 (2%)	2 (4%)	
NERVOUS SYSTEM			
#Brain/meninges	(50)	(50)	(50)
Hemorrhage			1 (2%)
#Brain	(50)	(50)	(50)
Hemorrhage	1 (2%)		
#Brain stem	(50)	(50)	(50)
Displacement, NOS	3 (6%)	2 (4%)	3 (6%)
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	1 (2%)
Inflammation, chronic		2 (4%)	
Cataract		5 (10%)	20 (40%)
Porphyrin pigmentation		1 (2%)	
*Eye/sclera	(50)	(50)	(50)
Mineralization	11 (22%)	20 (40%)	9 (18%)
*Eye/retina	(50)	(50)	(50)
Degeneration, NOS		21 (42%)	28 (56%)
*Harderian gland	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate	1 (2%)	10 (20%)	
Inflammation, chronic		8 (16%)	
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Osteosclerosis	1 (2%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
BODY CAVITIES			
*Thoracic cavity	(50)	(50)	(50)
Necrosis, fat		1 (2%)	
*Mediastinum	(50)	(50)	(50)
Hemorrhage		1 (2%)	
Inflammation, acute	1 (2%)		
*Abdominal cavity	(50)	(50)	(50)
Necrosis, fat	8 (16%)	6 (12%)	6 (12%)
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported			1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX C

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

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing	1		
Animals necropsied	49	50	50
Animals examined histopathologically	49	47	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Adenoma, NOS	1 (2%)		
Fibroma			2 (4%)
*Subcutaneous tissue	(49)	(50)	(50)
Hepatocellular carcinoma, metastatic	1 (2%)		
Sarcoma, NOS	1 (2%)	3 (6%)	1 (2%)
Fibrosarcoma	1 (2%)		2 (4%)
RESPIRATORY SYSTEM			
#Lung	(47)	(30)	(50)
Squamous cell carcinoma, metastatic		1 (3%)	
Hepatocellular carcinoma, metastatic	6 (13%)	1 (3%)	1 (2%)
Alveolar/bronchiolar adenoma	13 (28%)	7 (23%)	7 (14%)
Alveolar/bronchiolar carcinoma	2 (4%)	2 (7%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Malignant lymphoma, NOS	1 (2%)		1 (2%)
Malignant lymphoma, undifferentiated type	2 (4%)		1 (2%)
Malignant lymphoma, lymphocytic type		1 (2%)	2 (4%)
Malignant lymphoma, histiocytic type		1 (2%)	
Malignant lymphoma, mixed type	7 (14%)		4 (8%)
#Mesenteric lymph node	(48)	(20)	(49)
Malignant lymphoma, mixed type			2 (4%)
#Liver	(49)	(36)	(50)
Malignant lymphoma, lymphocytic type			1 (2%)
Malignant lymphoma, mixed type		1 (3%)	
#Small intestine	(47)	(19)	(47)
Malignant lymphoma, NOS		1 (5%)	
#Thymus	(32)	(11)	(33)
Malignant lymphoma, lymphocytic type		1 (9%)	
CIRCULATORY SYSTEM			
*Abdominal cavity	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)		
*Subcutaneous tissue	(49)	(50)	(50)
Hemangiosarcoma			1 (2%)
#Spleen	(48)	(20)	(48)
Hemangiosarcoma	1 (2%)	1 (5%)	
*Skeletal muscle	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)		
#Liver	(49)	(36)	(50)
Hemangiosarcoma	1 (2%)		
DIGESTIVE SYSTEM			
*Tongue	(49)	(50)	(50)
Squamous cell carcinoma		1 (2%)	
#Liver	(49)	(36)	(50)
Hepatocellular adenoma	7 (14%)	9 (25%)	10 (20%)
Hepatocellular carcinoma	15 (31%)	6 (17%)	5 (10%)
#Esophagus	(47)	(19)	(50)
Squamous cell papilloma	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Gastric serosa	(45)	(22)	(49)
Osteosarcoma, unclear primary or metastatic	1 (2%)		
#Forestomach	(45)	(22)	(49)
Squamous cell papilloma	1 (2%)	1 (5%)	2 (4%)
Squamous cell carcinoma	1 (2%)	1 (5%)	
#Small intestine	(47)	(19)	(47)
Adenocarcinoma, NOS	2 (4%)		
#Jejunum	(47)	(19)	(47)
Adenocarcinoma, NOS	1 (2%)		
URINARY SYSTEM			
#Kidney	(49)	(22)	(50)
Tubular cell adenoma			1 (2%)
ENDOCRINE SYSTEM			
#Adrenal/capsule	(45)	(18)	(49)
Adenoma, NOS	1 (2%)		
#Adrenal cortex	(45)	(18)	(49)
Adenoma, NOS			1 (2%)
#Adrenal medulla	(45)	(18)	(49)
Pheochromocytoma	2 (4%)		
#Pancreatic islets	(47)	(19)	(47)
Islet cell carcinoma		1 (5%)	
REPRODUCTIVE SYSTEM			
None			
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(50)	(50)
Adenoma, NOS	3 (6%)	4 (8%)	4 (8%)
Adenocarcinoma, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Mediastinum	(49)	(50)	(50)
Hepatocellular carcinoma, metastatic	1 (2%)	1 (2%)	1 (2%)
*Abdominal cavity	(49)	(50)	(50)
Hepatocellular carcinoma, metastatic	1 (2%)		
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic			1 (2%)
Small intestinal mesentery			
Adenocarcinoma, NOS	1		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	14	24	8
Moribund sacrifice			2
Terminal sacrifice	33	24	38
Dosing accident	2	2	2
Animal missing	1		
TUMOR SUMMARY			
Total animals with primary tumors**	43	29	35
Total primary tumors	69	41	51
Total animals with benign tumors	23	19	19
Total benign tumors	29	21	27
Total animals with malignant tumors	29	18	20
Total malignant tumors	39	20	24
Total animals with secondary tumors##	6	2	2
Total secondary tumors	9	3	3
Total animals with tumors-- uncertain primary or metastatic	1		
Total uncertain tumors	1		

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

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: VEHICLE CONTROL

ANIMAL NUMBER	C 0 5	C 1 4	C 2 5	C 1 1	C 3 2	C 0 2	C 2 0	C 2 2	C 2 3	C 1 8	C 0 9	C 0 7	C 5 0	C 2 1	C 2 9	C 4 2	C 1 3	C 0 1	C 0 3	C 0 4	C 0 6	C 0 8	C 1 0	C 1 2	C 1 5
WEEKS ON STUDY	0 5 1	0 5 4	0 5 6	0 6 2	0 6 6	0 7 3	0 7 5	0 7 7	0 8 3	0 8 7	0 9 0	0 9 1	0 9 4	0 9 5	0 9 6	0 9 8	1 0 1	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	N	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Subcutaneous tissue	+	+	+	+	+	N	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																X									
Sarcoma, NOS																									
Fibrosarcoma																X									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	M	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic								X								X					X				
Alveolar/bronchiolar adenoma												X			X	X		X			X	X			
Alveolar/bronchiolar carcinoma								X									X								
Trachea	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	M	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	M	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph nodes	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	–	+	+	+	+	–	–	+	M	–	–	–	–	–	–	+	+	+	+	–	–	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																			X						
Hepatocellular carcinoma		X				X	X	X						X		X					X				
Hemangiosarcoma																				X					
Bile duct	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	+	+	+	N	+	N	M	N	+	+	+	+	N	N	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	M	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach	+	–	+	+	+	+	–	+	+	M	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell carcinoma													X												
Osteosarcoma, unclear primary or metastatic																									
Small intestine	+	+	+	+	+	+	+	+	+	M	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																								X	X
Large intestine	+	–	+	–	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	M	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma																					X				
Thyroid	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Parathyroid	+	–	–	+	–	–	–	+	+	M	+	–	+	+	+	+	–	+	–	–	+	+	+	–	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																							X		
Adenocarcinoma, NOS															X										
MUSCULOSKELETAL SYSTEM																									
Muscle	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hemangiosarcoma																									
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hepatocellular carcinoma, metastatic																X									
Peritoneum	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hepatocellular carcinoma, metastatic																X									
Hemangiosarcoma												X													
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS											X														
Malignant lymphoma, undifferentiated type																	X	X							
Malignant lymphoma, mixed type																				X	X				

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	C016	C017	C019	C024	C026	C027	C028	C030	C031	C033	C034	C035	C036	C037	C038	C039	C040	C041	C043	C044	C045	C046	C047	C048	C049	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Adenoma, NOS				X																						1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Hepatocellular carcinoma, metastatic																										1
Sarcoma, NOS																									X	1
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hepatocellular carcinoma, metastatic		X												X							X					6
Alveolar/bronchiolar adenoma		X		X	X				X			X										X	X			13
Alveolar/bronchiolar carcinoma																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma								X																		1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	48
Thymus	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	32
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma	X						X			X	X	X	X													7
Hepatocellular carcinoma		X						X						X		X	X	X			X			X		15
Hemangiosarcoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Squamous cell papilloma																						X				1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Squamous cell papilloma																		X								1
Squamous cell carcinoma																										1
Osteosarcoma, unclear pri or metastatic		X																								1
Small intestine	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenocarcinoma, NOS							X									X										4
Large intestine	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	48
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	45
Adenoma, NOS																				X						1
Pheochromocytoma																				X						2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid	+	+	−	+	+	+	−	+	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	32
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	48
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS			X															X								3
Adenocarcinoma, NOS																										1
MUSCULOSKELETAL SYSTEM																										
Muscle	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Hemangiosarcoma																				X						1
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Hepatocellular carcinoma, metastatic																										1
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Hepatocellular carcinoma, metastatic																										1
Hemangiosarcoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Malignant lymphoma, NOS																										1
Malig. lymphoma, undifferentiated type																										2
Malignant lymphoma, mixed type						X						X		X			X		X							7

* Animals necropsied

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: LOW DOSE

ANIMAL NUMBER	C036	C007	C030	C116	C003	C025	C223	C228	C035	C118	C333	C400	C009	C119	C222	C339	C227	C226	C338	C334	C113	C117	C411	C110	C115
WEEKS ON STUDY	011	012	012	027	036	050	053	057	057	060	062	065	076	079	080	082	085	087	087	093	096	098	098	099	101
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N
Sarcoma, NOS													X				X								
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Squamous cell carcinoma, metastatic																				X					
Hepatocellular carcinoma, metastatic														X											
Alveolar/bronchiolar adenoma						X										X		X							X
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−
Hemangiosarcoma																		X							
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−
Thymus	+	+	−	−	+	−	+	+	+	−	−	+	−	−	−	+	+	−	+	−	−	−	−	−	−
Malignant lymphoma, lymphocytic type																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																				X					
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+
Hepatocellular adenoma							X																X		
Hepatocellular carcinoma							X							X	X									X	X
Malignant lymphoma, mixed type																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+
Gallbladder & common bile duct	+	+	N	N	+	+	N	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	+	+	−	−	+	−	−	−	−
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Stomach	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	+	−	−	−	−	+	−	+
Squamous cell papilloma																									
Squamous cell carcinoma						X																			
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−
Malignant lymphoma, NOS															X										
Large intestine	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	−	−	−	−	−	−	−
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−
Urinary bladder	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	−	−	−	−	−	−	−
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
Parathyroid	−	+	−	−	+	−	+	+	+	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−
Pancreatic islets	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	+	+	−	−	+	−	−	−	−
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS													X										X		
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hepatocellular carcinoma, metastatic														X											
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type																					X				
Malignant lymphoma, histiocytic type																							X		

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	C043	C001	C002	C004	C005	C006	C008	C011	C012	C014	C020	C021	C024	C029	C031	C032	C037	C042	C044	C045	C046	C047	C048	C049	C050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	101	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	N	N	N	N	N	+	N	N	N	N	N	N	N	+	N	N	N	N	+	N	N	N	N	N	*50
Sarcoma, NOS	X																									3
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	C	–	+	–	–	+	–	–	C	–	+	–	+	+	C	–	–	–	–	–	–	–	+	–	30
Squamous cell carcinoma, metastatic																										1
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma				X										X										X		7
Alveolar/bronchiolar carcinoma							X					X														2
Trachea	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	18
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
Spleen	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	20
Hemangiosarcoma																										1
Lymph nodes	–	C	–	+	–	–	+	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	20
Thymus	–	C	–	–	–	–	–	–	–	C	–	+	–	–	–	C	–	–	–	–	–	–	–	–	–	11
Malignant lymphoma, lymphocytic type												X														1
CIRCULATORY SYSTEM																										
Heart	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma																										1
Salivary gland	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
Liver	+	C	–	+	+	–	+	+	+	C	+	–	+	+	–	C	+	+	+	+	–	–	–	–	–	36
Hepatocellular adenoma				X	X			X					X				X		X	X						9
Hepatocellular carcinoma																		X								6
Malignant lymphoma, mixed type																				X						1
Bile duct	+	C	–	+	+	–	+	+	+	C	+	–	+	+	–	C	+	+	+	+	–	–	–	–	–	36
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	*50
Pancreas	–	C	–	–	–	–	–	–	–	C	–	–	+	–	–	C	–	–	–	–	–	+	–	+	–	19
Esophagus	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
Stomach	–	C	–	–	–	+	–	+	+	C	–	+	+	–	–	C	–	–	–	–	–	–	–	–	+	22
Squamous cell papilloma																									X	1
Squamous cell carcinoma																										1
Small intestine	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	+	–	–	–	–	–	–	–	–	19
Malignant lymphoma, NOS																										1
Large intestine	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	15
URINARY SYSTEM																										
Kidney	+	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	+	–	–	–	–	22
Urinary bladder	–	C	–	–	–	–	–	–	–	C	–	–	–	+	–	C	–	–	–	–	–	–	–	–	–	17
ENDOCRINE SYSTEM																										
Pituitary	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	16
Adrenal	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	18
Thyroid	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	20
Parathyroid	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	9
Pancreatic islets	–	C	–	–	–	–	–	–	–	C	–	–	+	–	–	C	–	–	–	–	–	+	–	+	–	19
Islet cell carcinoma																								X		1
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
Prostate	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
NERVOUS SYSTEM																										
Brain	–	C	–	–	–	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	19
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS			X																				X			4
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hepatocellular carcinoma, metastatic																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, histiocytic type																										1

* Animals necropsied

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: HIGH DOSE

ANIMAL NUMBER	C 2 0	C 4 0	C 1 3	C 0 3	C 2 4	C 2 9	C 0 2	C 1 8	C 4 1	C 3 0	C 3 1	C 0 1	C 0 4	C 0 5	C 0 6	C 0 7	C 0 8	C 0 9	C 1 0	C 1 1	C 1 2	C 1 4	C 1 5	C 1 6	C 1 7
WEEKS ON STUDY	0 2 3	0 5 9	0 6 3	0 6 4	0 6 4	0 6 6	0 7 7	0 8 6	0 8 8	0 9 0	1 0 3	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Fibroma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Sarcoma, NOS							X																		
Fibrosarcoma					X																		X		
Hemangiosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic							X																		
Alveolar/bronchiolar adenoma														X						X	X				X
Alveolar/bronchiolar carcinoma				X													X				X				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Thymus	+	+	+	−	+	−	−	+	−	−	+	+	+	+	+	−	+	+	−	+	−	+	−	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	−	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma													X	X											X
Hepatocellular carcinoma				X		X	X		X											X					
Malignant lymphoma, lymphocytic type																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	N	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																						X			
Small intestine	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma													X												
Urinary bladder	+	+	+	−	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																					X				
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	+	−	+	−	−	+	+	−	+	+	+	+	−	+	−	+	−	−	+	+	+	−	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																		X				X			
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hepatocellular carcinoma, metastatic							X																		
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alveolar/bronchiolar carcinoma, metastatic				X																					
Malignant lymphoma, NOS										X															
Malignant lymphoma, undifferentiated type								X																	
Malignant lymphoma, lymphocytic type												X													
Malignant lymphoma, mixed type																			X						

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	C 1 9	C 2 1	C 2 2	C 2 3	C 2 5	C 2 6	C 2 7	C 2 8	C 3 2	C 3 3	C 3 4	C 3 5	C 3 6	C 3 7	C 3 8	C 3 9	C 4 2	C 4 3	C 4 4	C 4 5	C 4 6	C 4 7	C 4 8	C 4 9	C 5 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																X					X					2
Subcutaneous tissue	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
Fibrosarcoma																										2
Hemangiosarcoma																				X						1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma							X									X			X							7
Alveolar/bronchiolar carcinoma																				X						4
Trachea	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type									X	X																2
Thymus	–	+	+	–	–	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	+	–	+	+	33
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma									X			X	X									X		X	X	10
Hepatocellular carcinoma	X																									5
Malignant lymphoma, lymphocytic type							X																			1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma													X													2
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																										1
Urinary bladder	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid	–	–	–	+	+	–	–	+	+	+	+	–	–	+	–	–	+	–	–	+	+	–	–	–	+	27
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS								X															X			4
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hepatocellular carcinoma, metastatic																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Alveolar/bronchiolar carcinoma, meta																										1
Malignant lymphoma, NOS																										1
Malig lymphoma, undifferentiated type																										1
Malignant lymphoma, lymphocytic type								X																		2
Malignant lymphoma, mixed type				X													X			X						4

* Animals necropsied

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	250 mg/kg	500 mg/kg
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	2/49 (4%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	5.8%	9.1%	6.8%
Terminal Rates (c)	1/33 (3%)	0/24 (0%)	1/39 (3%)
Week of First Observation	98	76	64
Life Table Tests (d)	P=0.462	P=0.404	P=0.546
Incidental Tumor Tests (d)	P=0.293	P=0.593	P=0.377
Cochran-Armitage Trend Test (d)	P=0.421		
Fisher Exact Test (d)		P=0.510	P=0.510
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	13/47 (28%)	(e) 7/30 (23%)	7/50 (14%)
Adjusted Rates (b)	36.7%		17.9%
Terminal Rates (c)	10/32 (31%)		7/39 (18%)
Week of First Observation	91		104
Life Table Test (d)			P=0.044N
Incidental Tumor Test (d)			P=0.075N
Fisher Exact Test (d)			P=0.079N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	2/47 (4%)	(e) 2/30 (7%)	4/50 (8%)
Adjusted Rates (b)	5.2%		9.7%
Terminal Rates (c)	0/32 (0%)		3/39 (8%)
Week of First Observation	77		64
Life Table Test (d)			P=0.402
Incidental Tumor Test (d)			P=0.253
Fisher Exact Test (d)			P=0.369
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	15/47 (32%)	(e) 9/30 (30%)	10/50 (20%)
Adjusted Rates (b)	40.0%		24.7%
Terminal Rates (c)	10/32 (31%)		9/39 (23%)
Week of First Observation	77		64
Life Table Test (d)			P=0.086N
Incidental Tumor Test (d)			P=0.176N
Fisher Exact Test (d)			P=0.134N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	0/49 (0%)	(e,f) 2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	0.0%		7.7%
Terminal Rates (c)	0/33 (0%)		3/39 (8%)
Week of First Observation			104
Life Table Test (d)			P=0.152
Incidental Tumor Test (d)			P=0.152
Fisher Exact Test (d)			P=0.125
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	7/49 (14%)	(e,f) 1/50 (2%)	6/50 (12%)
Adjusted Rates (b)	21.2%		15.4%
Terminal Rates (c)	7/33 (21%)		6/39 (15%)
Week of First Observation	104		104
Life Table Test (d)			P=0.370N
Incidental Tumor Test (d)			P=0.370N
Fisher Exact Test (d)			P=0.484N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	10/49 (20%)	(e,f) 5/50 (10%)	11/50 (22%)
Adjusted Rates (b)	28.3%		26.7%
Terminal Rates (c)	8/33 (24%)		9/39 (23%)
Week of First Observation	90		86
Life Table Test (d)			P=0.544N
Incidental Tumor Test (d)			P=0.576
Fisher Exact Test (d)			P=0.521

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	250 mg/kg	500 mg/kg
Circulatory System: Hemangiosarcoma			
Overall Rates (a)	4/49 (8%)	(e,f) 1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	11.4%		2.6%
Terminal Rates (c)	3/33 (9%)		1/39 (3%)
Week of First Observation	91		104
Life Table Test (d)			P=0.142N
Incidental Tumor Test (d)			P=0.144N
Fisher Exact Test (d)			P=0.175N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	7/49 (14%)	(e) 9/36 (25%)	10/50 (20%)
Adjusted Rates (b)	21.2%		25.6%
Terminal Rates (c)	7/33 (21%)		10/39 (26%)
Week of First Observation	104		104
Life Table Test (d)			P=0.436
Incidental Tumor Test (d)			P=0.436
Fisher Exact Test (d)			P=0.314
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	15/49 (31%)	(e) 6/36 (17%)	5/50 (10%)
Adjusted Rates (b)	37.2%		11.0%
Terminal Rates (c)	9/33 (27%)		1/39 (3%)
Week of First Observation	54		64
Life Table Test (d)			P=0.010N
Incidental Tumor Test (d)			P=0.017N
Fisher Exact Test (d)			P=0.010N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	22/49 (45%)	(e) 14/36 (39%)	15/50 (30%)
Adjusted Rates (b)	55.5%		34.5%
Terminal Rates (c)	16/33 (48%)		11/39 (28%)
Week of First Observation	54		64
Life Table Test (d)			P=0.053N
Incidental Tumor Test (d)			P=0.089N
Fisher Exact Test (d)			P=0.093N
Small Intestine: Adenocarcinoma			
Overall Rates (a)	3/47 (6%)	(e) 0/19 (0%)	0/47 (0%)
Adjusted Rates (b)	9.4%		0.0%
Terminal Rates (c)	3/32 (9%)		0/38 (0%)
Week of First Observation	104		
Life Table Test (d)			P=0.092N
Incidental Tumor Test (d)			P=0.092N
Fisher Exact Test (d)			P=0.121N
Harderian Gland: Adenoma			
Overall Rates (a)	3/49 (6%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	9.1%	13.8%	10.3%
Terminal Rates (c)	3/33 (9%)	2/24 (8%)	4/39 (10%)
Week of First Observation	104	76	104
Life Table Tests (d)	P=0.517	P=0.356	P=0.591
Incidental Tumor Tests (d)	P=0.456	P=0.404	P=0.591
Cochran-Armitage Trend Test (d)	P=0.435		
Fisher Exact Test (d)		P=0.511	P=0.511
Harderian Gland: Adenoma or Adenocarcinoma			
Overall Rates (a)	4/49 (8%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	11.6%	13.8%	10.3%
Terminal Rates (c)	3/33 (9%)	2/24 (8%)	4/39 (10%)
Week of First Observation	96	76	104
Life Table Tests (d)	P=0.478N	P=0.495	P=0.553N
Incidental Tumor Tests (d)	P=0.558	P=0.580	P=0.636
Cochran-Armitage Trend Test (d)	P=0.562N		
Fisher Exact Test (d)		P=0.631N	P=0.631N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	250 mg/kg	500 mg/kg
All Sites: Benign Tumors			
Overall Rates (a)	23/49 (47%)	19/50 (38%)	19/50 (38%)
Adjusted Rates (b)	63.7%	59.3%	48.7%
Terminal Rates (c)	20/33 (61%)	12/24 (50%)	19/39 (49%)
Week of First Observation	91	50	104
Life Table Tests (d)	P=0.084N	P=0.424	P=0.082N
Incidental Tumor Tests (d)	P=0.126N	P=0.472N	P=0.135N
Cochran-Armitage Trend Test (d)	P=0.212N		
Fisher Exact Test (d)		P=0.243N	P=0.243N
All Sites: Malignant Tumors			
Overall Rates (a)	29/49 (59%)	18/50 (36%)	20/50 (40%)
Adjusted Rates (b)	65.7%	47.4%	43.3%
Terminal Rates (c)	18/33 (55%)	5/24 (21%)	13/39 (33%)
Week of First Observation	54	50	64
Life Table Tests (d)	P=0.034N	P=0.250N	P=0.033N
Incidental Tumor Tests (d)	P=0.074N	P=0.008N	P=0.082N
Cochran-Armitage Trend Test (d)	P=0.035N		
Fisher Exact Test (d)		P=0.017N	P=0.044N
All Sites: All Tumors			
Overall Rates (a)	43/49 (88%)	29/50 (58%)	35/50 (70%)
Adjusted Rates (b)	97.7%	75.8%	76.0%
Terminal Rates (c)	32/33 (97%)	15/24 (63%)	28/39 (72%)
Week of First Observation	54	50	64
Life Table Tests (d)	P=0.015N	P=0.286N	P=0.010N
Incidental Tumor Tests (d)	P=0.024N	P=0.003N	P=0.019N
Cochran-Armitage Trend Test (d)	P=0.033N		
Fisher Exact Test (d)		P=0.001N	P=0.027N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 incidental tumor 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 is indicated by (N).
(e) Incomplete sampling of tissues
(f) Twenty spleens and 36 livers were examined microscopically.

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing	1		
Animals necropsied	49	50	50
Animals examined histopathologically	49	47	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Follicular cyst, NOS			1 (2%)
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)	
Inflammation, chronic suppurative			1 (2%)
Fibrosis	1 (2%)		
*Subcutaneous tissue	(49)	(50)	(50)
Inflammation, chronic		1 (2%)	1 (2%)
Fibrosis		1 (2%)	
RESPIRATORY SYSTEM			
*Nasal cavity	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)	1 (2%)	2 (4%)
*Maxillary sinus	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)		
*Larynx	(49)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
#Lung/bronchus	(47)	(30)	(50)
Inflammation, chronic			1 (2%)
#Lung	(47)	(30)	(50)
Congestion, NOS			1 (2%)
Edema, NOS	1 (2%)		
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, interstitial	11 (23%)	1 (3%)	11 (22%)
Foreign material, NOS	1 (2%)		
Pigmentation, NOS		1 (3%)	
Hyperplasia, focal	1 (2%)		1 (2%)
Histiocytosis	2 (4%)	1 (3%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(48)	(19)	(49)
Angiectasis	1 (2%)		
#Spleen	(48)	(20)	(48)
Atrophy, NOS		1 (5%)	
Depletion, lymphoid		1 (5%)	
Hyperplasia, lymphoid	4 (8%)		3 (6%)
Hematopoiesis	8 (17%)	2 (10%)	4 (8%)
#Lymph node	(48)	(20)	(49)
Hemorrhage	1 (2%)		
Hyperplasia, lymphoid			1 (2%)
Hematopoiesis	1 (2%)		
#Mandibular lymph node	(48)	(20)	(49)
Lymphocytic inflammatory infiltrate	1 (2%)		
Hemosiderosis	2 (4%)		
#Mediastinal lymph node	(48)	(20)	(49)
Hyperplasia, lymphoid			1 (2%)
#Hepatic lymph node	(48)	(20)	(49)
Hemorrhage	1 (2%)		
#Mesenteric lymph node	(48)	(20)	(49)
Congestion, NOS	1 (2%)		
Hemorrhage	2 (4%)		3 (6%)
Hyperplasia, lymphoid		1 (5%)	2 (4%)
#Liver	(49)	(36)	(50)
Hematopoiesis	1 (2%)		1 (2%)

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Peyer's patch	(47)	(19)	(47)
Hyperplasia, lymphoid			5 (11%)
*Rectum	(49)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Thymus	(32)	(11)	(33)
Embryonal duct cyst		1 (9%)	
Cyst, NOS			1 (3%)
Atrophy, NOS	2 (6%)		1 (3%)
CIRCULATORY SYSTEM			
*Multiple organs	(49)	(50)	(50)
Periarteritis			1 (2%)
#Bone marrow	(48)	(19)	(49)
Thrombosis, NOS	1 (2%)		
#Lymph node	(48)	(20)	(49)
Periarteritis			1 (2%)
#Heart	(49)	(19)	(50)
Mineralization		1 (5%)	
Inflammation, chronic focal		1 (5%)	
#Myocardium	(49)	(19)	(50)
Degeneration, NOS	1 (2%)		
*Mesentery	(49)	(50)	(50)
Perivasculitis			1 (2%)
Arteriosclerosis, NOS		1 (2%)	
#Testis	(48)	(19)	(50)
Periarteritis			1 (2%)
DIGESTIVE SYSTEM			
*Pulp of tooth	(49)	(50)	(50)
Inflammation, suppurative			1 (2%)
Inflammation, chronic suppurative			1 (2%)
*Gum	(49)	(50)	(50)
Inflammation, chronic suppurative	1 (2%)		
#Salivary gland	(49)	(19)	(47)
Lymphocytic inflammatory infiltrate	7 (14%)	5 (26%)	4 (9%)
#Liver	(49)	(36)	(50)
Lymphocytic inflammatory infiltrate	1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)
Peliosis hepatis			1 (2%)
Necrosis, NOS	1 (2%)		
Necrosis, focal		1 (3%)	
Necrosis, coagulative			1 (2%)
Infarct, NOS	1 (2%)		1 (2%)
Amyloidosis			1 (2%)
Cytoplasmic vacuolization	3 (6%)		4 (8%)
Basophilic cyto change	2 (4%)	3 (8%)	
Eosinophilic cyto change		2 (6%)	1 (2%)
Nuclei, abnormal number	8 (16%)	4 (11%)	32 (64%)
Angiectasis			1 (2%)
#Liver/centrilobular	(49)	(36)	(50)
Degeneration, NOS	1 (2%)		
Cytomegaly	23 (47%)	11 (31%)	38 (76%)
#Liver/Kupffer cell	(49)	(36)	(50)
Hyperplasia, NOS	1 (2%)		
#Bile duct	(49)	(36)	(50)
Hyperplasia, focal			1 (2%)
#Pancreas	(47)	(19)	(47)
Dilatation/ducts		1 (5%)	
Lymphocytic inflammatory infiltrate		1 (5%)	1 (2%)

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Pancreatic acinus	(47)	(19)	(47)
Atrophy, NOS	4 (9%)	1 (5%)	1 (2%)
#Stomach	(45)	(22)	(49)
Ulcer, NOS		1 (5%)	
Inflammation, chronic		1 (5%)	
Hyperplasia, epithelial		1 (5%)	
#Glandular stomach	(45)	(22)	(49)
Hyperplasia, epithelial			2 (4%)
#Forestomach	(45)	(22)	(49)
Epidermal inclusion cyst		1 (5%)	
Ulcer, NOS	1 (2%)	1 (5%)	
Ulcer, acute			1 (2%)
Inflammation, acute/chronic	1 (2%)		
Ulcer, chronic	1 (2%)		1 (2%)
Hyperplasia, epithelial	6 (13%)	4 (18%)	4 (8%)
Hyperplasia, focal	1 (2%)		
Hyperkeratosis	2 (4%)		1 (2%)
#Small intestine	(47)	(19)	(47)
Hyperplasia, adenomatous	1 (2%)	1 (5%)	
URINARY SYSTEM			
#Kidney	(49)	(22)	(50)
Mineralization	2 (4%)	3 (14%)	
Cyst, NOS			1 (2%)
Glomerulonephritis, NOS	5 (10%)		2 (4%)
Lymphocytic inflammatory infiltrate	9 (18%)	9 (41%)	12 (24%)
Inflammation, interstitial	4 (8%)		2 (4%)
Pyelonephritis, acute			1 (2%)
Fibrosis	1 (2%)		
Necrosis, NOS	1 (2%)		
Hemosiderosis	1 (2%)		
Metaplasia, osseous			1 (2%)
#Kidney/tubule	(49)	(22)	(50)
Dilatation, NOS	1 (2%)		
Cyst, NOS		1 (5%)	
Cytoplasmic vacuolization	1 (2%)		
#Urinary bladder	(49)	(17)	(45)
Calculus, gross observation only	1 (2%)		
Lymphocytic inflammatory infiltrate	3 (6%)		
Inflammation, suppurative	1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)		
Inflammation, chronic suppurative	1 (2%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(16)	(48)
Hyperplasia, focal	2 (4%)		1 (2%)
#Adrenal/capsule	(45)	(18)	(49)
Hyperplasia, focal	1 (2%)		
#Adrenal cortex	(45)	(18)	(49)
Hyperplasia, NOS		1 (6%)	
Hyperplasia, focal	2 (4%)		7 (14%)
#Thyroid	(48)	(20)	(50)
Follicular cyst, NOS	2 (4%)		1 (2%)
Hyperplasia, follicular cell	4 (8%)		
#Pancreatic islets	(47)	(19)	(47)
Hyperplasia, focal	2 (4%)		1 (2%)

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
*Penis	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)		
*Preputial gland	(49)	(50)	(50)
Dilatation/ducts	9 (18%)	3 (6%)	8 (16%)
Inflammation, suppurative			2 (4%)
Abscess, NOS	2 (4%)	1 (2%)	2 (4%)
Inflammation, chronic suppurative	4 (8%)		4 (8%)
Inflammation, granulomatous	1 (2%)		
#Prostate	(48)	(19)	(47)
Mineralization			1 (2%)
Lymphocytic inflammatory infiltrate		2 (11%)	6 (13%)
Inflammation, suppurative		1 (5%)	1 (2%)
Inflammation, chronic	1 (2%)		
Fibrosis		1 (5%)	1 (2%)
*Seminal vesicle	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)		
Inflammation, acute	1 (2%)		
Inflammation, chronic		1 (2%)	
Inflammation, granulomatous			1 (2%)
#Testis	(48)	(19)	(50)
Atrophy, NOS	2 (4%)		1 (2%)
*Epididymis	(49)	(50)	(50)
Spermatocele		1 (2%)	
Lymphocytic inflammatory infiltrate	1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)		
*Scrotum	(49)	(50)	(50)
Inflammation, chronic			1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Eye	(49)	(50)	(50)
Fibrosis		1 (2%)	
Cataract		1 (2%)	1 (2%)
Phthisis bulbi		1 (2%)	
*Harderian gland	(49)	(50)	(50)
Inflammation, chronic	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Skull	(49)	(50)	(50)
Hyperostosis			2 (4%)
BODY CAVITIES			
*Abdominal cavity	(49)	(50)	(50)
Hematoma, NOS		1 (2%)	
Necrosis, fat	1 (2%)	2 (4%)	2 (4%)

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(50)
Lymphocytic inflammatory infiltrate	19 (39%)	1 (2%)	25 (50%)
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		2	
Animal missing/no necropsy	1		
Necropsy perf/no histo performed		3	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX D

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

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
*Subcutaneous tissue	(50)	(50)	(50)
Trichoepithelioma		1 (2%)	
Sarcoma, NOS	2 (4%)	3 (6%)	3 (6%)
Neurofibrosarcoma	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(49)	(12)	(50)
Hepatocellular carcinoma, metastatic			1 (2%)
Alveolar/bronchiolar adenoma	4 (8%)		2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	2 (17%)	
Sarcoma, NOS, metastatic	1 (2%)	2 (17%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, NOS	1 (2%)		1 (2%)
Malignant lymphoma, undifferentiated type	1 (2%)	1 (2%)	
Malignant lymphoma, lymphocytic type	3 (6%)	2 (4%)	1 (2%)
Malignant lymphoma, histiocytic type			1 (2%)
Malignant lymphoma, mixed type	10 (20%)	11 (22%)	4 (8%)
#Spleen	(50)	(17)	(49)
Malignant lymphoma, mixed type		1 (6%)	1 (2%)
#Lymph node	(50)	(13)	(47)
Sarcoma, NOS, metastatic			1 (2%)
#Abdominal lymph node	(50)	(13)	(47)
Sarcoma, NOS, metastatic		1 (8%)	
#Liver	(50)	(50)	(49)
Malignant lymphoma, mixed type	1 (2%)	4 (8%)	
#Small intestine	(48)	(7)	(48)
Malignant lymphoma, mixed type	1 (2%)	1 (14%)	1 (2%)
#Ileum	(48)	(7)	(48)
Malignant lymphoma, mixed type		1 (14%)	
#Kidney	(49)	(7)	(49)
Malignant lymphoma, mixed type	1 (2%)		
#Thymus	(39)	(3)	(40)
Malignant lymphoma, lymphocytic type			1 (3%)
CIRCULATORY SYSTEM			
#Liver	(50)	(50)	(49)
Hemangiosarcoma	2 (4%)		
#Uterus	(50)	(35)	(49)
Hemangioma		2 (6%)	
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(49)
Hepatocellular adenoma	4 (8%)	2 (4%)	5 (10%)
Hepatocellular carcinoma			3 (6%)
#Forestomach	(48)	(18)	(49)
Squamous cell papilloma	2 (4%)	1 (6%)	2 (4%)
Squamous cell carcinoma		1 (6%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Duodenum	(48)	(7)	(48)
Adenomatous polyp, NOS	1 (2%)		
*Anus	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
URINARY SYSTEM			
#Kidney	(49)	(7)	(49)
Hepatocellular carcinoma, metastatic			1 (2%)
Sarcoma, NOS, metastatic		1 (14%)	
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(8)	(48)
Neuroblastoma	1 (2%)		
#Anterior pituitary	(49)	(8)	(48)
Carcinoma, NOS			1 (2%)
Adenoma, NOS	12 (24%)	5 (63%)	1 (2%)
#Adrenal/capsule	(49)	(3)	(48)
Adenoma, NOS	1 (2%)		
#Adrenal cortex	(49)	(3)	(48)
Adenoma, NOS			1 (2%)
#Adrenal medulla	(49)	(3)	(48)
Pheochromocytoma	1 (2%)		
#Thyroid	(50)	(3)	(49)
Follicular cell adenoma	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS		1 (2%)	1 (2%)
#Uterus	(50)	(35)	(49)
Sarcoma, NOS	1 (2%)		
Leiomyoma		1 (3%)	
Endometrial stromal polyp	1 (2%)		1 (2%)
#Ovary	(50)	(18)	(46)
Cystadenoma, NOS		1 (6%)	2 (4%)
Mixed tumor, benign	1 (2%)		
NERVOUS SYSTEM			
#Brain	(50)	(3)	(50)
Carcinoma, NOS, invasive			1 (2%)
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenoma, NOS	3 (6%)	1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*Vertebra	(50)	(50)	(50)
Sarcoma, NOS	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Sarcoma, NOS, metastatic	1 (2%)		
Sarcoma, NOS, unclear primary or metastatic			1 (2%)
*Mesentery	(50)	(50)	(50)
Sarcoma, NOS, metastatic	2 (4%)		

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Sarcoma, NOS, metastatic		1 (2%)	
Thigh			
Sarcoma, NOS		1	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	7	5	8
Moribund sacrifice	1		
Terminal sacrifice	42	44	42
Dosing accident		1	
TUMOR SUMMARY			
Total animals with primary tumors**	41	31	26
Total primary tumors	58	43	37
Total animals with benign tumors	24	11	12
Total benign tumors	31	14	16
Total animals with malignant tumors	26	25	19
Total malignant tumors	27	29	20
Total animals with secondary tumors##	2	3	4
Total secondary tumors	4	5	6
Total animals with tumors-- uncertain primary or metastatic			1
Total uncertain tumors			1

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

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: VEHICLE CONTROL

ANIMAL NUMBER	C 0 8	C 4 8	C 2 1	C 1 5	C 4 7	C 4 4	C 4 9	C 0 1	C 0 2	C 0 3	C 0 4	C 0 5	C 0 6	C 0 7	C 0 9	C 1 0	C 1 1	C 1 2	C 1 3	C 1 4	C 1 6	C 1 7	C 1 8	C 1 9	C 2 0
WEEKS ON STUDY	0 7 9	0 7 9	0 8 5	0 8 6	0 9 0	0 9 7	0 9 8	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS					X								X												
Neurofibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																								X	
Sarcoma, NOS, metastatic					X																				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																						X			
Hemangiosarcoma																									
Malignant lymphoma, mixed type																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	−	+	−	−	−	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenomatous polyp, NOS							X																		
Malignant lymphoma, mixed type																						X			
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																		X							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS								X	X			X			X					X		X			
Neuroblastoma																									X
Adrenal	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma									X																
Parathyroid	+	−	+	+	+	+	−	+	−	−	+	+	+	−	−	+	−	+	+	+	−	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	N	N	N	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS															X										
Endometrial stromal polyp																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+
Mixed tumor, benign																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS															X										
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS			X																						
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, metastatic					X																				
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, metastatic			X		X																				
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS	X																								
Malignant lymphoma, undifferentiated type												X													
Malignant lymphoma, lymphocytic type		X		X										X											
Malignant lymphoma, mixed type							X									X			X					X	

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	C 2 2 2	C 2 2 3	C 2 2 4	C 2 2 5	C 2 2 6	C 2 2 7	C 2 2 8	C 2 2 9	C 3 0 0	C 3 1 1	C 3 2 2	C 3 3 3	C 3 3 4	C 3 3 5	C 3 3 6	C 3 3 7	C 3 3 8	C 3 3 9	C 4 0 0	C 4 1 1	C 4 2 2	C 4 3 3	C 4 5 5	C 4 6 6	C 5 0 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										2
Neurofibrosarcoma									X																	1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma														X						X	X		X			4
Alveolar/bronchiolar carcinoma																										1
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+/	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+	–	+	+	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma											X				X					X						4
Hemangiosarcoma			X					X																		2
Malignant lymphoma, mixed type														X												1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma												X		X												2
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenomatous polyp, NOS																										1
Malignant lymphoma, mixed type																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS	X			X			X		X	X						X										12
Neuroblastoma																										1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS																				X						1
Pheochromocytoma																								X		1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
Parathyroid	–	–	–	–	+	+	+	+	–	–	–	+	–	+	–	+	+	–	–	+	+	+	–	+	–	29
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	*50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, NOS																										1
Endometrial stromal polyp								X																		1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mixed tumor, benign					X																					1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																X									X	3
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS																										1
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, metastatic																										1
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, metastatic																										2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																										1
Malig. lymphoma, undifferentiated type																										1
Malignant lymphoma, lymphocytic type																										3
Malignant lymphoma, mixed type		X		X		X							X								X	X				10

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: LOW DOSE

ANIMAL NUMBER	C030	C004	C021	C047	C040	C012	C001	C002	C003	C005	C006	C007	C008	C009	C010	C011	C013	C014	C015	C016	C017	C018	C019	C020	C022
WEEKS ON STUDY	058	079	084	092	098	102	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+
Trichoepithelioma				X																					
Sarcoma, NOS					X	X																			X
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	–	+	+	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	+	+
Alveolar/bronchiolar carcinoma												X													
Sarcoma, NOS, metastatic						X																			
Trachea	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	–	+
Malignant lymphoma, mixed type																									
Lymph nodes	+	+	+	–	+	+	–	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	+
Sarcoma, NOS, metastatic						X																			
Thymus	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DIGESTIVE SYSTEM																									
Salivary gland	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Malignant lymphoma, mixed type												X		X					X						
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	N	N	+	N	+	+	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+
Pancreas	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Esophagus	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	+	+	+	–	–	–	+	–	–	–	+	+	–	–	–	–	–	+	+	–	+	–	–	+	–
Squamous cell papilloma																									
Squamous cell carcinoma																									
Small intestine	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Malignant lymphoma, mixed type																									
Large intestine	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
URINARY SYSTEM																									
Kidney	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
Sarcoma, NOS, metastatic						X																			
Urinary bladder	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–
Adenoma, NOS												X								X					
Adrenal	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thyroid	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Parathyroid	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS		X																							
Uterus	+	+	+	–	–	–	+	–	–	+	+	+	–	+	+	–	+	+	+	+	+	+	+	+	–
Leiomyoma																				X					
Hemangioma		X																							
Ovary	+	+	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	+	+	–	+	+	–	+	–
Cystadenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, metastatic					X																				
Malignant lymphoma, undifferentiated type							X																		
Malignant lymphoma, lymphocytic type																									
Malignant lymphoma, mixed type			X			X		X								X					X		X		X
Thigh, NOS																									
Sarcoma, NOS																									

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	C 2 3	C 2 4	C 2 5	C 2 6	C 2 7	C 2 8	C 2 9	C 3 1	C 3 2	C 3 3	C 3 4	C 3 5	C 3 6	C 3 7	C 3 8	C 3 9	C 4 1	C 4 2	C 4 3	C 4 4	C 4 5	C 4 6	C 4 8	C 4 9	C 5 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Trichoepithelioma																										1
Sarcoma, NOS																										3
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
Alveolar/bronchiolar carcinoma									X																	2
Sarcoma, NOS, metastatic							X																			2
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Spleen	–	–	–	+	–	+	–	+	–	+	–	–	–	+	–	+	–	–	–	–	–	–	+	–	+	17
Malignant lymphoma, mixed type						X																				1
Lymph nodes	–	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
Sarcoma, NOS, metastatic																										1
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma						X																X				2
Malignant lymphoma, mixed type																		X								4
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	N	+	N	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	N	+	N	*50
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	6
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Stomach	–	–	–	+	–	+	+	–	–	–	–	–	–	–	+	–	+	+	–	–	+	–	–	+	–	18
Squamous cell papilloma																		X								1
Squamous cell carcinoma																	X									1
Small intestine	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	7
Malignant lymphoma, mixed type			X																		X					2
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Sarcoma, NOS, metastatic																										1
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
ENDOCRINE SYSTEM																										
Pituitary	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Adenoma, NOS		X				X						X														5
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenocarcinoma, NOS																										1
Uterus	+	+	+	–	+	+	–	–	+	–	+	+	+	–	+	+	+	–	+	+	+	+	–	+	+	35
Leiomyoma																										1
Hemangioma		X																								2
Ovary	+	–	–	–	–	–	–	–	+	–	–	–	+	–	+	–	+	–	+	+	–	–	–	–	+	18
Cystadenoma, NOS									X																	1
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS								X																		1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, metastatic																										1
Malig. lymphoma, undifferentiated type																										1
Malignant lymphoma, lymphocytic type				X																					X	2
Malignant lymphoma, mixed type							X			X						X							X			11
Thigh, NOS																										
Sarcoma, NOS							X																			1

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE: HIGH DOSE

ANIMAL NUMBER	C 2 8	C 3 0	C 0 3	C 4 9	C 4 8	C 0 7	C 1 5	C 0 1	C 0 2	C 0 4	C 0 5	C 0 6	C 0 8	C 0 9	C 1 0	C 1 1	C 1 2	C 1 3	C 1 4	C 1 6	C 1 7	C 1 8	C 1 9	C 2 0	C 2 1
WEEKS ON STUDY	0 3 1	0 4 3	0 6 5	0 9 5	0 9 6	0 9 8	1 0 3	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Squamous cell carcinoma																									
Subcutaneous tissue	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Sarcoma, NOS					X														X				X		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic						X																			
Alveolar/bronchiolar adenoma																									X
Sarcoma, NOS, metastatic					X														X						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Malignant lymphoma, mixed type																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+
Sarcoma, NOS, metastatic																			X						
Thymus	–	–	+	+	+	–	+	+	–	+	+	+	+	–	+	+	–	+	–	+	+	+	+	+	–
Malignant lymphoma, lymphocytic type																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Hepatocellular adenoma																X									
Hepatocellular carcinoma						X			X								X								
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Gallbladder & common bile duct	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Pancreas	+	+	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Squamous cell papilloma																	X				X				
Small intestine	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Malignant lymphoma, mixed type																									
Large intestine	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Rectum	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Squamous cell carcinoma							X																		
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Hepatocellular carcinoma, metastatic						X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Carcinoma, NOS																									
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Adenoma, NOS																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Follicular cell adenoma															X										
Parathyroid	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	–	+	–
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	N	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Adenocarcinoma, NOS																X									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Endometrial stromal polyp																	X								
Ovary	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	●	+	+	+	+	+	+	–
Cystadenoma, NOS																X									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive																									
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, unclear primary or metastatic																				X					
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS																									X
Malignant lymphoma, lymphocytic type			X																						
Malignant lymphoma, histiocytic type							X																		
Malignant lymphoma, mixed type										X															

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	C22	C23	C24	C25	C26	C27	C29	C31	C32	C33	C34	C35	C36	C37	C38	C39	C40	C41	C42	C43	C44	C45	C46	C47	C50	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma														X												1
Subcutaneous tissue	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										3
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma															X											2
Sarcoma, NOS, metastatic																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type											X															1
Lymph nodes	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Sarcoma, NOS, metastatic																										1
Thymus	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	40
Malignant lymphoma, lymphocytic type									X																	1
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma	X	X								X		X														5
Hepatocellular carcinoma																										3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																										2
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Malignant lymphoma, mixed type												X														1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, NOS		X																								1
Adenoma, NOS										X																1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS											X															1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma																										1
Parathyroid	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	−	+	+	+	37
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endometrial stromal polyp																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	46
Cystadenoma, NOS																								X		2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, invasive		X																								1
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																								X		1
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, unclear primary or meta																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																										1
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type	X															X	X									4

* Animals necropsied

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	500 mg/kg	1,000 mg/kg
Subcutaneous Tissue: Sarcoma or Neurofibrosarcoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	6.7%	6.5%	6.7%
Terminal Rates (c)	2/43 (5%)	1/44 (2%)	2/43 (5%)
Week of First Observation	90	98	96
Life Table Tests (d)	P=0.577N	P=0.646N	P=0.657N
Incidental Tumor Tests (d)	P=0.567N	P=0.635	P=0.561
Cochran-Armitage Trend Test (d)	P=0.583		
Fisher Exact Test (d)		P=0.661	P=0.661
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	4/49 (8%)	(e) 0/12 (0%)	2/50 (4%)
Adjusted Rates (b)	9.5%		4.7%
Terminal Rates (c)	4/42 (10%)		2/43 (5%)
Week of First Observation	104		104
Life Table Test (d)			P=0.326N
Incidental Tumor Test (d)			P=0.326N
Fisher Exact Test (d)			P=0.329N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	5/49 (10%)	(e) 2/12 (17%)	2/50 (4%)
Adjusted Rates (b)	11.9%		4.7%
Terminal Rates (c)	5/42 (12%)		2/43 (5%)
Week of First Observation	104		104
Life Table Test (d)			P=0.207N
Incidental Tumor Test (d)			P=0.207N
Fisher Exact Test (d)			P=0.210N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	3/50 (6%)	(e,f) 2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	6.3%		4.4%
Terminal Rates (c)	1/43 (2%)		1/43 (2%)
Week of First Observation	79		65
Life Table Test (d)			P=0.509N
Incidental Tumor Test (d)			P=0.762
Fisher Exact Test (d)			P=0.500N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	13/50 (26%)	(e,f) 18/50 (36%)	6/50 (12%)
Adjusted Rates (b)	29.5%		14.0%
Terminal Rates (c)	12/43 (28%)		6/43 (14%)
Week of First Observation	98		104
Life Table Test (d)			P=0.063N
Incidental Tumor Test (d)			P=0.053N
Fisher Exact Test (d)			P=0.063N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	18/50 (36%)	(e,f) 21/50 (42%)	10/50 (20%)
Adjusted Rates (b)	38.1%		22.1%
Terminal Rates (c)	14/43 (33%)		8/43 (19%)
Week of First Observation	79		65
Life Table Test (d)			P=0.072N
Incidental Tumor Test (d)			P=0.091N
Fisher Exact Test (d)			P=0.059N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	5/49 (10%)
Adjusted Rates (b)	9.3%	4.5%	11.9%
Terminal Rates (c)	4/43 (9%)	2/44 (5%)	5/42 (12%)
Week of First Observation	104	104	104
Life Table Tests (d)	P=0.410	P=0.326N	P=0.485
Incidental Tumor Tests (d)	P=0.410	P=0.326N	P=0.485
Cochran-Armitage Trend Test (d)	P=0.413		
Fisher Exact Test (d)		P=0.339N	P=0.487

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	500 mg/kg	1,000 mg/kg
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	3/49 (6%)
Adjusted Rates (b)	0.0%	0.0%	6.9%
Terminal Rates (c)	0/43 (0%)	0/44 (0%)	2/42 (5%)
Week of First Observation			98
Life Table Tests (d)	P=0.037	(g)	P=0.122
Incidental Tumor Tests (d)	P=0.054	(g)	P=0.159
Cochran-Armitage Trend Test (d)	P=0.036		
Fisher Exact Test (d)		(g)	P=0.117
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	8/49 (16%)
Adjusted Rates (b)	9.3%	4.5%	18.5%
Terminal Rates (c)	4/43 (9%)	2/44 (5%)	7/42 (17%)
Week of First Observation	104	104	98
Life Table Tests (d)	P=0.108	P=0.326N	P=0.170
Incidental Tumor Tests (d)	P=0.125	P=0.326N	P=0.195
Cochran-Armitage Trend Test (d)	P=0.108		
Fisher Exact Test (d)		P=0.339N	P=0.168
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	12/49 (24%)	(e) 5/8 (63%)	1/48 (2%)
Adjusted Rates (b)	27.9%		2.4%
Terminal Rates (c)	12/43 (28%)		1/41 (2%)
Week of First Observation	104		104
Life Table Test (d)			P=0.002N
Incidental Tumor Test (d)			P=0.002N
Fisher Exact Test (d)			P=0.002N
Anterior Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	12/49 (24%)	(e) 5/8 (63%)	2/48 (4%)
Adjusted Rates (b)	27.9%		4.9%
Terminal Rates (c)	12/43 (28%)		2/41 (5%)
Week of First Observation	104		104
Life Table Test (d)			P=0.006N
Incidental Tumor Test (d)			P=0.006N
Fisher Exact Test (d)			P=0.004N
Harderian Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	7.0%	2.3%	2.3%
Terminal Rates (c)	3/43 (7%)	1/44 (2%)	1/43 (2%)
Week of First Observation	104	104	104
Life Table Tests (d)	P=0.201N	P=0.297N	P=0.305N
Incidental Tumor Tests (d)	P=0.201N	P=0.297N	P=0.305N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.309N	P=0.309N
All Sites: Benign Tumors			
Overall Rates (a)	24/50 (48%)	11/50 (22%)	12/50 (24%)
Adjusted Rates (b)	54.5%	23.7%	27.9%
Terminal Rates (c)	23/43 (53%)	9/44 (20%)	12/43 (28%)
Week of First Observation	98	79	104
Life Table Tests (d)	P=0.006N	P=0.005N	P=0.009N
Incidental Tumor Tests (d)	P=0.010N	P=0.007N	P=0.008N
Cochran-Armitage Trend Test (d)	P=0.007N		
Fisher Exact Test (d)		P=0.006N	P=0.011N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	500 mg/kg	1,000 mg/kg
All Sites: Malignant Tumors			
Overall Rates (a)	26/50 (52%)	25/50 (50%)	19/50 (38%)
Adjusted Rates (b)	53.0%	52.0%	40.4%
Terminal Rates (c)	20/43 (47%)	21/44 (48%)	15/43 (35%)
Week of First Observation	79	79	65
Life Table Tests (d)	P=0.121N	P=0.464N	P=0.142N
Incidental Tumor Tests (d)	P=0.194N	P=0.559	P=0.241N
Cochran-Armitage Trend Test (d)	P=0.096N		
Fisher Exact Test (d)		P=0.500N	P=0.114N
All Sites: All Tumors			
Overall Rates (a)	41/50 (82%)	31/50 (62%)	26/50 (52%)
Adjusted Rates (b)	83.6%	63.3%	55.3%
Terminal Rates (c)	35/43 (81%)	26/44 (59%)	22/43 (51%)
Week of First Observation	79	79	65
Life Table Tests (d)	P=0.004N	P=0.036N	P=0.005N
Incidental Tumor Tests (d)	P=0.006N	P=0.040N	P=0.007N
Cochran-Armitage Trend Test (d)	P=0.001N		
Fisher Exact Test (d)		P=0.022N	P=0.002N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 incidental tumor 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 is indicated by (N).
(e) Incomplete sampling of tissues
(f) Seventeen spleens were examined microscopically.
(g) No P value is reported because no tumors were observed in the 500 mg/kg and vehicle control groups.

TABLE D4. HISTORICAL INCIDENCE OF ANTERIOR PITUITARY GLAND TUMORS IN FEMALE B6C3F$_1$ MICE ADMINISTERED CORN OIL BY GAVAGE (a)

Study	Incidence in Vehicle Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Microbiological Associates			
d-Limonene	12/49	0/49	12/49
Benzyl alcohol	10/48	0/48	10/48
TOTAL	22/97 (22.7%)	0/97 (0.0%)	22/97 (22.7%)
Overall Historical Incidence			
TOTAL	(b) 373/1,798 (20.7%)	(c) 23/1,798 (1.3%)	(b,c) 396/1,798 (22.0%)
SD (d)	9.57%	2.48%	9.84%
Range (e)			
High	20/49	5/47	21/49
Low	2/44	0/49	2/44

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes 38 chromophobe adenomas and 1 acidophil adenoma
(c) Includes six adenocarcinomas, NOS
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE

	Vehicle Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Ulcer, NOS		1 (2%)	
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Inflammation, suppurative	14 (28%)	1 (2%)	4 (8%)
Inflammation, chronic suppurative	1 (2%)		
#Lung/bronchiole	(49)	(12)	(50)
Hyperplasia, epithelial	1 (2%)		2 (4%)
#Lung	(49)	(12)	(50)
Inflammation, interstitial	2 (4%)	1 (8%)	8 (16%)
Foreign material, NOS		1 (8%)	
Hyperplasia, focal		1 (8%)	
Histiocytosis		1 (8%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(48)	(3)	(47)
Myelofibrosis	1 (2%)		
Myelopoiesis	1 (2%)		
#Spleen	(50)	(17)	(49)
Hyperplasia, NOS		1 (6%)	
Hyperplasia, lymphoid	4 (8%)	2 (12%)	8 (16%)
Hematopoiesis	6 (12%)	7 (41%)	6 (12%)
Myelopoiesis	1 (2%)		
#Lymph node	(50)	(13)	(47)
Fibrosis	1 (2%)		
Hemosiderosis	1 (2%)		
#Mandibular lymph node	(50)	(13)	(47)
Edema, NOS	1 (2%)		
Hyperplasia, lymphoid	1 (2%)		
#Mediastinal lymph node	(50)	(13)	(47)
Hyperplasia, lymphoid			1 (2%)
#Mesenteric lymph node	(50)	(13)	(47)
Congestion, NOS		1 (8%)	1 (2%)
Edema, NOS	1 (2%)		
Hemorrhage			1 (2%)
Hematopoiesis		1 (8%)	
Myelopoiesis			1 (2%)
*Tibia	(50)	(50)	(50)
Myelofibrosis	1 (2%)		
#Liver	(50)	(50)	(49)
Hematopoiesis	2 (4%)		
Myelopoiesis	1 (2%)	1 (2%)	1 (2%)
#Adrenal	(49)	(3)	(48)
Myelopoiesis			1 (2%)
#Thymus	(39)	(3)	(40)
Hyperplasia, lymphoid	3 (8%)		4 (10%)
CIRCULATORY SYSTEM			
#Lung	(49)	(12)	(50)
Perivasculitis			1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
#Heart	(50)	(3)	(50)
Mineralization			1 (2%)
Lymphocytic inflammatory infiltrate		1 (33%)	
#Heart/atrium	(50)	(3)	(50)
Thrombosis, NOS			1 (2%)
#Myocardium	(50)	(3)	(50)
Inflammation, suppurative			1 (2%)
Inflammation, acute/chronic			1 (2%)
Degeneration, NOS	1 (2%)		
*Superior mesenteric vein	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
#Pancreas	(44)	(6)	(46)
Periarteritis			1 (2%)
#Uterus	(50)	(35)	(49)
Periarteritis			1 (2%)
#Ovary	(50)	(18)	(46)
Thrombus, fibrin		1 (6%)	
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(4)	(49)
Lymphocytic inflammatory infiltrate	4 (8%)	2 (50%)	3 (6%)
Inflammation, chronic		1 (25%)	
Focal cellular change			1 (2%)
#Liver	(50)	(50)	(49)
Congenital malformation, NOS	1 (2%)		
Lymphocytic inflammatory infiltrate	1 (2%)	20 (40%)	2 (4%)
Inflammation, suppurative		3 (6%)	4 (8%)
Necrosis, NOS			1 (2%)
Infarct, NOS	1 (2%)		
Amyloidosis	1 (2%)		
Cytoplasmic vacuolization	1 (2%)	1 (2%)	2 (4%)
Basophilic cyto change	2 (4%)	3 (6%)	1 (2%)
#Liver/hepatocytes	(50)	(50)	(49)
Hypertrophy, NOS	1 (2%)		
*Gallbladder	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate		2 (4%)	
#Bile duct	(50)	(50)	(49)
Cyst, NOS	1 (2%)		
#Pancreas	(44)	(6)	(46)
Dilatation/ducts			3 (7%)
Lymphocytic inflammatory infiltrate			1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)
Inflammation, chronic suppurative		1 (17%)	
Atrophy, NOS			1 (2%)
#Forestomach	(48)	(18)	(49)
Ulcer, NOS			2 (4%)
Inflammation, acute/chronic	1 (2%)		
Hyperplasia, epithelial	13 (27%)	11 (61%)	10 (20%)
#Small intestine	(48)	(7)	(48)
Amyloidosis			3 (6%)
URINARY SYSTEM			
#Kidney	(49)	(7)	(49)
Lymphocytic inflammatory infiltrate	2 (4%)	2 (29%)	7 (14%)
Inflammation, interstitial	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
Pyelonephritis, chronic	1 (2%)		
Fibrosis, focal	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney (Continued)	(49)	(7)	(49)
Amyloidosis			1 (2%)
Hemosiderosis	1 (2%)		
Atrophy, NOS	1 (2%)		
Metaplasia, osseous	1 (2%)		1 (2%)
#Urinary bladder	(50)	(2)	(48)
Lymphocytic inflammatory infiltrate	3 (6%)		7 (15%)
Inflammation, chronic	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(8)	(48)
Hyperplasia, focal			1 (2%)
#Anterior pituitary	(49)	(8)	(48)
Hyperplasia, focal	16 (33%)		17 (35%)
Angiectasis	6 (12%)		1 (2%)
#Adrenal	(49)	(3)	(48)
Congestion, NOS		1 (33%)	
#Adrenal cortex	(49)	(3)	(48)
Lipoidosis	1 (2%)		
#Thyroid	(50)	(3)	(49)
Follicular cyst, NOS			2 (4%)
Inflammation, chronic	2 (4%)		2 (4%)
Hyperplasia, follicular cell	6 (12%)		5 (10%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Inflammation, chronic			1 (2%)
*Mammary acinus	(50)	(50)	(50)
Hyperplasia, NOS	1 (2%)		
#Uterus	(50)	(35)	(49)
Hemorrhage		1 (3%)	
Hematoma, NOS		1 (3%)	
Inflammation, suppurative		3 (9%)	
Abscess, NOS			1 (2%)
Inflammation, chronic	1 (2%)		
#Uterus/endometrium	(50)	(35)	(49)
Hyperplasia, cystic	43 (86%)	31 (89%)	43 (88%)
#Ovary	(50)	(18)	(46)
Cyst, NOS	12 (24%)	10 (56%)	12 (26%)
Hematoma, NOS	1 (2%)		
Hemorrhagic cyst			1 (2%)
Abscess, NOS		1 (6%)	
Inflammation, chronic	1 (2%)		
Inflammation, chronic suppurative			2 (4%)
Angiectasis		1 (6%)	1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Cataract	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF *d*-LIMONENE (Continued)

	Vehicle Control	Low Dose	High Dose
MUSCULOSKELETAL SYSTEM			
*Maxilla	(50)	(50)	(50)
Hyperostosis	1 (2%)		
BODY CAVITIES			
*Abdominal cavity	(50)	(50)	(50)
Steatitis	1 (2%)		
Necrosis, fat			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate	33 (66%)		34 (68%)
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX E

GENETIC TOXICOLOGY OF

d-LIMONENE

TABLE E1. MUTAGENICITY OF *d*-LIMONENE 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	160 ± 4.1	121 ± 15.4	147 ± 8.4	150 ± 6.6	166 ± 6.4	151 ± 11.6
	0.3	--	132 ± 3.8	--	--	--	--
	1	138 ± 1.2	117 ± 9.4	--	--	--	--
	3	128 ± 7.0	131 ± 4.2	--	--	--	--
	10	135 ± 15.4	122 ± 7.5	138 ± 7.5	136 ± 10.7	159 ± 8.4	--
	33	Toxic	129 ± 4.6	135 ± 4.1	125 ± 4.5	175 ± 8.6	153 ± 21.0
	100	Toxic	--	136 ± 8.8	138 ± 12.5	151 ± 10.9	143 ± 1.8
	333	--	--	144 ± 2.3	110 ± 9.9	169 ± 9.5	129 ± 13.6
	1,000	--	--	132 ± 11.0	(c) 105 ± 9.6	160 ± 7.3	(c) 112 ± 21.1
	3,333	--	--	--	--	--	133 ± 2.5
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	396 ± 9.0	410 ± 27.1	2,207 ± 108.8	1,401 ± 53.4	986 ± 15.6	601 ± 37.7
TA1535	0	19 ± 2.7	24 ± 4.2	33 ± 3.8	24 ± 5.0	26 ± 0.3	24 ± 4.3
	0.3	--	14 ± 4.3	--	--	--	--
	1	12 ± 3.2	15 ± 0.6	--	--	--	--
	3	20 ± 4.4	13 ± 2.1	--	--	--	--
	10	22 ± 2.2	17 ± 2.3	26 ± 0.3	28 ± 1.5	27 ± 4.5	--
	33	(c) 4 ± 1.5	(c) 0 ± 0.0	25 ± 1.7	21 ± 2.2	28 ± 4.4	31 ± 1.9
	100	Toxic	--	20 ± 1.8	24 ± 3.3	24 ± 4.8	20 ± 2.6
	333	--	--	21 ± 5.0	19 ± 4.5	21 ± 2.5	24 ± 3.5
	1,000	--	--	(c) 13 ± 5.0	Toxic	24 ± 2.0	26 ± 0.5
	3,333	--	--	--	--	--	25 ± 4.4
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	320 ± 28.5	406 ± 4.0	548 ± 20.4	309 ± 8.7	186 ± 0.6	163 ± 12.2
TA1537	0	8 ± 2.9	4 ± 1.5	5 ± 0.9	5 ± 0.9	8 ± 0.3	5 ± 1.2
	0.3	--	5 ± 0.3	--	--	--	--
	1	6 ± 1.5	3 ± 1.2	--	--	--	--
	3	9 ± 2.3	3 ± 0.6	--	--	--	--
	10	8 ± 0.9	6 ± 1.7	6 ± 1.5	10 ± 2.7	7 ± 0.3	--
	33	(c) 4 ± 0.6	(c) 4 ± 0.7	9 ± 2.6	6 ± 0.7	13 ± 0.7	5 ± 0.9
	100	Toxic	--	6 ± 1.0	6 ± 0.9	8 ± 2.3	7 ± 1.5
	333	--	--	7 ± 1.9	6 ± 1.5	12 ± 2.0	7 ± 3.2
	1,000	--	--	7 ± 2.7	(c) 6 ± 2.8	10 ± 2.2	4 ± 1.2
	3,333	--	--	--	--	--	10 ± 2.0
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	212 ± 5.5	172 ± 18.5	493 ± 20.1	506 ± 3.4	277 ± 46.3	193 ± 11.6
TA98	0	23 ± 2.9	18 ± 1.2	44 ± 7.3	32 ± 2.3	34 ± 4.7	32 ± 0.6
	0.3	--	18 ± 1.7	--	--	--	--
	1	22 ± 4.3	21 ± 4.7	--	--	--	--
	3	23 ± 5.5	17 ± 4.6	--	--	--	--
	10	17 ± 2.2	23 ± 2.0	36 ± 7.0	31 ± 3.5	40 ± 7.2	--
	33	13 ± 3.6	(c) 13 ± 4.3	32 ± 3.0	26 ± 3.0	37 ± 4.7	39 ± 1.2
	100	Toxic	--	36 ± 8.0	27 ± 5.8	46 ± 2.0	34 ± 1.8
	333	--	--	30 ± 1.7	28 ± 3.9	48 ± 1.5	26 ± 3.1
	1,000	--	--	32 ± 2.6	(c) 20 ± 2.1	53 ± 5.9	16 ± 8.4
	3,333	--	--	--	--	--	(c) 14 ± 8.1
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	752 ± 46.8	728 ± 67.6	1,850 ± 215.9	1,276 ± 33.1	510 ± 36.9	380 ± 16.6

TABLE E1. MUTAGENICITY OF *d*-LIMONENE IN *SALMONELLA TYPHIMURIUM* (Continued)

(a) Study performed at SRI International. The detailed protocol is presented by Haworth et al. (1983). Cells and study compound or solvent (95% ethanol) 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) Slight toxicity
(d) 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 E2. INDUCTION OF TFT RESISTANCE BY *d*-LIMONENE IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

Compound	Concentration (µl/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
-S9					
Trial 1					
Ethanol (d)		81.0 ± 3.6	100.0 ± 2.5	81.0 ± 6.0	33.5 ± 1.9
d-Limonene	0.01	73.0 ± 2.0	97.3 ± 6.4	41.3 ± 7.7	19.0 ± 3.6
	0.02	85.3 ± 2.7	92.3 ± 3.4	36.7 ± 6.5	14.7 ± 3.3
	0.03	99.3 ± 6.5	88.3 ± 4.2	55.0 ± 6.0	18.7 ± 3.0
	0.04	79.7 ± 4.3	75.0 ± 16.7	76.0 ± 2.1	32.0 ± 1.2
	0.05	75.7 ± 6.3	46.7 ± 8.4	32.7 ± 3.4	14.3 ± 0.3
	0.06	77.3 ± 4.3	64.7 ± 8.0	82.7 ± 3.2	36.0 ± 3.1
Methyl methanesulfonate	5 µg/ml	67.7 ± 2.6	57.7 ± 2.7	425.3 ± 74.8 (e)	208.3 ± 28.9
Trial 2					
Ethanol (d)		79.5 ± 2.0	100.0 ± 3.9	76.5 ± 4.5	32.0 ± 1.5
d-Limonene	0.03	96.0 ± 4.6	101.3 ± 5.2	117.7 ± 0.7	41.0 ± 1.7
	(f) 0.04	85.5 ± 15.5	88.5 ± 1.5	113.5 ± 23.5	44.0 ± 1.0
	0.05	90.7 ± 3.7	85.7 ± 6.9	127.7 ± 19.6	46.7 ± 5.6
	0.06	78.3 ± 2.4	82.7 ± 14.7	130.7 ± 9.5	(e) 56.0 ± 5.0
	0.08	83.3 ± 6.4	95.7 ± 8.0	124.3 ± 17.8	(e) 49.7 ± 4.1
	0.1	80.0 ± 4.0	103.0 ± 2.5	96.0 ± 10.7	40.7 ± 6.5
Methyl methanesulfonate	5 µg/ml	73.3 ± 0.3	45.7 ± 7.2	552.7 ± 86.8 (e)	252.0 ± 40.3
Trial 3					
Ethanol (d)		73.0 ± 2.7	100.0 ± 14.7	68.8 ± 7.9	31.5 ± 3.2
d-Limonene	5	59.3 ± 6.2	142.3 ± 28.4	61.3 ± 2.7	35.3 ± 2.4
	10	79.3 ± 3.9	126.0 ± 4.4	53.7 ± 7.2	23.0 ± 4.0
	20	68.7 ± 11.3	120.3 ± 20.5	69.3 ± 3.4	35.3 ± 5.4
	30	63.7 ± 0.3	120.3 ± 4.8	60.7 ± 2.9	31.7 ± 1.8
	(f) 40	78.5 ± 9.5	169.0 ± 3.0	76.5 ± 1.5	33.0 ± 3.0
	50	Lethal	--	--	--
Methyl methanesulfonate	5 µg/ml	66.3 ± 7.5	98.7 ± 13.7	183.7 ± 17.2 (e)	93.0 ± 5.9
Trial 4					
Ethanol (d)		102.3 ± 6.5	100.0 ± 4.8	100.8 ± 4.2	33.5 ± 2.6
d-Limonene	5	78.0 ± 11.0	76.7 ± 13.0	77.7 ± 1.8	34.3 ± 4.1
	(f) 10	86.0 ± 6.0	81.5 ± 16.5	82.0 ± 4.0	32.0 ± 1.0
	20	83.7 ± 11.3	74.3 ± 6.2	85.3 ± 3.3	35.7 ± 5.7
	(f) 30	59.0 ± 29.0	29.5 ± 11.5	93.0 ± 10.0	(e) 74.0 ± 42.0
	40	109.7 ± 1.2	50.7 ± 18.7	86.3 ± 8.2	26.3 ± 2.2
	(g) 50	100.0 ± 10.0	51.0 ± 1.0	57.0 ± 4.0	19.5 ± 3.5
	60	Lethal	--	--	--
Methyl methanesulfonate	5 µg/ml	57.7 ± 5.8	36.3 ± 3.3	493.0 ± 17.6 (e)	292.7 ± 35.5

TABLE E2. INDUCTION OF TFT RESISTANCE BY *d*-LIMONENE IN MOUSE L5178Y LYMPHOMA CELLS (Continued)

Compound	Concentration (µl/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
+S9 (h)					
Trial 1					
Ethanol (d)		76.8 ± 3.8	100.0 ± 6.4	167.0 ± 5.7	73.0 ± 4.9
d-Limonene	10	72.3 ± 10.8	71.7 ± 12.5	77.3 ± 2.4	37.7 ± 6.3
	20	72.3 ± 2.0	81.3 ± 4.3	75.3 ± 0.3	34.7 ± 0.9
	30	85.0 ± 3.5	88.7 ± 3.4	95.3 ± 2.9	37.7 ± 1.8
	40	78.7 ± 7.9	63.3 ± 18.8	113.3 ± 11.9	49.7 ± 9.0
	(f) 50	68.0 ± 6.0	51.5 ± 1.5	118.0 ± 5.0	58.5 ± 7.5
	(g) 60	82.5 ± 12.5	62.0 ± 19.0	97.0 ± 2.0	40.0 ± 5.0
	80	Lethal	--	--	--
Methylcholanthrene	2.5 µg/ml	65.3 ± 14.2	42.3 ± 8.0	757.7 ± 73.6 (e)	410.3 ± 50.6
Trial 2					
Ethanol (f)		115.5 ± 0.5	100.0 ± 12.0	230.0 ± 48.0	66.5 ± 13.5
d-Limonene	10	83.3 ± 5.2	86.7 ± 7.2	150.3 ± 40.4	60.3 ± 16.4
	20	83.3 ± 9.2	84.3 ± 6.5	135.7 ± 12.7	56.0 ± 9.2
	30	94.3 ± 11.6	75.0 ± 5.9	190.3 ± 22.5	68.3 ± 8.2
	40	85.3 ± 11.8	70.7 ± 15.3	157.0 ± 25.6	67.0 ± 20.4
	50	78.7 ± 0.3	31.0 ± 7.5	205.0 ± 16.9	87.3 ± 7.1
	(g) 60	88.0 ± 13.0	40.0 ± 14.0	263.5 ± 37.5	100.0 ± 0.0
	80	Lethal	--	--	--
Methylcholanthrene	2.5 µg/ml	66.3 ± 3.2	21.7 ± 1.2	608.0 ± 68.1 (e)	304.0 ± 21.7
Trial 3					
Ethanol (d)		81.5 ± 3.6	100.0 ± 6.6	146.8 ± 10.7	60.5 ± 4.0
d-Limonene	30	85.0 ± 17.1	100.7 ± 33.2	140.0 ± 16.4	59.0 ± 13.3
	40	95.3 ± 9.2	86.0 ± 2.1	123.3 ± 7.8	43.7 ± 2.8
	50	80.3 ± 10.4	82.7 ± 10.6	108.7 ± 6.9	46.0 ± 3.6
	60	67.7 ± 14.5	74.3 ± 18.5	138.3 ± 7.2	77.7 ± 22.7
	80	84.7 ± 5.4	78.0 ± 5.5	145.7 ± 24.3	57.7 ± 10.7
	(f) 100	95.0 ± 3.0	109.0 ± 12.0	130.0 ± 8.0	46.0 ± 4.0
Methylcholanthrene	(f) 2.5 µg/ml	98.0 ± 15.0	61.0 ± 14.0	606.5 ± 28.5 (e)	212.0 ± 42.0

(a) Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses are tested in triplicate, unless otherwise indicated; the average for the tests is presented in the table. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (Tft) for selection of Tft-resistant cells, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

(b) Mean ± standard error of replicate trials for approximately 3×10^6 cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered mutagenic. If only one of these responses is significant, the call is "questionable"; the absence of both trend and peak response results in a "negative" call.

(c) Mutant fraction (frequency) is a ratio of the Tft-resistant cells to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.

(d) Data presented are the average of four tests.

(e) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.

(f) Data presented are the average of two tests.

(g) Data presented are for two tests; the dose in the third test was lethal.

(h) Tests conducted with metabolic activation were performed as described in (a) except that S9, prepared from the liver of Aroclor 1254-induced F344 rats, was added at the same time as the study chemical and/or solvent (ethanol).

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY *d*-LIMONENE (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
−S9 (c)								
Trial 1--Summary: Negative								
Dimethyl sulfoxide		50	1,050	389	0.37	7.8	26.5	--
d-Limonene	16.2	50	1,050	447	0.43	8.9	26.5	114.1
	54	50	1,051	463	0.44	9.3	26.5	119.2
	162	50	1,051	457	0.43	9.1	26.5	116.7
Mitomycin C	0.001	50	1,048	701	0.67	14.0	26.5	179.5
	0.01	10	211	341	1.62	34.1	26.5	437.2
Trial 2--Summary: Weakly positive								
Dimethyl sulfoxide		50	1,049	366	0.35	7.3	26.5	--
d-Limonene	30	50	1,049	407	0.39	8.1	26.5	111.0
	50	50	1,046	405	0.39	8.1	(d) 30.5	111.0
	100	50	1,041	475	0.46	9.5	(d) 30.5	130.1
Mitomycin C	0.001	50	1,046	476	0.46	9.5	26.5	130.1
	0.01	10	210	252	1.20	25.2	26.5	345.2
Trial 3--Summary: Negative								
Dimethyl sulfoxide		50	1,048	345	0.33	6.9	26.5	--
d-Limonene	15	50	1,049	343	0.33	6.9	26.5	100.0
	30	50	1,048	349	0.33	7.0	26.5	101.4
	50	50	1,046	406	0.39	8.1	(d) 30.5	117.4
Mitomycin C	0.001	50	1,051	516	0.49	10.3	26.5	149.3
	0.01	10	209	230	1.10	23.0	26.5	333.3
+S9 (e)								
Trial 1--Summary: Negative								
Dimethyl sulfoxide		50	1,047	398	0.38	8.0	26.0	--
d-Limonene	16.2	50	1,048	404	0.39	8.1	26.0	101.3
	54	50	1,049	399	0.38	8.0	26.0	100.0
	162	50	1,045	394	0.38	7.9	26.0	98.8
Cyclophosphamide	0.4	50	1,046	620	0.59	12.4	26.0	155.0
	2.5	10	210	405	1.93	40.5	26.0	506.3

(a) Study performed at Bioassay Systems Corporation. 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 (dimethyl sulfoxide) as described in (c) or (e) 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) SCEs/cell in treated culture expressed as a percent of the SCEs/cell in the control culture
(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) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.
(e) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Then cells were 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.

TABLE E4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY *d*-LIMONENE (a)

	−S9 (b)					+S9 (c)			
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time 10.5 h					Harvest time 12.0 h (d)				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	100	4	0.04	4.0		100	7	0.07	4.0
d-Limonene					*d*-Limonene				
10	100	2	0.02	2.0	50	100	0	0.00	0.0
30	100	5	0.05	2.0	150	100	4	0.04	4.0
100	100	6	0.06	6.0	500	100	5	0.05	5.0
Summary: Negative					Summary: Negative				
Mitomycin C					Cyclophosphamide				
5	50	52	1.04	50.0	50	50	26	0.52	40.0

(a) Study performed at Bioassay Systems Corporation. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as indicated in (b) or (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, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) 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 (dimethyl sulfoxide) 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 chemically induced cell cycle delay, incubation time before addition of colcemid was lengthened to provide sufficient metaphases at harvest.

APPENDIX F

SENTINEL ANIMAL PROGRAM

APPENDIX F. 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 and 15 F344/N rats 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. 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
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai (6, 12 mo)	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) Sendai (18 mo)	MHV (mouse hepatitis virus) *M. pul.* (*Mycoplasma pulmonis*)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus)	*M. pul.*

Results

Results are presented in Table F1.

TABLE F1. MURINE ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR GAVAGE STUDIES OF *d*-LIMONENE (a)

Interval (months)	Number of Animals	Positive Serologic Reaction for
RATS		
6	10/10	Sendai
12	8/10	Sendai
18	9/9	RCV
	8/9	Sendai
	9/9	*M. pul.* (b)
MICE		
6	10/10	Sendai
12	6/10	Sendai
18	8/9	*M. pul.*
	8/9	Sendai

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing; samples were sent to Microbiological Associates (Bethesda, MD) for determination of antibody titers. Due to an oversight, the laboratory did not collect blood samples at 24 months.
(b) Further evaluation of this assay indicated that it was not specific for *M. pulmonis*, and these results were considered to be false positive.

APPENDIX G

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

Pelleted Diet: December 1980 to January 1983

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

TABLE G1. 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 G2. VITAMINS AND MINERALS IN NIH 07 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 G3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	23.85 ± 0.78	22.7-25.3	24
Crude fat (percent by weight)	5.02 ± 0.44	4.2-5.7	24
Crude fiber (percent by weight)	3.31 ± 0.23	2.9-3.8	24
Ash (percent by weight)	6.44 ± 0.44	5.7-7.43	24
Amino Acids (percent of total diet)			
Arginine	1.323 ± 0.830	1.21-1.39	4
Cystine	0.310 ± 0.099	0.218-0.400	4
Glycine	1.155 ± 0.069	1.06-1.21	4
Histidine	0.572 ± 0.030	0.530-0.603	4
Isoleucine	0.910 ± 0.033	0.881-0.944	4
Leucine	1.949 ± 0.065	1.85-1.99	4
Lysine	1.275 ± 0.076	1.20-1.37	4
Methionine	0.422 ± 0.187	0.306-0.699	4
Phenylalanine	0.909 ± 0.167	0.665-1.04	4
Threonine	0.844 ± 0.029	0.824-0.886	4
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.631 ± 0.094	0.566-0.769	4
Valine	1.11 ± 0.050	1.05-1.17	4
Essential Fatty Acids (percent of total diet)			
Linoleic	2.44	2.37-2.52	3
Linolenic	0.274	0.256-0.308	3
Arachidonic	0.008		1
Vitamins			
Vitamin A (IU/kg)	10,917 ± 1,876	8,210-15,000	24
Vitamin D (IU/kg)	4,650	3,000-6,300	2
α-Tocopherol (ppm)	41.53 ± 7.52	31.1-48.9	4
Thiamine (ppm)	16.80 ± 2.0	14.0-21.0	(b) 23
Riboflavin (ppm)	7.5 ± 0.96	6.1-8.2	4
Niacin (ppm)	85.0 ± 14.2	65.0-97.0	4
Pantothenic acid (ppm)	29.3 ± 4.6	23.0-34.0	4
Pyridoxine (ppm)	7.6 ± 1.5	5.6-8.8	4
Folic acid (ppm)	2.8 ± 0.88	1.8-3.7	4
Biotin (ppm)	0.27 ± 0.05	0.21-0.32	4
Vitamin B_{12} (ppb)	21.0 ± 11.9	11.0-38.0	4
Choline (ppm)	3,302.0 ± 120.0	3,200.0-3,430.0	4
Minerals			
Calcium (percent)	1.25 ± 0.15	1.08-1.69	24
Phosphorus (percent)	0.98 ± 0.06	0.88-1.10	24
Potassium (percent)	0.862 ± 0.100	0.772-0.974	3
Chloride (percent)	0.546 ± 0.100	0.442-0.635	4
Sodium (percent)	0.311 ± 0.038	0.258-0.350	4
Magnesium (percent)	0.169 ± 0.133	0.151-0.181	4
Sulfur (percent)	0.316 ± 0.070	0.270-0.420	4
Iron (ppm)	447.0 ± 57.3	409.0-523.0	4
Manganese (ppm)	90.6 ± 8.20	81.7-95.5	4
Zinc (ppm)	53.6 ± 5.27	46.1-58.6	4
Copper (ppm)	10.77 ± 3.19	8.09-15.39	4
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.81 ± 0.28	1.44-2.09	4
Cobalt (ppm)	0.68 ± 0.14	0.49-0.80	4

(a) One to four batches of feed analyzed for nutrients reported in this table were manufactured during 1983-85.
(b) One batch (7/22/81) not analyzed for thiamine

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.48 ± 0.17	<0.29-1.06	24
Cadmium (ppm) (a)	<0.10		24
Lead (ppm)	1.00 ± 0.74	0.42-3.37	24
Mercury (ppm) (a)	< 0.05		24
Selenium (ppm)	0.29 ± 0.07	0.13-0.40	24
Aflatoxins (ppb) (a,b)	<10	<5.0-<10.0	24
Nitrate nitrogen (ppm) (c)	9.22 ± 3.62	3.8-17.0	24
Nitrite nitrogen (ppm) (c)	2.16 ± 1.53	0.4-6.9	24
BHA (ppm) (d)	6.68 ± 4.95	<0.4-17.0	24
BHT (ppm) (d)	3.45 ± 2.56	0.9-12.0	24
Aerobic plate count (CFU/g) (e)	40,557 ± 29,431	4,900-88,000	23
Aerobic plate count (CFU/g) (f)	77,617 ± 183,824	4,900-930,000	24
Coliform (MPN/g) (g)	16.6 ± 22.9	<3-93	22
Coliform (MPN/g) (h)	80.2 ± 236.3	<3-1,100	24
E. coli (MPN/g) (i)	<3		24
Total nitrosamines (ppb) (j,k)	4.63 ± 4.19	<0.8-18.5	21
Total nitrosamines (ppb) (j,l)	27.15 ± 64.35	0.8-273.2	24
N-Nitrosodimethylamine (ppb) (j,k)	3.43 ± 3.96	0.8-16.5	21
N-Nitrosodimethylamine (ppb) (j,l)	25.71 ± 64.90	0.8-272	24
N-Nitrosopyrrolidine (ppb)	1.05 ± 0.49	0.3-2.9	24
Pesticides (ppm)			
α-BHC (a,m)	<0.01		24
β-BHC (a)	<0.02		24
γ-BHC-Lindane (a)	<0.01		24
δ-BHC (a)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (a)	<0.01		24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (n)	<0.05	0.09 (8/26/81)	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCBs (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (n)	<0.1	0.2 (4/27/81)	24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (o)	0.10 ± 0.07	<0.05-0.27	24
Endosulfan I (a,p)	<0.01		14
Endosulfan II (a,p)	<0.01		14
Endosulfan sulfate (a,p)	<0.03		14

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) Mean, standard deviation, and range exclude one high value of 930,000 obtained for the batch produced on 12/22/82 (CFU = colony forming unit).
(f) Mean, standard deviation, and range include the high value listed in footnote (e).
(g) Mean, standard deviation, and range exclude one high value of 1,100 obtained for the batch produced on 12/16/80 and one high value of 460 obtained for the batch produced on 9/23/82 (MPN = most probable number).
(h) Mean, standard deviation, and range include the high values listed in footnote (g).
(i) All values were less than 3 MPN/g.
(j) All values were corrected for percent recovery.
(k) Mean, standard deviation, and range exclude three very high values in the range of 115-273.2 ppb for batches produced on 1/26/81, 2/23/81, and 4/27/81.
(l) Mean, standard deviation, and range include the very high values given in footnote (k).
(m) BHC = hexachlorocyclohexane or benzene hexachloride
(n) There was one observation above the detection limit; the value and date it was obtained are given under the range.
(o) Thirteen batches contained more than 0.05 ppm.
(p) Analysis started on 12/23/81

APPENDIX H

AUDIT SUMMARY

APPENDIX H. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and draft (June 1987) of NTP Technical Report No. 347 for the 2-year studies of *d*-limonene in rats and mice were audited for the National Institute of Environmental Health Sciences (NIEHS) at the National Toxicology Program (NTP) Archives during July and October 1987 by Program Resources, Inc. 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 husbandry, environmental conditions, dosing, external masses, mortality, animal identification, and serology.
(3) Body weight and clinical observation data for a random 10% sample of the animals in each study group.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning disposition codes, condition codes, tissue accountability, correlation of masses or clinical signs recorded at the last inlife observation with gross observations and microscopic diagnoses, and correlations between gross observations and microscopic diagnoses.
(6) All wet tissue bags for inventory and wet tissues from a random 20% sample of the animals in all study groups, plus other relevant cases to verify animal identity and to examine for untrimmed potential lesions.
(7) Blocks and slides of tissues from a random 20% sample of animals from each study group to examine for proper match, preservation, and inventory.
(8) Correlation between original microscopic observations and tabulated pathology diagnoses for a random 10% sample of study animals to verify computer data entry.
(9) Correlation between the data, results, and procedures for the 2-year studies presented in the draft of the Technical Report and the records available at the NTP Archives.

Inlife procedures and events were documented adequately by the archival records, with a few exceptions. The disposition of surplus animals, net weight of *d*-limonene received, chemical usage, and allocation of animal cages to racks by study group were not documented. The dose preparation and analysis and animal dosing records were present; they showed that mixtures of *d*-limonene in corn oil were prepared and administered to animals throughout the studies according to protocols. Dose analysis results for some dates were recalculated in the original data; the audit confirmed the correctness of the calculations. Other audit findings were evaluated by NTP staff and considered to be of no significance to the interpretation of the studies. For example, 11 rats (2 vehicle control, 3 low dose, and 4 high dose males and 2 low dose females) and 12 mice (10 vehicle control and 2 high dose males; all preputial gland masses) had clinical observations recorded during the last month of life which were not noted either on the necropsy record form or as already trimmed by review of residual tissues. Also, the inlife records indicated gavage death for 10 rats (1 vehicle control, 1 low dose, and 1 high dose male and 7 high dose females) and 4 mice (3 low dose and 1 high dose males), whereas the necropsy disposition code indicated natural death; however, the audit checked postmortem records and found no conclusive evidence that these animals died early because of gavage procedures.

Audit of the pathology specimens showed that single wet tissue bags were available for all but three rats and one mouse and that histology (but not animal) numbers were incorrect on the labels for seven rats. An ear tag was present and correct in the residual tissues for 93/101 rats and 84/105 mice examined. The ear tag was missing from the tissue bags for 4 rats and 21 mice; however, comparison of residual tissues with necropsy records and followup examination of identifiers in bags for animals in other study groups with the same number corroborated the identification of each animal with a missing ear tag. The ear tags for four rats did not agree with the animal number on the bag. For two of these, the necropsy record form adequately documented the mistagging and confirmed the correct identity of the animal. The audit findings for the remaining two rats (high dose males) indicated that

the labels for their wet tissue bags had been switched at final bagging but that histopathology for each had otherwise been performed properly (necropsy observations, slides, blocks, and microscopic observations for each animal were internally consistent for the wet tissues identified by each respective ear tag rather than by bag label). The audit also identified a variety of untrimmed potential lesions in nontarget organs (5/101 rats and 1/105 mice examined) and gross observations that lacked corresponding microscopic diagnoses (eight in rats, four in mice) which, when evaluated by NTP staff, were judged to be relatively minor and to have no adverse impact on interpretation of the pathology data.

Full details of these and other audit findings are presented in the audit reports, which are on file at the NIEHS and were reviewed by NTP staff when the study interpretations were prepared. In conclusion, the data and results presented in the draft (June 1987) of the Technical Report for the 2-year gavage studies of *d*-limonene are supported by the records at the NTP Archives.

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