NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of alpha,beta-Thujone (CAS No. 76231-76-0) in F344/N Rats and B6C3F1 Mice (Gavage Studies)

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF α,β-THUJONE

(CAS NO. 76231-76-0)

IN F344/N RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

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SUMMARY

Background

α,β-Thujone is a component of the essential oils of some plants including wormwood, sage, and cedar. It is used in herbal medicines, food and flavoring, and notably as the principal ingredient of the liqueur absinthe. We studied the effects of α,β-thujone on male and female rats and mice to identify potential toxic or cancer-related hazards.

Method

We deposited solutions containing α,β-thujone in methylcellulose through a tube directly into the stomach to groups of 50 male and female rats and mice five days per week for two years. Exposed rats received either 12.5, 25, or 50 milligrams of α,β-thujone per kilogram of body weight, and mice received 3, 6, 12, or 25 mg/kg. Control animals received methylcellulose with no chemical added by the same method. At the end of the study, tissues from more than 40 sites were examined for every animal.

Results

All male and female rats receiving 50 mg/kg α,β-thujone died before the end of the study. All of those animals, and most receiving 25 mg/kg, experienced seizures. In male rats there was an increased incidence of cancers of the preputial gland and a slight increase in the incidence of pheochromocytomas of the adrenal gland. Nearly all male and female mice receiving 25 mg/kg α,β-thujone experienced seizures, and all of the female mice receiving 25 mg/kg died before the end of the study. No increases in cancers were observed in female rats or in male or female mice.

Conclusions

We conclude that α,β-thujone caused cancers of the preputial gland in male rats, and an increase in adrenal gland tumors in male rats may have been related to α,β-thujone administration. There was no increase in cancer incidence in female rats or male or female mice. Seizures were seen in almost all rats and mice receiving the highest doses of α,β-thujone.

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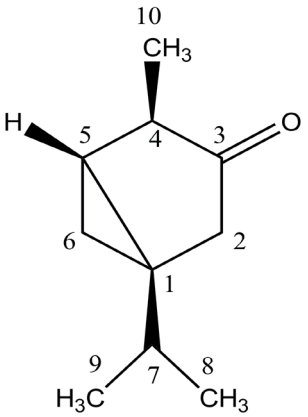
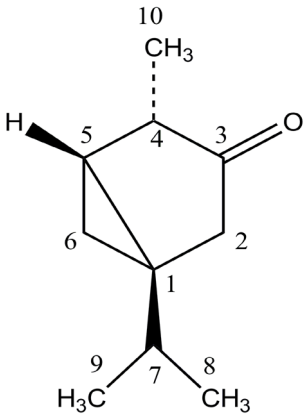
ABSTRACT



α-Thujone



β-Thujone

α,β-THUJONE

CAS No. 76231-76-0

Chemical Formula: $C_{10}H_{16}O$ Molecular Weight: 152.23

Synonyms: α-Thujone: (1S, 4R, 5R)-(–)-3-thujanone; (–)-isothujone; (–)-3-isothujone; l-thujone; (–)-thujone; *trans*-thujone
β-Thujone: (1S, 4S, 5R)-(+)-3-thujanone; d-isothujone; (+)-3-thujone; (+)-thujone; *cis*-thujone
Trade name: See ERRATUM

Thujone is a monoterpene ketone that exists in two stereoisomeric forms: (–)-3-isothujone, or α-thujone, and (+)-3-thujone, or β-thujone. Thujone occurs in nature as a mixture of α- and β-isomers and is the primary constituent of essential oils derived from a variety of plants. Although thujone itself is banned as a food additive in the United States, it appears in many other natural flavoring substances that are approved food additives and flavorings. α-Thujone was nominated for study by the National Cancer Institute based on the potential for widespread human exposure through consumption of herbs and essential oils containing thujone. Two-week toxicity studies were conducted with α-thujone and an α,β-thujone mixture (71%, 12%, respectively). The α,β-thujone mixture (70%, 11%, respectively) was selected for subsequent 3-month and 2-year studies because thujone occurs naturally as a mixture and human exposure is to the mixture. Male and female F344/N rats and B6C3F1 mice were administered α-thujone or α,β-thujone for 2 weeks and α,β-thujone for 3 months or 2 years by gavage. Genetic toxicology studies of α-thujone and α,β-thujone were conducted in *Salmonella typhimurium* and *Escherichia coli* and mouse peripheral blood erythrocytes.

2-WEEK STUDY OF α-THUJONE IN RATS

Groups of five male and five female rats were administered 0, 1, 3, 10, 30, or 100 mg α-thujone/kg body weight in 0.5% methylcellulose by gavage for 16 days. All male rats survived to the end of the study; three 100 mg/kg female rats died before the end of the study. Mean body weights of dosed rats were similar to those of the vehicle controls. The thymus weights of

100 mg/kg females were significantly less than those of the vehicle controls. Clinical findings included convulsions/seizures in three 100 mg/kg females. No gross or histologic findings were attributed to administration of α-thujone.

2-Week Study of α,β-Thujone in Rats

Groups of five male and five female rats were administered 0, 1, 3, 10, 30, or 100 mg α,β-thujone/kg body weight in 0.5% methylcellulose by gavage for 16 days. One 100 mg/kg male rat died before the end of the study. Final mean body weights and body weight gains of 10 mg/kg male rats were significantly less than those of the vehicle control group. No convulsions/seizures were observed in rats administered α,β-thujone. No gross or histologic findings were attributed to α,β-thujone administration.

2-Week Study of α-Thujone in Mice

Groups of five male and five female mice were administered 0, 1, 3, 10, 30, or 100 mg α-thujone/kg body weight in 0.5% methylcellulose by gavage for 17 days. Four 100 mg/kg males and all five 100 mg/kg females died before the end of the study. Mean body weights of surviving mice were similar to those of the vehicle controls. Clinical findings included seizures and tremors in 100 mg/kg males and hyperactivity in 100 mg/kg male and female mice. No gross or histologic findings were attributed to administration of α-thujone.

2-Week Study of α,β-Thujone in Mice

Groups of five male and five female mice were administered 0, 1, 3, 10, 30, or 100 mg α,β-thujone/kg body weight in 0.5% methylcellulose by gavage for 17 days. All 100 mg/kg male mice and two 100 mg/kg female mice died before the end of the study. Final mean body weights of surviving dosed mice were similar to those of the vehicle control groups; the mean body weight gains of 3 mg/kg males and 100 mg/kg females were significantly greater than those of the vehicle control groups. No seizures or tremors were observed in mice administered α,β-thujone. There were no gross or histologic findings associated with α,β-thujone administration.

3-Month Study of α,β-Thujone in Rats

Groups of 10 male and 10 female rats were administered 0, 12.5, 25, 50, 75, or 100 mg α,β-thujone/kg body weight in 0.5% methylcellulose by gavage for 14 weeks. Administered doses of α,β-thujone were lower than target concentrations as indicated by after dosing sample analysis. Additional clinical pathology groups of 10 male and 10 female rats were administered the same doses for 3 or 24 days. Two male and eight female 75 mg/kg rats and eight male and nine female 100 mg/kg rats died before the end of the study. Final mean body weights and body weight gains of 50 and 75 mg/kg females were significantly increased. Seizures were observed in male rats administered 50 mg/kg or greater and in female rats administered 25 mg/kg or greater. All but one of the early deaths occurred in animals that had previously been observed to have seizures. Thymus weights were significantly decreased in 75 and 100 mg/kg males.

Incidences of congestion and/or hemorrhage of the brain were increased in 75 mg/kg females and 100 mg/kg males and females. The incidences of pigmentation in the brain were also increased in 50 mg/kg or greater females. Changes in the pituitary gland were increased with α,β-thujone administration in female rats, and included atrophy of the pars distalis and dilatation of Rathke's cleft. Congestion, hemorrhage, and/or edema were observed in several other organs of rats that died before the end of the study. The incidences of renal tubule mineralization were significantly increased in all dosed groups of female rats.

3-Month Study of α,β-Thujone in Mice

Groups of 10 male and 10 female mice were administered 0, 6.25, 12.5, 25, 50, or 75 mg α,β-thujone/kg body weight in 0.5% methylcellulose by gavage for 14 weeks. Administered doses of α,β-thujone were lower than target concentrations as indicated by after dosing sample analysis. All 75 mg/kg mice and nine male and seven female 50 mg/kg mice died before the end of the study. Mean body weights of surviving dosed mice were similar to those of the vehicle controls. Seizures were observed in 50 and 75 mg/kg male mice and in female mice administered 25 mg/kg or greater. Early deaths of 50 and 75 mg/kg mice occurred in animals that had previously been observed to have seizures. The incidences of lung congestion in 75 mg/kg males and females and lung hemorrhage in 50 mg/kg males were generally

significantly greater than those in the vehicle control group.

2-YEAR STUDY OF α,β-THUJONE IN RATS

Groups of 50 male and 50 female rats were administered 0, 12.5, 25, or 50 mg α,β-thujone/kg body weight in 0.5% methylcellulose by gavage for up to 105 weeks. All of the 50 mg/kg male and female rats died before the end of the study, and survival of 25 mg/kg males and females was significantly less than that of the respective vehicle control group. Mean body weights of all dosed groups were generally within 10% of those of the vehicle control groups throughout the study. Seizures occurred in all 50, most 25, and a few 12.5 mg/kg rats.

Incidences of preputial gland carcinoma and adenoma or carcinoma (combined) in male rats occurred with positive trends, and the combined incidence in 25 mg/kg males was significantly greater than that in the vehicle control group. The incidence of benign pheochromocytoma of the adrenal medulla was significantly increased in 25 mg/kg male rats.

The incidences of necrosis and pigmentation of the brain were significantly increased in 50 mg/kg males, and the incidence of pigmentation was significantly increased in 50 mg/kg females. Pigmentation was localized to macrophages and was consistent with hemosiderin. Lower incidences of these brain lesions also occurred in rats administered 12.5 or 25 mg/kg. In female rats, increased incidences of atrophy of the pars distalis and dilatation of Rathke's cleft in the pituitary gland were observed in the 50 mg/kg and 25 and 50 mg/kg groups, respectively. In the spleen, the incidences of pigmentation in 25 and 50 mg/kg males and 50 mg/kg females were significantly increased. The incidences of mineralization of the kidney were significantly increased in all dosed groups of males.

2-YEAR STUDY OF α,β-THUJONE IN MICE

Groups of 50 male and 50 female mice were administered 0, 3, 6, 12, or 25 mg α,β-thujone/kg body weight in 0.5% methylcellulose by gavage for up to 105 weeks. Survival of male and female mice in the 25 mg/kg groups was significantly less than that of the vehicle controls. Mean body weights of all dosed groups of males and of females administered 12 mg/kg or less were within 10% of those of the vehicle control groups throughout the study. Mean body weights of 25 mg/kg females were less than those of the vehicle controls after week 29. Most male and all female 25 mg/kg mice had seizures. No neoplasms or nonneoplastic lesions were attributed to α,β-thujone administration.

SINGLE-DOSE TOXICOKINETIC STUDIES OF α-THUJONE AND α,β-THUJONE

Single-dose toxicokinetic studies of α-thujone and α,β-thujone were conducted in male and female F344/N rats and B6C3F1 mice following intravenous and oral gavage administration. Intravenous doses of α-thujone and α,β-thujone, respectively, were 1.6 and 3.0 mg/kg for rats and 3.2 and 6.0 mg/kg for mice. Gavage doses of α-thujone and α,β-thujone, respectively, were 25 and 50 mg/kg for rats and 40 and 80 mg/kg for mice. α-Thujone absorption was rapid and independent of dose, species, and sex following gavage administration of either formulation. In general, elimination was faster in mice than in rats. The bioavailability was higher in female rats compared to male rats following administration of either formulation although a sex difference was not observed in mice. Female rats and male and female mice showed greater than proportional increases in bioavailability with increasing dose following administration of α-thujone and α,β-thujone, respectively, which is likely due to saturation of elimination kinetics. Following administration of both formulations, α-thujone was distributed to the brain; females generally had higher brain/plasma ratios than males in both species.

GENETIC TOXICOLOGY

Neither α,β-thujone nor α-thujone was mutagenic in bacterial tester strains (*S. typhimurium* and *E. coli*) when testing was conducted with or without exogenous metabolic activation provided by rat or hamster liver S9 mix. *In vivo*, daily exposure by gavage to α,β-thujone for 3 months did not result in an increase in micronucleated erythrocytes in the peripheral blood of male mice. However, female mice had a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-month study. No significant changes in the percentage of reticulocytes among total erythrocytes was seen in either male or female mice at the end of the 3-month study, suggesting that α,β-thujone did not induce bone marrow toxicity.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of α,β-thujone in male F344/N rats based on increased incidences of preputial gland neoplasms; increased incidences of benign pheochromocytoma of the adrenal medulla may have been related to administration of α,β-thujone in male F344/N rats administered 12.5 or 25 mg/kg. There was *no evidence of carcinogenic activity* of α,β-thujone in female F344/N rats administered 12.5 or 25 mg/kg. There was *no evidence of carcinogenic activity* of α,β-thujone in male or female B6C3F1 mice administered 3, 6, or 12 mg/kg.

Administration of α,β-thujone for 2 years resulted in increased incidences of seizures in F344/N rats and B6C3F1 mice and increased incidences of nonneoplastic lesions in the brain and spleen of male and female F344/N rats, the kidney of male F344/N rats, and the pituitary gland of female F344/N rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Peer Review Panel comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis Studies of α,β-Thujone and Genetic Toxicology Studies of α-Thujone and α,β-Thujone

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in methylcellulose by gavage	0, 12.5, 25, or 50 mg/kg	0, 12.5, 25, or 50 mg/kg	0, 3, 6, 12, or 25 mg/kg	0, 3, 6, 12, or 25 mg/kg
Body weights	Dosed groups generally within 10% of the vehicle control group	Dosed groups generally within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	25 mg/kg group 16% less than the vehicle control group after week 29
Survival rates	27/50, 25/50, 17/50, 0/50	35/50, 33/50, 19/50, 0/50	40/50, 42/50, 41/50, 37/50, 14/50	37/50, 33/50, 40/50, 41/50, 0/50
Clinical findings	Seizures: (1/50, 5/50, 43/50, 50/50)	Seizures: (1/50, 3/50, 47/50, 50/50)	Seizures: (0/50, 0/50, 0/50, 0/50, 44/50)	Seizures: (1/50, 1/50, 0/50, 0/50, 50/50)
Nonneoplastic effects	Brain: necrosis (0/50, 0/50, 1/50, 3/50); pigmentation (0/50, 1/50, 0/50, 3/50) Spleen: pigmentation (19/50, 24/50, 30/49, 46/48) Kidney: mineralization (17/48, 33/48, 41/44, 38/49)	Brain: pigmentation (1/50, 3/50, 5/50, 19/50) Pituitary gland: pars distalis, atrophy (0/50, 0/49, 2/49, 12/48); Rathke's cleft, dilatation (7/50, 1/49, 13/49, 26/48) Spleen: pigmentation (39/48, 40/49, 39/48, 45/50)	None	None
Neoplastic effects[a]	Preputial gland: carcinoma (1/49, 0/49, 5/50); adenoma or carcinoma (3/49, 1/49, 9/50)	None	None	None
Equivocal findings[a]	Adrenal medulla: benign pheochromocytoma (6/50, 8/50, 12/49)	None	None	None
Level of evidence of carcinogenic activity	Some evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Bacterial gene mutations:				
α,β-thujone		Negative in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 with and without S9		
α -thujone		Negative in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 and in *E. coli* strain WP2*uvrA*/pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative in males and positive in females		

[a] Neoplastic incidences are not presented for 50 mg/kg male rats due to 100% mortality.

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 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 cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report 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 five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to 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 chemical-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 chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-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.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

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. Such consideration 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:

- 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 incidence 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 other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- 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.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on α,β-thujone on January 26, 2011, 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 in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW PANEL COMMENTS

On January 26, 2011, the draft Technical Report on the toxicology and carcinogenesis studies of α,β-thujone received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. C.R. Blystone, NIEHS, presented the details of the studies on α-thujone and α,β-thujone to the panel. Thujone is a monoterpene found in several plant species. It exists in nature as a mixture of α and β stereoisomeric forms, and is used in herbal medicines, food and beverage flavorings, cosmetic products, and repellents. α-Thujone is the principal component of absinthe and has been identified as a γ-aminobutyric acid receptor antagonist. Thujone was nominated for study by the National Cancer Institute based on concerns of widespread exposure and the lack of toxicity and carcinogenicity data. Two-week, 3-month, and 2-year toxicity and carcinogenicity studies were conducted in F344/N male and female rats and B6C3F1 male and female mice, as well as single-dose toxicokinetic studies in both genders of both species and genetic toxicology studies. An α,β-thujone mixture was selected for subchronic and chronic testing because it represents a common human exposure. The proposed conclusions were *some evidence* of carcinogenic activity of α,β-thujone in male F344/N rats and *no evidence* of carcinogenic activity of α,β-thujone in female F344/N rats or male or female B6C3F1 mice.

Dr. Dorman, first primary reviewer, wondered about the cells of origin of the preputial gland tumors. He asked about urinary excretion, as related to the possibility that there may be a grooming effect in the animals, resulting in an atypical preputial exposure to the compound. He was concerned about the high level of contamination of the study compound with another compound with unknown toxicological characteristics and felt that the NTP should address that concern in its discussion in the report. He also expressed concern about lack of attention to the difference between nominal exposures and actual exposures in the study, in that the findings would be skewed significantly as a result. He felt that there should have been more detail in the report regarding seizures, with a grading system and more information about clinical signs.

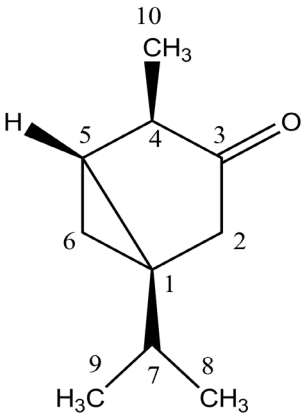
Dr. Birt, second primary reviewer, felt the report was clearly presented and easy to read, and that the study had been well designed and carefully conducted. She had no scientific criticisms, but several suggestions about information that could be added to the report including the nature of the source (synthesized or isolated) of the compounds, data supporting the comments on alterations in diestrus, and inclusion of time on the study with different groups so that the reduced exposure time is reflected with the lesion data.

Dr. Wilson, third primary reviewer, had no arguments with the report's proposed conclusions regarding carcinogenicity. He suggested further discussion about the potential mechanistic connection to 5-HT activity, which was mentioned briefly in the report.

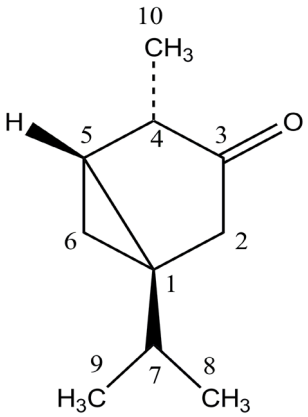
Dr. D.E. Malarkey, NIEHS, explained that the preputial gland is a modified sebaceous gland with squamous cells lining the ducts and the cell of origin for preputial gland neoplasms is likely a glandular epithelial, squamous, or stem cell. Regarding Dr. Dorman's question about contamination, Dr. Blystone replied that the bulk of the chemical came from cedar wood, and that in such natural products other chemicals are often present. There was further discussion of Dr. Dorman's question regarding nominal versus actual dosing, as he recommended that reference to that issue be brought forward into the report's abstract.

Following subsequent discussion about several details concerning the studies' methodologies, the panel considered the proposed conclusions. Dr. Dorman moved to accept the conclusions as written. Dr. Birt seconded the motion, which passed with a vote of seven yes, one no, and one abstention. Dr. Barlow voted against the motion, suggesting that the passage regarding pheochromocytoma should have been related to the *some evidence* language rather than being characterized as *may have been related*.

INTRODUCTION



α-Thujone



β-Thujone

α,β-THUJONE

CAS No. 76231-76-0

Chemical Formula: $C_{10}H_{16}O$ Molecular Weight: 152.23

Synonyms: α-Thujone: (1S, 4R, 5R)-(–)-3-thujanone; (–)-isothujone; (–)-3-isothujone; l-thujone; (–)-thujone; *trans*-thujone
β-Thujone: (1S, 4S, 5R)-(+)-3-thujanone; d-isothujone; (+)-3-thujone; (+)-thujone; *cis*-thujone
Trade name: See ERRATUM

CHEMICAL AND PHYSICAL PROPERTIES

Thujone is a monoterpene ketone that exists in two stereoisomeric forms: (–)-3-isothujone, or α-thujone, and (+)-3-thujone, or β-thujone. It is a structural isomer of camphor. Thujone is a colorless oil with a specific gravity of 0.9109 to 0.9135 at 25° C and is soluble in alcohol and many other organic solvents; it is insoluble in water (*Merck*, 2006). Thujone has an odor similar to menthol (Albert-Puleo, 1978) and exhibits low volatility and high viscosity (Holstege *et al.*, 2002).

PRODUCTION, USE, AND HUMAN EXPOSURE

Thujone occurs in nature as a mixture of α- and β-isomers (Albert-Puleo, 1978). Thujone can be isolated from natural oils with bisulfate or via fractional distillation and crystallization, and it can be synthesized commercially (Albert-Puleo, 1978).

Thujone is the primary constituent of essential oils derived from a variety of plants including wormwood (*Artemisia absinthium*), Roman wormwood (*Artemisia pontica*), mugwort (*Artemisia vulgaris*), sage (*Salvia officinalis*), clary (*Salvia sclarea*), tansy (*Tanacetum vulgare*), white or yellow cedar (cedarleaf oil, *Thuja occidentalis*), and *Juniperus* and *Cedris* species (Albert-Puleo, 1978; IPCS, 1981; Holstege *et al.*, 2002). Absinthol, tanacetone, and asalviol are terms that have been applied to the thujone extracted from *Artemisia absinthium, Salvia officinalis*, and *Tanacetum vulgare*, respectively (Albert-Puleo, 1978). Essential oils derived from the individual plant species vary in thujone content and in the ratios of α- to β-thujone. For example, α-thujone levels are higher in sage and thuja, whereas β-thujone levels are higher in wormwood and

tansy (Albert-Puleo, 1978; IPCS, 1981). *Thuja occidentalis*, commonly known as Arbor vitae or white cedar, is a native European tree cultivated in North America (Naser *et al.*, 2005). The fresh plant leaves contain 0.6% essential oil composed of 65% thujone, 8% isothujone, 8% fenchone, 5% sabines, 2% α-pinene, and smaller quantities of other monoterpenes (Naser *et al.*, 2005). Thujone is the major constituent in the essential oil from the dried herbal substance *Thujae occidentalis herba* and consists of 85% α-thujone and 15% β-thujone.

Essential oils containing thujone are used in herbal medicines, as flavorings in food and beverages, in cosmetic products, and as rodent and mite repellants. Thujone has been found to effectively kill the western corn rootworm (Lee *et al.*, 1997). Essential oils containing thujone have been used in traditional medicine as an abortifacient, for the induction of menstruation, to treat digestive problems, as an antihelmintic, as an antimalarial agent, and to treat fever and the common cold (Albert-Puleo, 1978; Arnold, 1989; Ishida *et al.*, 1989; Naser *et al.*, 2005; Lachenmeier *et al.*, 2006a). A review article published by Naser *et al.* (2005) summarized several German publications that reported that *Thuja occidentalis* has been used clinically to treat acute and chronic infections of the upper respiratory tract and as an adjuvant to antibiotics in bacterial infections such as bronchitis, angina, pharyngitis, otitis media, and sinusitis.

In France, the mean and 97.5 percentile daily intakes of thujone were estimated to be 15.6 and 44.3 µg/kg body weight per day, respectively, and the intakes in the United Kingdom were estimated to be 3.9 and 14.2 µg/kg body weight per day, respectively (SCF, 2003). The major dietary contribution to intake appears to be consumption of sage, sage-flavored products, and alcoholic beverages.

The wormwood plant (*Artemisia absinthium L.*) is a shrub that is indigenous in Central Europe and Asia. The plant parts above ground are harvested when the flowers are opening and, after drying, are used to produce the bitter spirit absinthe (Lachenmeier *et al.*, 2006a). Thujone is the main component of wormwood oil, composing 40% to 90% of the essential oil. Wormwood also contains a number of other compounds including *cis*-chrysanthenyl acetate, *cis*-chrysanthenol, *cis*-epoxy-ocimene, sabinyl acetate, or bornyl acetate as principal components in addition to terpene lactone bitter substances such as absinthin. The concentration of β-thujone is usually higher than that of α-thujone and represents 70% to 90% of the total thujone content. The highest content of thujone is found from June to July, while the highest content of bitter compounds is found in September (Lachenmeier *et al.*, 2006a).

α-Thujone is the principal ingredient of the European liqueur absinthe, which was made from wormwood oil and was widely consumed in the nineteenth century. Although wormwood and alcohol were the primary ingredients of absinthe, the drink also contained other dried herbs including anise, fennel, hyssop, lemon balm (melissa), angelica, star anise, dittany, juniper, nutmeg, and veronica (Arnold, 1989; Lachenmeier *et al.*, 2006a). The turbidity of absinthe is caused by the presence of numerous terpenes including thujone from wormwood, pinocamphone from hyssop, fenchone from fennel, and citral from lemon balm (Arnold, 1989). The chronic abuse of absinthe was associated with a syndrome called absinthism that was characterized by hallucinations, convulsions/seizures, mania, and psychosis (Padosch *et al.*, 2006). As a result, absinthe was banned in most of the world in the 1900s except in a few countries, including Spain and the Czech Republic. Thujone was believed to be the agent responsible for the clinical signs of absinthism. A number of review articles have been published in the last decade that argue that alcohol or other constituents of absinthe were more likely responsible for the adverse effects of absinthe consumption (Lachenmeier *et al.*, 2006a,b; Padosch *et al.*, 2006).

The thujone content of historic absinthe is largely unknown but has been speculated to contain up to 260 mg of thujone per liter (Strang *et al.*, 1999; Hutton, 2002; Lachenmeier *et al.*, 2006b; Padosch *et al.*, 2006) or 360 mg/L (Bonkovsky *et al.*, 1992; Meschler and Howlett, 1999). In 2005, Lachenmeier *et al.* (2006b) recreated three 1899 recipes that required the highest amount of wormwood and evaluated the α- and β-thujone content with gas chromatography-mass spectrometry. The highest thujone content of the three absinthe samples was 4.3 mg/L consisting of 0.8 mg/L α-thujone and 3.5 mg/L β-thujone. Three vintage bottles of absinthe, including a 1930s Pernod Tarragona, contained 1.7 to 9.4 mg thujone/L. These results are similar to an earlier study reporting that a 1900 Pernod absinthe contained 6 mg/L (Hutton, 2002). Lachenmeier *et al.* (2006b) also presented the results of four studies that reported the thujone content of current commercially available absinthe and demonstrated that the majority of the samples did not exceed the European Union thujone maximum limit of 35 mg/L, and more than half contained less than 2 mg/L.

Sage oil, Dalmation is produced from the dried leaves of wild *Salvia officinalis* L. Sage oil, Dalmation is used extensively as a flavor material for liqueurs, canned meats, sauces, pickles, sausages, and as a fragrance in

fine perfumes (*Kirk-Othmer*, 2000). Sage oil and Spanish sage oil are also used as fragrance components in soaps, detergents, creams, lotions, and perfumes with a maximum use level of 0.89% reported for perfumes (Leung, 1980). Wormwood oil has been used as a fragrance component in soaps, detergents, creams, lotions, and perfumes with maximum use levels of 0.01% in detergents and 0.25% in perfumes (Leung, 1980). Cedarleaf oil is used for the scenting of perfumes and in some consumer products (*Kirk-Othmer*, 2000).

REGULATORY STATUS

Due to its toxicity, the amount of thujone allowed in food or beverages is regulated in many countries. In the United States, thujone is banned as a food additive. However, the compound appears in many approved food additives and flavorings. Natural flavoring substances such as artemisia (wormwood), white cedar (leaf and twigs), oak moss, tansy, and yarrow are permitted to be used as food additives, as long as the finished products are thujone free (FDA, 2010). Other herbs that contain thujone, such as clary and sage, are on the Food and Drug Administration's list of Substances Generally Recognized as Safe (21 CFR, Part 182).

Maximum levels of thujone (α- and β-) allowed in the European Union are 0.5 mg/kg in foodstuffs and beverages, 5 mg/kg in alcoholic beverages with not more than 25% alcohol by volume, 10 mg/kg in alcoholic beverages with more than 25% alcohol by volume, 25 mg/kg in foodstuffs containing preparations based on sage, and 35 mg/kg in bitters (SCF, 2003; EEC, 2010). Thujone may not be added as such to foodstuffs or flavorings but may be present in foodstuffs either naturally or following the addition of flavorings prepared from natural raw materials.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

After oral administration of a mixture of α- and β-thujone (ratio 9:2), metabolites arising from the reduction of the carbonyl group to secondary alcohols were identified in the urine of rabbits (Ishida *et al.*, 1989). A similar reductive pathway was observed following incubation of α-thujone with rabbit liver cytosol (Höld *et al.*, 2000). Site specificity and species differences in metabolism of the thujone diastereoisomers were observed in mouse, rat, and human liver microsomes and in mice and rats treated *in vivo*. Microsomes from male albino Swiss Webster mice in the presence of NADPH produced 7-hydroxy-α-thujone and 4-hydroxy-β-thujone as major metabolites from α- and β-thujone, respectively (Höld *et al.*, 2000, 2001). Other metabolites observed in this system were 2-hydroxy-α-thujone, 4-hydroxy-α-thujone, 4-hydroxy-β-thujone, and 7,8-dehydro-α-thujone from α-thujone, and 2-hydroxy-β-thujone, 4-hydroxy-α-thujone, 7-hydroxy-β-thujone, and 7,8-dehydro-β-thujone from β-thujone. Although the pattern of metabolism was somewhat similar in microsomes from albino rats, humans, and mice, 2-hydroxythujones were not observed in microsomal incubations from rats and humans as they were in mice. Rodent *in vitro* microsomal systems generally predicted the *in vivo* metabolism with some exceptions. Major metabolites observed in male albino Swiss Webster mouse urine following oral administration of 40 mg/kg α- or β-thujone were the glucuronide conjugates of 2-hydroxy-α-thujone and 7-hydroxy-β-thujone, respectively. The major metabolite observed in male albino rats following oral administration of 40 mg/kg of either α- or β-thujone was the glucuronide conjugate of 4-hydroxy-α-thujone. Although the 7,8-dehydro metabolites were observed *in vitro* in microsomes from male mice, rats, and humans, 4,10-dehydro metabolites were observed in the urine of rats and mice. The formation of 2-hydroxythujone was specific to mice and was detected only following administration of α-thujone (Höld *et al.*, 2001). In male albino Swiss Webster mice treated intraperitoneally with α-thujone, 7-hydroxy-α-thujone was detected in the brain along with several other hydroxy metabolites (Höld *et al.*, 2000). Based on these observations, the scheme presented in Figure 1 is proposed for the metabolism of α- and β-thujones in rodents.

Humans

No information on the absorption, distribution, metabolism, or excretion of α- and β-thujone in humans was found in the literature.

TOXICITY

Experimental Animals

The oral LD_{50} for thujone (isomer not specified) was reported to be 192 mg/kg body weight for the rat, 230 mg/kg for the mouse, and 396 mg/kg for the guinea pig (IPCS, 1981). The oral LD_{50} for α-thujone was 250 mg/kg in the dog (IPCS, 1981) and 500 mg/kg in the rat (RTECS, 1997). The oral LD_{50} for β-thujone was 250 mg/kg in the mouse (IPCS, 1981). The intravenous LD_{50} for α-thujone in the rabbit is stated to be 0.031 mg/kg (RTECS, 1997). The signs associated with acute intoxication are epileptiform convulsions preceded by general vasodilation, hypotension, slower

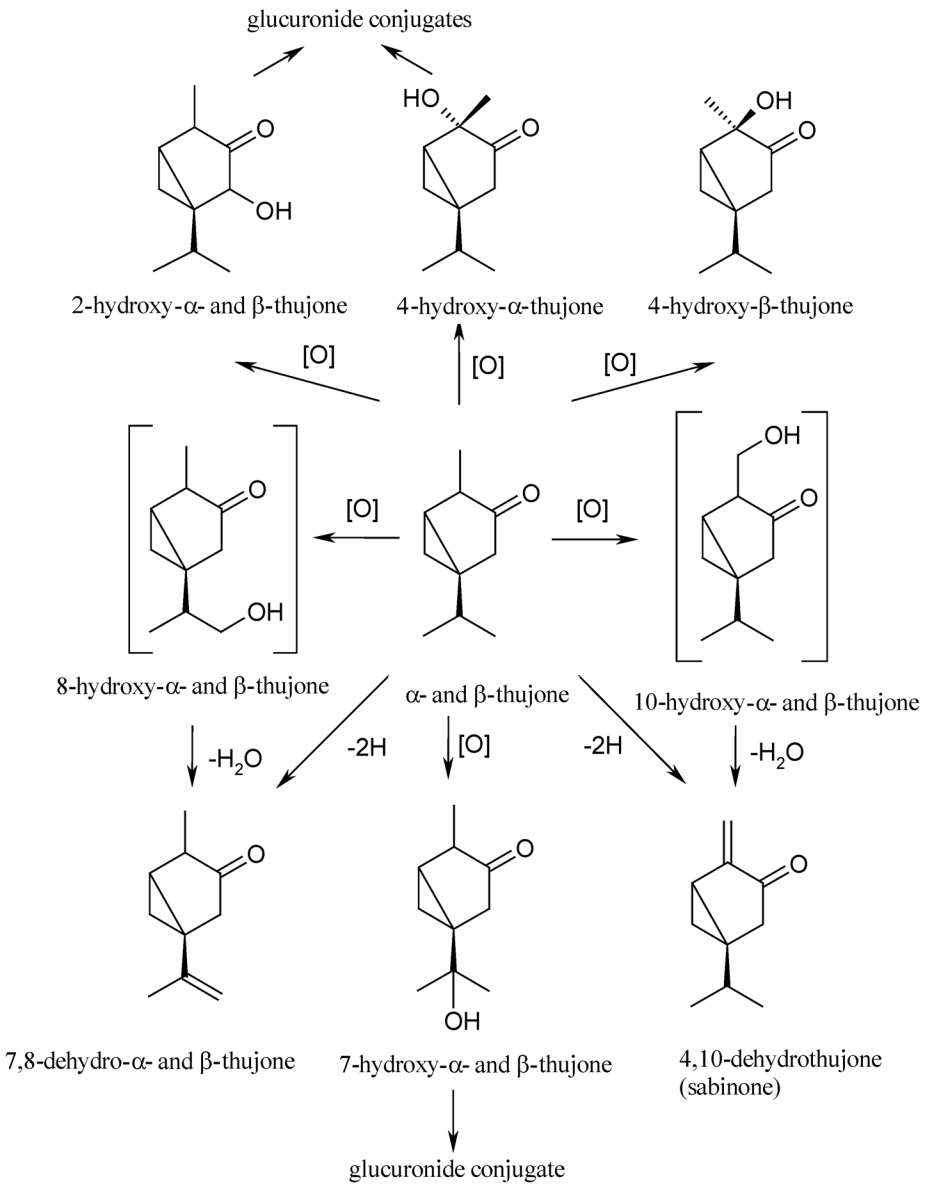


FIGURE 1
Proposed Metabolic Scheme for α,β-Thujone in Rodents Based on *in vitro* and *in vivo* Data (adapted from Höld *et al.*, 2001)

cardiac rhythm, and increased respiratory amplitude (IPCS, 1981).

A commercial mixture of α- and β-thujone was administered to male and female weanling rats (strain unspecified) by gavage at doses of 12.5, 25, or 50 mg/kg body weight for 13 weeks (IPCS, 1981). At the highest dose, 60% of the females and 37% of the males died. Convulsions were frequently observed. The no-observed-adverse-effect level (NOAEL) for convulsions in the males was 12.5 mg/kg, but a NOAEL could not be established for females in this study. No effects were observed on body weight gain, hematology, or histopathologic evaluations.

Thujone (mixture undefined) was administered to male and female rats (strain unspecified) by gavage at doses of 0, 5, 10, or 20 mg/kg body weight, 6 days per week for 14 weeks (IPCS, 1981). Convulsions were observed after dosing in high dose males and females, and three females and one male died in convulsions. The NOAEL for convulsions was reported to be 10 mg/kg in males and 5 mg/kg in females. No treatment-related effects were observed for body weight gain or organ weights. No treatment-related histopathologic lesions were observed.

Gilani and Janbaz (1995) found that an aqueous-menthanolic extract of *Artemisia absinthium* protected male Swiss mice and albino Wistar rats against acetaminophen and carbon tetrachloride-induced hepatotoxicity. Pretreatment of animals with wormwood extract reduced mortality and liver damage partly through the inhibition of microsomal drug metabolizing enzymes.

Undiluted tansy oil (*Tanacetum vulgare*) and wormwood oil (*Artemisia absinthium*) were not skin irritants in hairless mice but were mildly irritating to rabbit skin in a 24-hour patch test (IPCS, 1981). No phototoxic effects were found in hairless mice or swine. Thujone (97% α-, 3% β-) produced an increase in porphyrin production in primary cultures of chick embryo liver cells leading to accumulation of copro- and protoporphyrins (Bonkovsky *et al.*, 1992). Thujone induced 5-aminolevulinic acid synthase, the rate-limiting enzyme of hepatic heme synthesis, and benzphetamine demethylase, a measure of P450-dependent mixed-function oxidase activity. It also induced heme oxygenase by a heme-dependent mechanism. The results indicated that thujone could pose a threat to individuals with underlying defects in hepatic heme synthesis (acute prophyrias).

Humans

Undiluted tansy oil and wormwood oil were not irritants in humans in a 48-hour patch test (IPCS, 1981).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of thujone in humans or experimental animals was found in the literature.

NEUROTOXICITY

Experimental Animals

Based on a structural similarity between thujone and Δ^9-tetrahydrocannabinol, the active component in marijuana, and similar behavioral effects including hallucinations, it was hypothesized that both drugs bind to the CB_1 cannabinoid receptor (Del Castillo *et al.*, 1975). The antinociceptive (pain blocking) activity of α-thujone was evaluated in male white mice after subcutaneous exposure using the hot-plate and Nilsen tests (Perrine *et al.*, 1972). The Nilsen test measures vocalization following application of electrical pulsations to the tail as the pain stimulus. In the hot-plate test, α-thujone was found to be equipotent with codeine and Δ^9-tetrahydrocannabinol. Less antinociceptive activity was observed in the Nilsen test. A racemic mixture was approximately half as active as α-thujone, suggesting that most or all of the activity is due to this isomer. β-Thujone was inactive in both tests up to dose levels of 100 mg/kg (Rice and Wilson, 1976). Subsequently, Meschler and Howlett (1999) demonstrated that thujone exhibited low affinity for brain cannabinoid receptors. Thujone did not exhibit cannabimimetic behavioral effects in Sprague-Dawley rats including immobility, analgesia, and decreased locomotor activity, similar to levonantradol, a potent cannabinoid agonist.

In male and female Wistar rats, intraperitoneal injections of increasing doses (number and dose not specified) of thujone induced electrocortical seizures associated with myoclonic activity (Millet *et al.*, 1981). The convulsant and lethal effects appeared together at 0.2 mL/kg.

Studies on the mechanism of the neurotoxicity of α-thujone indicate that it is a γ-aminobutyric acid (GABA) type A receptor antagonist and modulates the GABA-gated chloride channel (Höld *et al.*, 2000; Olsen, 2000; Dettling *et al.*, 2004). α-Thujone acts like many naturally occurring and synthetic convulsive agents blocking GABA-mediated inhibition which has an

excitatory effect on the brain (Padosch *et al.*, 2006). Four lines of evidence demonstrating that thujone acts as a $GABA_A$ receptor antagonist were provided by the study conducted by Höld *et al.* (2000). First, the signs of thujone poisoning and the protection by benzodiazepines and barbiturates resemble those of the GABA antagonist picrotoxinin. The intraperitoneal LD_{50} of α-thujone in male albino Swiss Webster mice was approximately 45 mg/kg body weight with 0% mortality at 30 mg/kg and 100% mortality at 60 mg/kg. Mortality was preceded by tonic/clonic convulsions. Intraperitoneal administration of diazepam, phenobarbital, or ethanol before a 100 mg/kg dose of α-thujone protected against lethality. Second, a strain of *Drosophila* resistant to chloride channel blockers was also resistant to α-thujone. Third, α-thujone competitively inhibited the binding of a radioactive convulsant to the picrotoxin site on $GABA_A$ receptors in mammalian brain membranes. Finally, thujone reversibly blocked $GABA_A$ receptor chloride currents in mammalian neurons. The toxicity of thujone appears to be due to the parent compound, and metabolism leads to detoxification. Brain levels of the major 7-hydroxy metabolite were much higher and more persistent than α-thujone, but this metabolite was less toxic to mice and *Drosophila*. Likewise, α-thujone was more toxic than β-thujone to mice and *Drosophila* (Höld *et al.*, 2000).

α-Thujone also appears to inhibit 5-HT_3 receptors, but the authors did not investigate whether the inhibition of serotonergic responses contributed to the psychotropic actions of α-thujone (Deiml *et al.*, 2004). α-Thujone does not appear to block the ligand-gated ion channel directly. In homomeric receptors, α-thujone enhanced the inherent channel-blocking potency of the natural ligand 5-HT. In heteromeric receptors, α-thujone recruited an additional channel-blocking component of the agonist. The authors suggest that receptor desensitization results in reduced 5-HT_3 receptor activity.

Humans

There are several anecdotal and case study reports of the acute effects of essential oils containing thujone causing seizures in humans (Millet *et al.*, 1981, Weisbord *et al.*, 1997; Burkhard *et al.*, 1999; Strang *et al.*, 1999). In most cases, the doses are not well documented. Clinical intoxication following ingestion of commercial preparations of essential oils of cedar, sage, tansy, thuja, and wormwood is characterized by tonic and/or clonic convulsions that generally regress spontaneously (Millet *et al.*, 1981; Burkhard *et al.*, 1999). However, the cumulative effect of consumption was shown in the case of a 50-year old woman who took 20 drops of undiluted thuja oil twice a day for 5 days uneventfully, but 30 minutes after the tenth dose suffered a tonic seizure (Millet *et al.*, 1981). A commercial preparation of hyssop, an herbal ingredient in absinthe that contains the monoterpene pinocamphon, also caused tonic/clonic seizures in humans (Millet *et al.*, 1981).

In another case, a man drank about 10 mL of essential oil of wormwood assuming it was absinthe liqueur (Weisbord *et al.*, 1997). He was found several hours later agitated, incoherent, and disoriented. Paramedics noted tonic and clonic seizures that apparently led to rhabdomyolysis and subsequent acute renal failure. He improved with treatment and had no further symptoms or changes in serum clinical chemistry parameters.

Dettling *et al.* (2004) conducted a study to determine the effects of thujone on attention performance and mood. Twenty-five healthy subjects (15 males and 10 females) consumed three drinks containing 16 g of alcohol/L but different amounts of α-thujone (0, 10, or 100 mg/L). The amount of liquid consumed depended on the weight of the subject with a goal of attaining a maximum blood alcohol concentration of 0.05% for each subject. The results showed that consumption of alcohol containing 100 mg thujone/L had a negative effect on attention performance that was not observed when the subjects consumed alcohol alone or alcohol containing 10 mg thujone/L. In addition, the high concentration of thujone temporarily counteracted the anxiolytic effect of alcohol.

CARCINOGENICITY

No carcinogenicity studies in experimental animals or epidemiology studies in humans were found in the literature.

GENETIC TOXICITY

The published mutagenicity data for thujones is limited to a single study that examined both the mutagenic and antimutagenic activities of an α-β-thujone mixture (94.48:3.50) in several strains of *Salmonella typhimurium* and *Escherichia coli*, with and without induced rat liver S9 (Vuković-Gacić *et al.*, 2006). In addition to this thujone mixture, the related monoterpenes D,L-camphor, 1,8-cineole, limonene, and essential oil of sage were tested for mutagenic and antimutagenic activity. Standard bacterial mutagenicity studies were conducted with multiple concentrations of all compounds in *S. typhimurium* strains TA98, TA100, and TA102, with and without S9; no activity was observed with any of the test articles, including the thujone mixture. Antimutagenic activity was evaluated for essential oil of sage and the monoterpenes, including

an α/β-thujone mixture, in three strains of *E. coli* and in the yeast *Saccharomyces cerevisiae D7* following exposure to ultraviolet radiation. Dose-related reductions in the number of ultraviolet-induced mutants were observed with essential oil of sage, α-β-thujone mixture, 1,8-cineole, and camphor; in contrast, limonene showed no antimutagenic potential in either bacteria or yeast.

The lack of mutagenicity demonstrated by these compounds in the Vuković-Gacić *et al.* (2006) study is consistent with results of NTP bacterial and mammalian cell mutagenicity studies with α-β-thujone, 1,8-cineole, camphor, or limonene (Haworth *et al.*, 1983; Galloway *et al.*, 1987; Anderson *et al.*, 1990; Myhr *et al.*, 1990; NTP, 2010). Furthermore, camphor, 1,8-cineole, citral, and citronellal (all monoterpenes) were reported to be nonmutagenic when tested in *S. typhimurium* strains TA97a, TA98, TA100, and TA102, with and without induced rat liver S9 (Gomes-Carneiro *et al.*, 1998).

α-Fenchone, present in the α-β-thujone mixture at 16%, was examined by the NTP for evidence of bacterial mutagenicity and induction of micronuclei in erythrocytes of rats (NTP, 2010). No evidence of mutagenicity was seen with α-fenchone in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, with or without hamster or rat liver S9 metabolic activation. Results of the *in vivo* rat bone marrow micronucleus test were judged to be equivocal, based on a small increase in micronucleated polychromatic (immature) erythrocytes observed in a single trial at the highest dose administered (2,500 mg/kg body weight per day for 3 days by intraperitoneal injection), which resulted in a significant trend test (P=0.007).

STUDY RATIONALE

α-Thujone was nominated for study by the National Cancer Institute based on the potential for widespread human exposure through consumption of herbs and essential oils containing thujone. Comparative 2-week toxicity studies were conducted with α-thujone and an α,β-thujone mixture. Since the toxicity results were similar between the compounds, with the exception of convulsions/seizures observed following α-thujone administration and because thujone occurs in nature as a mixture of α and β isomers and human exposure is to the mixture, the α,β-thujone mixture was selected for subsequent 3-month and 2-year studies in F344/N rats and B6C3F1 mice. Oral gavage was selected as the route of exposure to mimic human exposure through the consumption of foods, beverages, and herbal medicines.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

α-Thujone

α-Thujone was obtained from Fluka Chemical Corporation (Milwaukee, WI) in one lot (10825/1). Identity and purity analyses were conducted by the analytical chemistry laboratory (Battelle Columbus Operations, Chemistry Support Services, Columbus, OH); the study laboratory (BioReliance Corporation, Rockville, MD) performed identity analyses (Appendix I). Galbraith Laboratories, Inc. (Knoxville, TN), performed Karl Fischer titration and elemental analyses. Reports on analyses performed in support of the α-thujone studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, colorless liquid, was identified as α-thujone by infrared spectroscopy, proton and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and optical rotation analyses. Water content of the bulk chemical was determined by Karl Fisher titration. The purity of lot 10825/1 was determined by elemental analyses and gas chromatography (GC) with flame ionization detection (FID).

Karl Fischer titration indicated an average water content of 0.04%, and elemental analyses for carbon and hydrogen were generally consistent with the theoretical values for α-thujone. GC/FID indicated one major peak and three minor peaks with areas greater than 0.1% of the total peak area. The overall purity of lot 10825/1 was determined to be approximately 99%.

To ensure stability, the bulk chemical was stored at approximately 5° C under a headspace of inert gas in amber glass bottles sealed with Teflon®-lined lids. Analyses of the bulk chemical before and after the 2-week studies were conducted by the study laboratory using GC/FID, and no degradation of the bulk chemical was detected.

α,β-Thujone

α,β-Thujone was obtained from Fluka Chemical Corporation (Milwaukee, WI) in five lots (341637/1 196, 350864/1 897, 288156/1 493, 350864/1 14698, and 431314/1 44801). The analytical chemistry laboratory combined and homogenized lots 341637/1 196, 350864/1 897, 288156/1 493, and 350864/1 14698 and assigned a new lot number (121698) to the resulting mixture. Lot 121698 was used in the 2-week studies conducted by BioReliance Corporation. Lot 431314/1 44801 was homogenized by the analytical chemistry laboratory and reassigned lot number E58/L-2 by the study laboratory (Southern Research Institute, Birmingham, AL) and was used in the 3-month and 2-year studies. Identity and purity analyses were performed by the analytical chemistry laboratory and the study laboratories. Galbraith Laboratories, Inc., performed Karl Fischer titration and elemental analyses. Reports on analyses performed in support of the α,β-thujone studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a light-yellow liquid, was identified as α,β-thujone by infrared (IR) spectroscopy, proton- and ^{13}C-NMR spectroscopy, GC coupled with mass spectrometry (MS), and optical rotation analyses. Water content of the bulk chemical was determined by Karl Fisher titration. Purity was determined by elemental analyses and GC/FID, GC/MS, and GC/IR analyses.

For lot 121698, Karl Fischer titration indicated an average water content of 0.17% and elemental analyses for carbon, hydrogen, and oxygen were generally consistent with theoretical values, with oxygen content slightly higher than expected. GC/FID indicated two major peaks and six impurities greater than 0.1% of the total peak area. The combined results of the purity analyses were determined to be approximately 71% α-thujone, 12% β-thujone, 13% fenchone, 3% camphor, and approximately 1% unidentified impurities. The overall purity was determined to be approximately 83% α,β-thujone, consistent with the manufacturer's Certificate of Analysis.

For lot E58/L-2, Karl Fischer titration indicated an average water content of 0.10% and elemental analyses for carbon, hydrogen, and oxygen were generally consistent with theoretical values, with oxygen content slightly higher than expected. GC/FID indicated two major peaks and five impurities greater than 0.1% of the total peak area. The combined results of the purity analyses were determined to be approximately 70% α-thujone, 11% β-thujone, 16% fenchone, 2% camphor, and 0.5% unidentified impurities. The overall purity

was determined to be approximately 81% α,β-thujone, consistent with the manufacturer's Certificate of Analysis.

To ensure stability, the bulk chemical was stored in amber glass bottles under a headspace of inert gas sealed with Teflon®-lined lids at approximately 5° C. Periodic reanalyses of the bulk chemical were performed by the study laboratories using GC/FID before and after all of the studies and approximately every 6 months during the 2-year studies. No degradation of the bulk chemical was detected.

Methylcellulose

Methylcellulose was obtained in one lot (984735) from Fisher Scientific (Pittsburgh, PA) for the 2-week studies and in one lot (31K0155) from Sigma-Aldrich (St. Louis, MO) for the 3-month and 2-year studies. Aqueous 0.5% methylcellulose was chosen as an alternative to corn oil at a time when it was considered important to minimize oil in the overall animal diet following the change to the NTP-2000 feed. The identity of each lot was confirmed by IR spectroscopy. The average methoxyl contents were 29.13% and 30.0% for lots 984735 and 31K0155, respectively, which are in the acceptable range for methylcellulose.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing α-thujone or α,β-thujone with 0.5% methylcellulose in deionized water. Studies to determine the resuspendability and gavagability of a 30 mg/mL dose formulation, homogeneity of 0.05 and 30 mg/mL dose formulations, and stability of a 0.05 mg/mL dose formulation were performed by the analytical chemistry laboratory using GC/FID; the study laboratories also performed homogeneity studies of 0.1 and 20 mg/mL dose formulations using GC/FID. Dose formulations were confirmed to be resuspendable by vigorous stirring for 10 minutes prior to analysis, and homogeneity and gavagability were confirmed. Stability of the dose formulations was confirmed for at least 47 (α-thujone) or 42 (α,β-thujone) days for dose formulations stored in sealed amber glass bottles at room temperature or 5° C and for at least 3 hours under simulated animal room conditions. The analytical method for formulation analyses required extraction of thujones with ethyl acetate using propiophenone as an internal standard and GC/FID analysis of the extract.

Periodic analyses of the dose formulations of α-thujone and α,β-thujone were conducted by the study laboratories using GC/FID. Throughout the 2-week and 3-month studies, difficulties were experienced by the study laboratory with resuspension of the formulations for analyses, a problem not seen at the analytical chemistry laboratory and apparently due to scale-up to large formulations and use over a dosing period. This issue was particularly noticeable in the analysis of samples from the animal rooms. During the 2-week studies, the dose formulations were analyzed two (mice and α-thujone rats) or three (α,β-thujone rats) times. Of the α-thujone dose formulations analyzed, five of seven for rats and five of seven for mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; one of eight for rats and one of eight for mice were within 10% of the target concentrations. Of the α,β-thujone dose formulations analyzed for the 2-week studies, four of five for rats and all five for mice were within 10% of the target concentrations (Table I4); one of five animal room samples for rats and none of five for mice were within 10% of the target concentrations. Additional formulation and animal room analyses were added during the 3-month studies to help resolve the formulation analysis problem. During the 3-month studies, the dose formulations were analyzed at least monthly; animal room samples were also analyzed (Table I5). Of the dose formulations analyzed, all 20 for rats and all 19 for mice were within 10% of the target concentrations; 11 of 20 animal room samples for rats and none of the 19 animal room samples for mice were within 10% of the target concentrations. A set of special experiments was undertaken prior to the beginning of the 2-year study to resolve the issue of low animal room concentrations. These experiments involved changing parameters such as dosing bottles and stir bar sizes, shaking the formulations and not stirring them, and minimization of air exposure during dosing. Parameters that proved most important were those that minimized adsorption to container walls and losses due to volatility. The use of Teflon® containers and keeping dosing bottles covered as much as possible overcame most of the problem. During the 2-year studies, the dose formulations were analyzed approximately every 8 weeks; animal room samples were also analyzed (Table I6). Of the dose formulations analyzed, all 94 for rats and all 122 for mice were within 10% of the target concentrations; 100 of 114 animal room samples for rats and 110 of 169 animal room samples for mice were within 10% of the target concentrations.

2-WEEK STUDIES

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old.

Rats and mice were quarantined for 11 days and were 5 to 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Groups of five male and five female rats and mice were administered α-thujone or α,β-thujone in 0.5% methylcellulose by gavage at doses of 0, 1, 3, 10, 30, or 100 mg/kg, 5 days per week for 16 (rats) or 17 (mice) days; dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice. Feed and water were available *ad libitum*. Rats and female mice were housed four to five per cage; male mice were housed individually. Clinical findings were recorded daily for rats and mice. The animals were weighed initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus of rats and mice were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on early death animals and on all vehicle control animals, 100 mg/kg α-thujone male and female rats and mice and α,β-thujone male and female rats and mice, 30 mg/kg α-thujone female rats and male mice, and 30 mg/kg α,β-thujone male mice. Microscopic findings were read down to a no-effect level. Table 1 lists the tissues and organs examined.

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to α,β-thujone and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 12 (female rats), 13 (male rats), 14 (male mice), or 15 (female mice) days and were 5 to 6 (rats) or 6 to 7 (mice) weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female vehicle control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were administered α,β-thujone in 0.5% methylcellulose by gavage at doses of 0, 6.25 (mice), 12.5, 25, 50, 75, or 100 (rats) mg/kg, 5 days per week for 14 weeks; dosing volumes were 5 mL/kg (rats) or 10 mL/kg (mice). Additional clinical pathology groups of 10 male and 10 female rats were administered the same doses for 24 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. Core study animals were weighed initially, on day 2 (female mice), on day 3 (male rats and male mice), on day 4 (female rats), weekly thereafter, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with a CO_2/O_2 mixture, and blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 25 and from core study rats and mice at the end of the study for hematology and clinical chemistry (rats). Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Hematology analyses and reticulocyte counts were performed using an ADVIA 120 Hematology System Analyzer (Bayer, Inc., Tarrytown, NY) with reagents supplied by the manufacturer, Fisher Scientific (Norcross, GA), or Sigma Diagnostics (St. Louis, MO). Platelet and erythrocyte morphologies were evaluated using light microscopy. Blood samples for clinical chemistry were placed in tubes containing no anticoagulant. Clinical chemistry analyses were conducted using a Hitachi 911 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) with reagents from the manufacturer or from Sigma Diagnostics. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study animals administered 0, 6.25 (mice), 12.5, 25, or 50 (rats) mg/kg. Animals from the top two dose groups (75 and 100 mg/kg) were not selected for these evaluations because of excessive mortality. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for

sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus of core study rats and mice were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control, 50 (female), 75, and 100 mg/kg rats and vehicle control, 25, 50, and 75 mg/kg mice. The adrenal cortex of rats and male mice; the brain, kidney, and stomach of rats; the ovary of female rats and mice; and the uterus of female mice were examined to a no-effect level. The spinal cord, sciatic nerve, and muscle were examined in vehicle control rats and mice, 100 mg/kg rats, and 75 mg/kg mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats were administered α,β-thujone in 0.5% methylcellulose by gavage at doses of 0, 12.5, 25, or 50 mg/kg, 5 days per week for up to 105 weeks. Groups of 50 male and 50 female mice were administered α,β-thujone in 0.5% methylcellulose by gavage at doses of 0, 3, 6, 12, or 25 mg/kg, 5 days per week for up to 105 weeks. Dosing volumes were 5 mL/kg (rats) or 10 mL/kg (mice).

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. The animals were quarantined for 11 (rats) or 12 (mice) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Male rats were housed three per cage; female rats and mice were housed five per cage, and male mice were housed individually. Feed and water were available *ad libitum*. Cages were changed twice weekly (rats and female mice) or weekly (male mice). Racks were changed every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks. Animals were weighed initially, on day 4 (males), on day 5 (females), then weekly for 13 weeks, every 4 weeks thereafter until the end of the studies (mice) or through day 648 (male rats) or day 649 (female rats), then every 2 weeks (rats), and at the end of the studies (rats and mice).

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified; and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the adrenal medulla and preputial gland of male rats, the Harderian gland of male and female mice, and the spleen of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of α-Thujone and α,β-Thujone

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
BioReliance Corporation (Rockville, MD)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
11 days	Rats: 12 (females) or 13 (males) days Mice: 14 (males) or 15 (females) days	Rats: 11 Mice: 12
Age When Studies Began		
5-6 weeks	Rats: 5-6 weeks Mice: 6-7 weeks	5-6 weeks
Date of First Dose		
April 23, 2001 (α-thujone) May 7, 2001 (α,β-thujone)	Rats: August 12 (females) or 13 (males), 2002 Mice: August 14 (males) or 15 (females), 2002	Rats: June 9, 2003 Mice: June 30, 2003
Duration of Dosing		
5 days/week for 16 (rats) or 17 (mice) days	5 days/week for 14 weeks	5 days/week for 104 to 105 weeks
Date of Last Dose		
May 8 (rats) or 9 (mice), 2001 (α-thujone); May 22 (rats) or 23 (mice), 2001 (α,β-thujone)	Rats: November 11 (females) or 12 (males), 2002 Mice: November 13 (males) or 14 (females), 2002	Rats: June 5-7, 2005 Mice: June 26-30, 2005
Necropsy Dates		
May 9 (rats) or 10 (mice), 2001 (α-thujone); May 23 (rats) or 24 (mice), 2001 (α,β-thujone)	Rats: November 12 (females) or 13 (males), 2002 Mice: November 14 (males) or 15 (females), 2002	Rats: June 6-8, 2005 Mice: June 27-July 1, 2005
Age at Necropsy		
8-9 weeks	19-20 weeks	109-111 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	50 males and 50 females

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of α-Thujone and α,β-Thujone

2-Week Studies	3-Month Studies	2-Year Studies
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage Rats: 4 to 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 2-week studies, except wafer form	Same as 3-month studies
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant, Washington, DC) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Tap water (Birmingham, AL, municipal water supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice weekly for rats and female mice or weekly for male mice	Same as 2-week studies, except Maywood, NJ	Same as 3-month studies
Bedding Irradiated heat-treated Sani-Chip Hardwood (P.J. Murphy Forest Products, Montville, NJ), changed twice weekly for rats and female mice or weekly for male mice	Same as 2-week studies	Same as 2-week studies
Rack Filters Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks	Remay spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Same as 3-month studies
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 2-week studies, except Maywood, NJ	Same as 3-month studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of α-Thujone and α,β-Thujone

2-Week Studies	3-Month Studies	2-Year Studies
Doses		
0, 1, 3, 10, 30, or 100 mg/kg in 0.5% methylcellulose (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice)	Rats: 0, 12.5, 25, 50, 75, or 100 mg/kg in 0.5% methylcellulose (dosing volume 5 mL/kg) Mice: 0, 6.25, 12.5, 25, 50, or 75 mg/kg in 0.5% methylcellulose (dosing volume 10 mL/kg)	Rats: 0, 12.5, 25, or 50 mg/kg in 0.5% methylcellulose (dosing volume 5 mL/kg) Mice: 0, 3, 6, 12, or 25 mg/kg in 0.5% methylcellulose (dosing volume 10 mL/kg)
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; core study animals were weighed initially, on day 2 (female mice), on day 3 (male rats and male mice), on day 4 (female rats), weekly thereafter, and at the end of the studies; clinical findings for core study animals were recorded weekly.	Observed twice daily; animals were weighed initially, on day 4 (males), on day 5 (females), then weekly for 13 weeks, every 4 weeks thereafter until the end of the studies (mice) or through day 648 (male rats) or day 649 (female rats), then every 2 weeks (rats), and at the end of the studies (rats and mice) Clinical findings were recorded every 4 weeks.
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed.	Necropsies were performed on the core study animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of special study rats on days 4 and 25 and from core study animals at the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, platelet, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; and large unstained cell concentration ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of α-Thujone and α,β-Thujone

2-Week Studies	3-Month Studies	2-Year Studies
Histopathology Complete histopathology was performed on 0 and 100 mg/kg α-thujone male and female rats and mice and α,β-thujone male and female rats and mice, 30 mg/kg α-thujone female rats and male mice, and 30 mg/kg α,β-thujone male mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung with mainstem bronchus, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, sciatic nerve, skin, spinal cord, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on 0, 50 (female), 75, and 100 mg/kg core study rats and 0, 25, 50, and 75 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung with mainstem bronchus, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the spinal cord, sciatic nerve, and muscle were examined in vehicle control rats and mice, 100 mg/kg rats, 75 mg/kg mice, and all animals that died early. Target tissues were examined to a no-effect level in the remaining dose groups.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung with mainstem bronchus, lymph nodes (mandibular, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology None	At the end of the core studies, spermatid and sperm samples were collected from male rats in the 0, 12.5, 25, and 50 mg/kg groups and from male mice in the 0, 6.25, 12.5, and 25 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats administered 0, 12.5, 25, or 50 mg/kg and from female mice administered 0, 6.25, 12.5, or 25 mg/kg.	None

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 found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B3, C1, C3, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology and clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values or statistical outliers were eliminated

from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the four stages: proestrus, estrus, metestrus, and diestrus, with provisions for extended stays within each stage or for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of α-thujone and α,β-thujone was assessed by testing the ability of the chemicals to induce mutations in various bacterial tester strains; the ability of α,β-thujone to induce increases in the frequency of micronucleated erythrocytes in mouse peripheral blood was also tested. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and

chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

2-WEEK STUDY OF α-THUJONE

All male rats survived to the end of the study; three 100 mg/kg female rats died before the end of the study (Table 2). Final mean body weights and body weight gains of dosed rats were similar to those of the vehicle controls. There were no significant differences in organ weights of dosed males compared to the vehicle weights of dosed males compared to the vehicle controls (Table G1). The absolute and relative thymus weights of 100 mg/kg females were significantly less than those of the vehicle controls. Clinical findings included convulsions/seizures in three 100 mg/kg females on day 15. No gross or histologic findings were attributed to administration of α-thujone.

TABLE 2
Survival and Body Weights of Rats in the 2-Week Gavage Study of α-Thujone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	99 ± 2	177 ± 4	78 ± 3	
1	5/5	99 ± 3	177 ± 4	78 ± 2	100
3	5/5	91 ± 2	160 ± 3	69 ± 2	90
10	5/5	96 ± 3	171 ± 7	76 ± 5	97
30	5/5	87 ± 2*	171 ± 4	84 ± 3	96
100	5/5	95 ± 5	168 ± 6	73 ± 3	95
Female					
0	5/5	85 ± 3	123 ± 3	39 ± 2	
1	5/5	81 ± 3	122 ± 3	41 ± 2	99
3	5/5	85 ± 4	124 ± 2	39 ± 2	100
10	5/5	79 ± 2	120 ± 3	41 ± 2	97
30	5/5	86 ± 3	124 ± 3	38 ± 1	100
100	2/5[c]	85 ± 4	125 ± 4	32 ± 3	101

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 17 days/number initially in group

[c] Days of death: 8, 8, 15

2-WEEK STUDY OF α,β-THUJONE

One 100 mg/kg male rat died before the end of the study; all other rats survived to the end of the study (Table 3). Final mean body weights and body weight gains of 10 mg/kg male rats were significantly less than those of the vehicle control group. There were no treatment-related changes in organ weights; significant organ weight changes in 10 mg/kg males were associated with decreased body weights (Table G2). One male rat in the 10 mg/kg group was noted as thin on day 17; there were no other clinical findings. No convulsions/seizures were observed in animals administered α,β-thujone. No gross or histologic findings were attributed to α,β-thujone administration.

Dose Selection Rationale: Because thujone occurs in nature as a mixture and human exposure is to the mixture, the α,β-thujone mixture was selected for the 3-month gavage study rather than α-thujone. Based on the minimal mortality observed at 100 mg/kg and the lack of toxicity at 30 mg/kg in the 2-week study of α,β-thujone, α,β-thujone doses selected for the 3-month gavage study in rats were 12.5, 25, 50, 75, and 100 mg/kg.

TABLE 3
Survival and Body Weights of Rats in the 2-Week Gavage Study of α,β-Thujone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	105 ± 3	174 ± 6	70 ± 3	
1	5/5	109 ± 4	180 ± 6	72 ± 3	104
3	5/5	99 ± 5	172 ± 8	74 ± 7	99
10	5/5	106 ± 5	141 ± 5**	35 ± 6**	81
30	5/5	100 ± 3	168 ± 6	68 ± 4	96
100	4/5[c]	107 ± 6	170 ± 4	68 ± 6	98
Female					
0	5/5	100 ± 3	134 ± 3	33 ± 1	
1	5/5	93 ± 5	128 ± 3	34 ± 2	96
3	5/5	99 ± 3	131 ± 3	32 ± 1	98
10	5/5	93 ± 3	133 ± 2	40 ± 3	99
30	5/5	96 ± 4	130 ± 5	34 ± 2	97
100	5/5	97 ± 4	130 ± 5	33 ± 3	97

** Significantly different (P≤0.01) from the vehicle control group by Dunnett's test

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 17 days/number initially in group

[c] Day of death: 10

3-MONTH STUDY OF α,β-THUJONE

Two male and eight female 75 mg/kg rats and eight male and nine female 100 mg/kg rats died before the end of the study (Table 4). The final mean body weights and body weight gains of 50 and 75 mg/kg females were significantly greater than those of the vehicle controls (Table 4 and Figure 2). Seizures were observed in all male rats administered 75 or 100 mg/kg and in three males administered 50 mg/kg. In the male rats, seizures occurred at weeks 9, 13, and 14 in the 50 mg/kg dose group and during weeks 4 to 14 in the 75 and 100 mg/kg groups. Seizures were observed in six, 10, and nine females administered 50, 75, or 100 mg/kg, respectively, and in one female rat administered 25 mg/kg. Seizures occurred during week 9 in the 25 mg/kg group and during weeks 5 to 13 in the 50 mg/kg or greater groups. All but one of the early deaths occurred in animals that had previously been observed to have seizures.

TABLE 4
Survival and Body Weights of Rats in the 3-Month Gavage Study of α,β-Thujone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	117 ± 2	339 ± 13	222 ± 12	
12.5	10/10	117 ± 1	347 ± 2	230 ± 3	102
25	10/10	116 ± 2	344 ± 4	228 ± 4	101
50	10/10	117 ± 1	339 ± 4	222 ± 3	100
75	8/10[c]	118 ± 1	338 ± 7	220 ± 7	100
100	2/10[d]	117 ± 1	323 ± 5	208 ± 4	95
Female					
0	10/10	95 ± 1	186 ± 3	91 ± 3	
12.5	10/10	94 ± 1	184 ± 3	90 ± 2	99
25	10/10	95 ± 1	183 ± 3	89 ± 3	99
50	10/10	94 ± 1	209 ± 4**	115 ± 4**	112
75	2/10[e]	95 ± 1	218 ± 3**	124 ± 0**	117
100	1/10[f]	94 ± 1	233	133	125

** Significantly different (P≤0.01) from the vehicle control group by Williams's test

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 3 months/number initially in group

[c] Weeks of death: 8, 9

[d] Weeks of death: 8, 11, 12, 12, 12, 13, 13, 14

[e] Weeks of death: 7, 7, 7, 7, 9, 9, 10, 13

[f] Weeks of death: 3, 5, 6, 6, 6, 12, 12, 13, 13

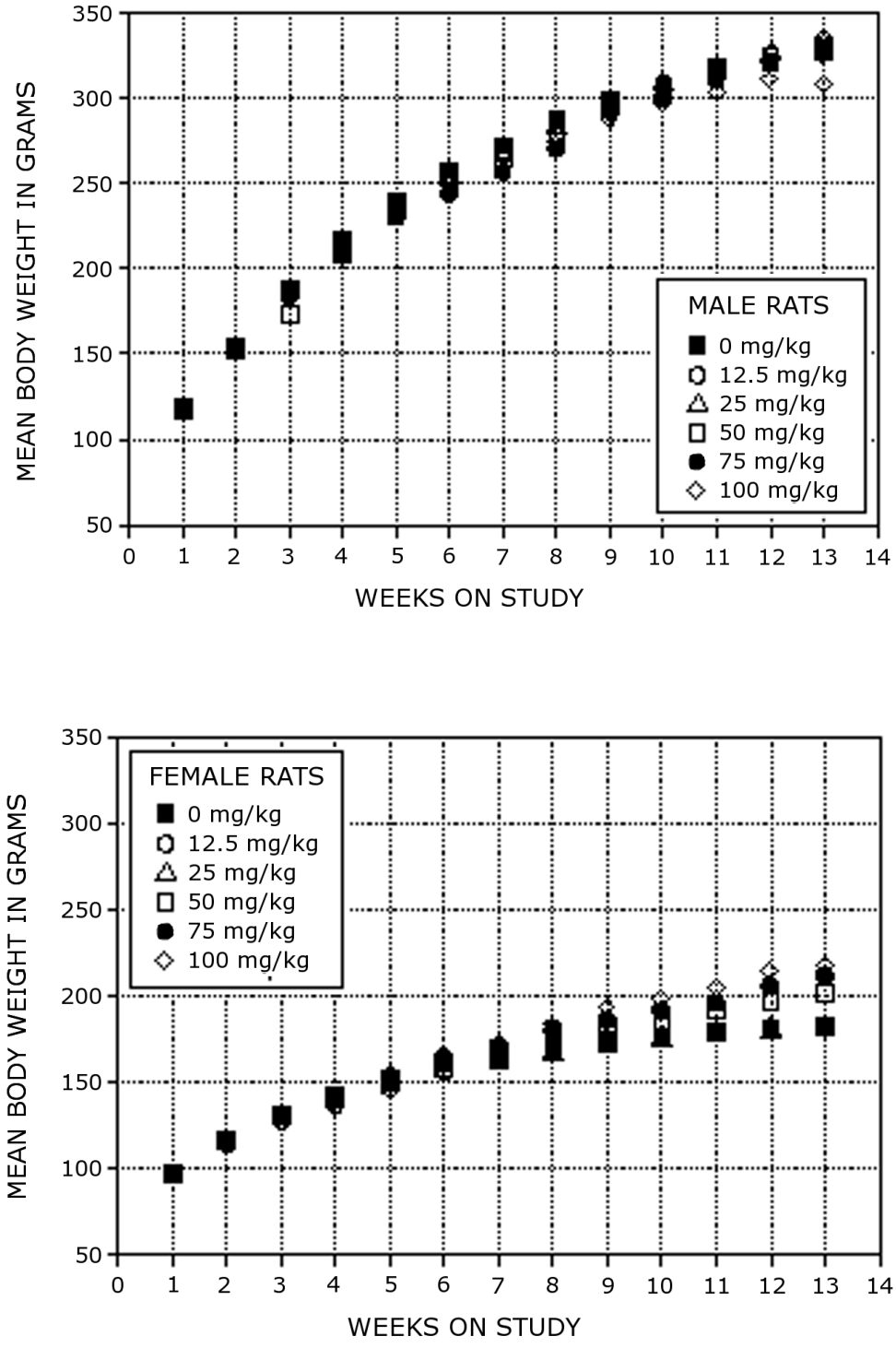


FIGURE 2
Growth Curves for Rats Administered α,β-Thujone by Gavage for 3 Months

Hematology and clinical chemistry data for the 3-month rat study are presented in Table F1. Differences in hematology and clinical chemistry parameters were not considered to be related to administration of α,β-thujone.

Absolute and relative thymus weights were significantly decreased in 75 and 100 mg/kg males; absolute and relative liver weights were significantly increased in 50 and 75 mg/kg females (Table G3).

There were no significant differences in sperm parameters of male rats administered 12.5, 25, or 50 mg/kg α,β-thujone when compared to the vehicle controls (Table H1). There were no significant differences in the proportion of regular cycling females or in the average length of estrous cycle for female rats administered 12.5, 25, or 50 mg/kg α,β-thujone when compared to the vehicle controls (Table H2). However, when female rats were tested for estrous cycle transitions, those in the highest dose group (50 mg/kg) were found to be more likely to remain in extended diestrus than controls, although they were slightly less likely to transition into diestrus (Table H2).

Incidences of congestion and/or hemorrhage of the brain were increased in 75 mg/kg females and 100 mg/kg males and females (Table 5). The incidences of pigmentation in the brain were also increased in 50 mg/kg or greater females. Hemorrhage was most prominent in the meninges and third ventricle (Plates 1 and 2). Golden brown pigment, consistent with hemosiderin, was present within macrophages in and around the choroid plexus in the third ventricle (Plate 3).

Changes in the pituitary gland, including atrophy of the pars distalis and dilatation of Rathke's cleft, were also increased with α,β-thujone administration in female rats (Table 5). Rathke's cleft dilatation was characterized by a widening of Rathke's cleft and filling of the space with eosinophilic proteinaceous material (Plate 4). Atrophy of the pars distalis occurred as a band of stromal collapse subjacent to the third ventricle.

Congestion, hemorrhage, and/or edema were observed in several other organs of rats that died before the end of the study. In females, these included edema and hemorrhage in the glandular stomach, congestion in the lung, and hemorrhage in the thymus (Table 5). Congestion in the lung and spleen and hemorrhage in the thymus were observed in males. The cause of the congestion and hemorrhage is unknown; congestion may simply be a result of the early death animals not being exsanguinated, or it may have been secondary to repeated seizures.

The incidences of renal tubule mineralization were significantly increased in all groups of females administered α,β-thujone, and the incidences of nephropathy were slightly increased in females administered 50 mg/kg or greater (Table 5). Mineralization was present in the corticomedullary region as basophilic amorphous material within the tubules.

Dose Selection Rationale: Based on decreased survival of 75 and 100 mg/kg rats in the 3-month study, α,β-thujone doses selected for the 2-year gavage study in rats were 12.5, 25, and 50 mg/kg. The kidney lesions observed in the 50 mg/kg females were not considered dose limiting. Although infrequent seizures were observed in a few males and females administered 50 mg/kg and one female administered 25 mg/kg, they were not expected to adversely affect survival in a 2-year study.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Male						
Brain[a]	10	10	10	10	10	10
Congestion[b]	0	0	0	0	2 (2.0)[c]	6** (1.5)
Hemorrhage	0	0	0	0	2 (2.0)	4* (1.5)
Pituitary Gland	10	0	10	10	10	10
Pars Distalis, Atrophy	0		0	0	1 (2.0)	2 (2.5)
Rathke's Cleft, Dilatation	1 (1.0)		0	1 (1.0)	3 (1.0)	4 (1.8)
Lung	10	0	0	0	10	10
Congestion	0				1 (2.0)	8** (1.6)
Spleen	10	0	0	0	10	10
Congestion	0				0	4* (2.3)
Thymus	10	0	0	0	10	10
Hemorrhage	1 (2.0)				4 (1.3)	6* (1.8)
Female						
Brain	10	10	10	10	10	10
Congestion	0	0	0	0	3 (1.7)	8** (1.5)
Hemorrhage	0	0	0	0	4* (1.5)	3 (2.0)
Pigmentation	0	0	1 (1.0)	6** (1.2)	5* (1.0)	4* (1.3
Pituitary Gland	10	10	10	10	10	10
Pars Distalis, Atrophy	0	0	0	0	3 (2.7)	3 (3.0)
Rathke's Cleft, Dilatation	0	0	1 (1.0)	5* (1.0)	4* (1.8)	5* (2.0)
Glandular Stomach	10	10	10	10	10	10
Edema	0	0	0	0	1 (2.0)	3 (2.0)
Hemorrhage	0	0	0	0	0	2 (1.0)
Lung	10	0	0	10	10	10
Congestion	0			0	2 (2.0)	4* (1.8)
Thymus	10	0	0	10	10	10
Hemorrhage	0			0	4* (1.0)	4* (1.5)
Kidney	10	10	10	10	10	10
Renal Tubule, Mineralization	3 (1.0)	8* (1.0)	8* (1.0)	8* (1.0)	10** (1.0)	9** (1.0)
Nephropathy	0	0	1 (1.0)	3 (2.0)	2 (1.0)	3 (1.0)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2-YEAR STUDY OF α,β-THUJONE

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 3). All of the 50 mg/kg male and female rats died before the end of the study, and survival of 25 mg/kg males and females was significantly less than that of the respective vehicle control group. Most of the early deaths occurred in rats that had previously been observed to have seizures.

TABLE 6
Survival of Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	2	0	1	0
Moribund	14	21	13	1
Natural deaths	7	4	19	49
Animals surviving to study termination	27[e]	25	17	0
Percent probability of survival at end of study[b]	56	50	35	0
Mean survival (days)[c]	662	681	622	351
Survival analysis[d]	P=0.019	P=0.754	P=0.027	P<0.001
Female				
Animals initially in study	50	50	50	50
Moribund	6	9	10	5
Natural deaths	9	8	21	45
Animals surviving to study termination	35	33	19	0
Percent probability of survival at end of study	70	66	38	0
Mean survival (days)	689	669	601	131
Survival analysis	P=0.001	P=0.770	P=0.001	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dose group columns. The 50 mg/kg group was excluded from the trend test due to 100% mortality.
[e] Includes one animal that died during the last week of the study

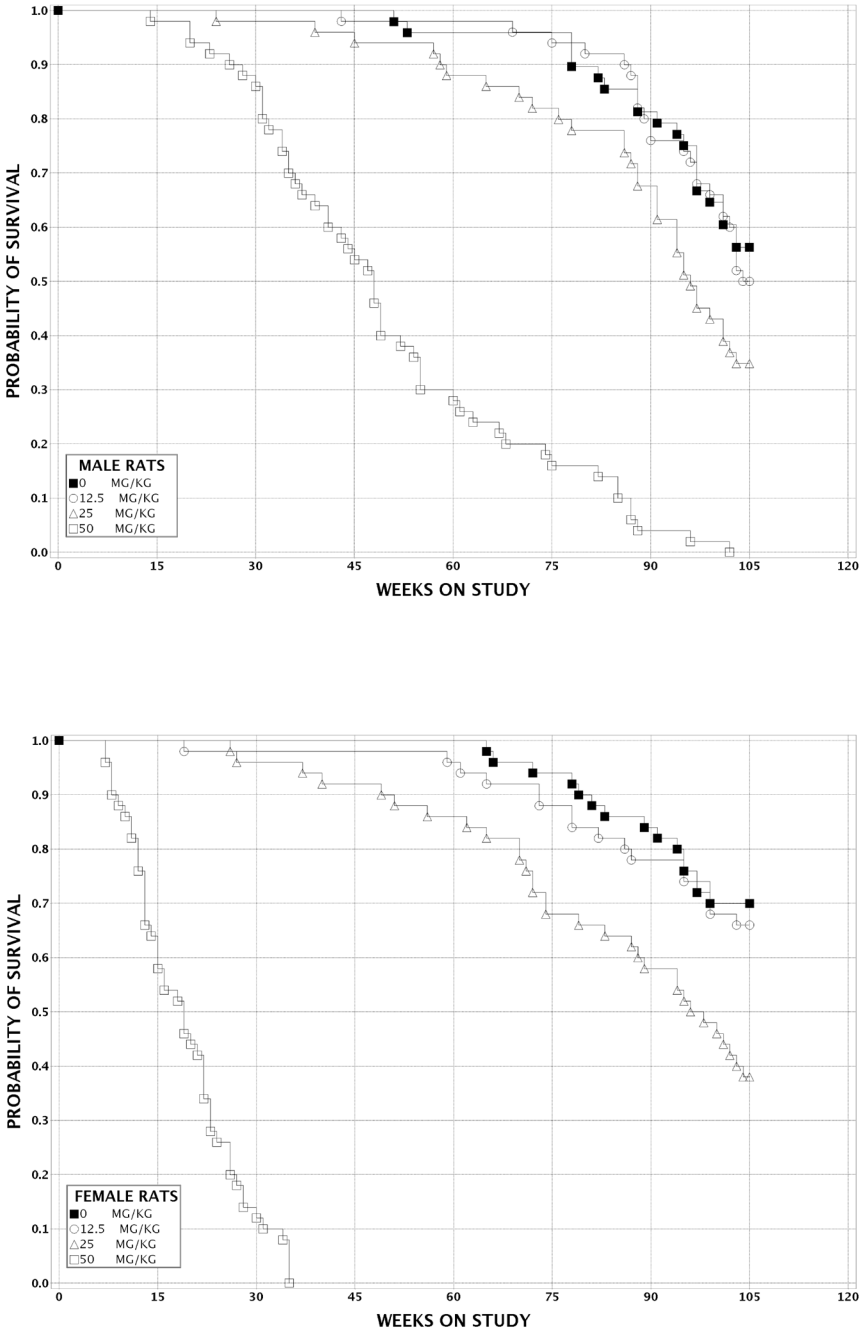


FIGURE 3
Kaplan-Meier Survival Curves for Rats Administered α,β-Thujone by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of all dosed groups were generally within 10% of those of the vehicle control groups throughout the study (Tables 7 and 8; Figure 4). Seizures occurred in all 50 mg/kg rats and most 25 mg/kg rats (43/50 males, 47/50 females). Fewer 12.5 mg/kg rats had seizures (5/50 males, 3/50 females). Both the incidences and the time of appearance of the first seizure were dose related in male and female rats. Seizures first occurred on days 694, 612, 109, and 73 in male rats in the vehicle control, 12.5, 25, and 50 mg/kg groups, respectively. In female rats, seizures first occurred on days 304, 408, 47, and 21 for the vehicle control, 12.5, 25, and 50 mg/kg groups, respectively. Seizures in one vehicle control male rat and one vehicle control female rat were considered incidental; similar seizures have been observed in a low number of control F344/N rats in other NTP studies.

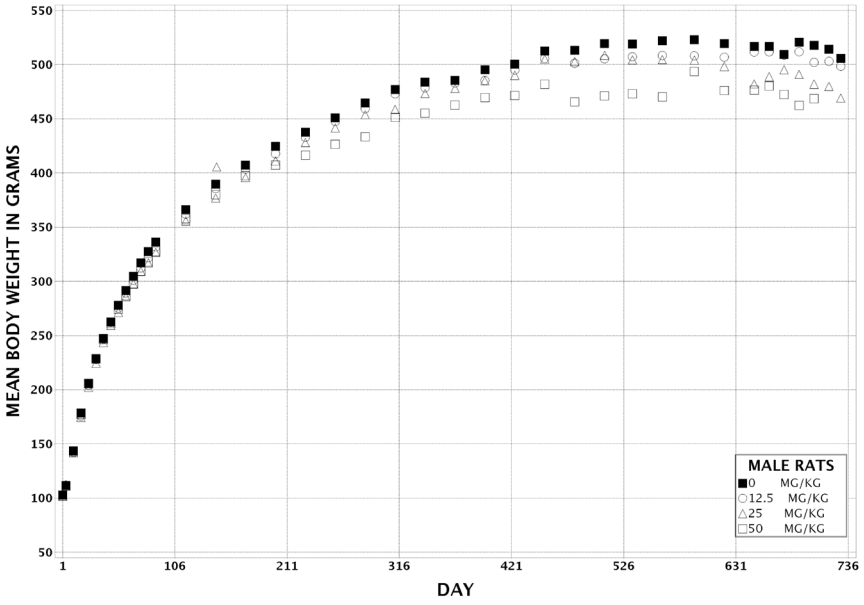
TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control		12.5 mg/kg			25 mg/kg			50 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	103	50	103	100	50	102	99	50	102	100	50
4	112	50	112	100	50	111	99	50	112	100	50
11	144	50	142	99	50	142	99	50	144	100	50
18	179	50	176	99	50	175	98	50	177	99	50
25	206	50	204	99	50	202	98	50	206	100	50
32	229	50	228	100	50	225	98	50	228	100	50
39	247	50	247	100	50	244	99	50	247	100	50
46	263	50	262	100	50	259	99	50	261	99	50
53	278	50	275	99	50	272	98	50	275	99	50
60	292	50	289	99	50	286	98	50	287	98	50
67	305	50	301	99	50	298	98	50	298	98	50
74	317	50	313	99	50	309	98	50	310	98	50
81	327	50	322	98	50	317	97	50	318	97	50
88	336	50	332	99	50	327	97	50	328	98	50
116	366	50	362	99	50	356	97	50	358	98	49
144	390	50	386	99	50	377	97	47[a]	380	98	47
145						406		3[a]			
172	407	49	404	99	50	396	97	49	398	98	46
200	425	49	418	98	50	411	97	49	407	96	44
228	438	49	433	99	50	428	98	49	417	95	39
256	451	49	448	99	50	442	98	49	427	95	34
284	465	49	459	99	50	454	98	48	433	93	30
312	477	49	473	99	49	459	96	48	451	95	27
340	484	49	479	99	49	473	98	47	455	94	20
368	486	47	483	100	49	478	99	47	463	95	19
396	495	46	485	98	49	485	98	46	470	95	15
424	501	46	495	99	49	490	98	44	471	94	13
452	513	46	506	99	49	505	99	43	482	94	12
480	513	46	501	98	48	503	98	43	466	91	10
508	519	46	505	97	48	509	98	40	471	91	10
534	519	46	507	98	47	505	97	39	473	91	8
562	522	43	509	97	46	505	97	38	470	90	8
592	523	41	508	97	46	504	96	38	494	94	5
620	520	39	507	98	40	498	96	33	476	92	2
648	517	38	512	99	38	482	93	30	477	92	2
662	517	36	512	99	37	489	95	26	480	93	2
676	509	35	509	100	36	495	97	24	472	93	1
690	521	31	512	98	33	491	94	21	463	89	1
704	518	29	502	97	31	482	93	20	469	91	1
718	514	27	503	98	26	480	93	17			
Mean for weeks											
1-13	238		236	99		234	98		235	99	
14-52	434		429	99		420	97		414	95	
53-103	513		504	98		494	96		473	92	

[a] The number of animals weighed this day was less than the number of animals surviving.

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control		12.5 mg/kg			25 mg/kg			50 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	91	50	91	99	50	90	98	50	92	100	50
5	100	50	99	100	50	98	98	50	100	100	50
12	115	50	116	101	50	113	99	50	116	101	50
19	131	50	131	101	50	129	99	50	131	100	50
26	143	50	142	99	50	140	98	50	141	99	50
33	152	50	150	99	50	149	98	50	151	99	50
40	161	50	158	98	50	156	97	50	159	99	50
47	167	50	165	99	50	163	98	50	167	100	48
54	172	50	170	99	50	168	98	50	171	100	45
61	177	50	175	99	50	173	98	50	175	99	44
68	180	50	178	99	50	176	97	50	181	100	43
75	186	50	184	99	50	181	97	50	185	99	41
82	188	50	186	99	50	181	97	50	190	101	38
89	192	50	188	98	50	185	97	50	193	101	33
117	202	50	197	97	50	195	96	50	212	105	27
145	208	50	204	98	49	202	97	50	232	112	21
173	215	50	213	99	49	210	98	50	242	113	13
201	222	50	219	99	49	215	97	48	247	111	7
229	230	50	229	99	49	221	96	48	257	112	5
257	237	50	235	99	49	227	96	47			
285	245	50	242	99	49	233	95	46			
313	253	50	250	99	49	241	95	46			
341	261	50	259	100	49	245	94	45			
369	267	50	265	99	49	250	94	44			
397	281	50	275	98	49	262	93	43			
425	290	50	286	99	47	271	94	43			
453	306	49	304	100	46	288	94	41			
481	302	48	307	102	46	288	95	41			
509	310	47	312	101	44	294	95	36			
535	316	47	320	101	44	301	95	34			
563	320	45	324	101	42	305	95	33			
593	325	43	330	102	41	310	95	32			
621	325	43	329	101	39	312	96	29			
649	324	41	330	102	39	315	97	29			
663	328	38	333	102	37	320	97	26			
677	330	36	331	100	37	319	97	25			
691	335	35	339	101	34	318	95	24			
705	332	35	341	103	34	314	95	22			
719	335	35	343	102	34	317	95	20			
Mean for weeks											
1-13	154		152	99		150	97		154	100	
14-52	230		228	99		221	96		238	103	
53-103	314		317	101		299	95				



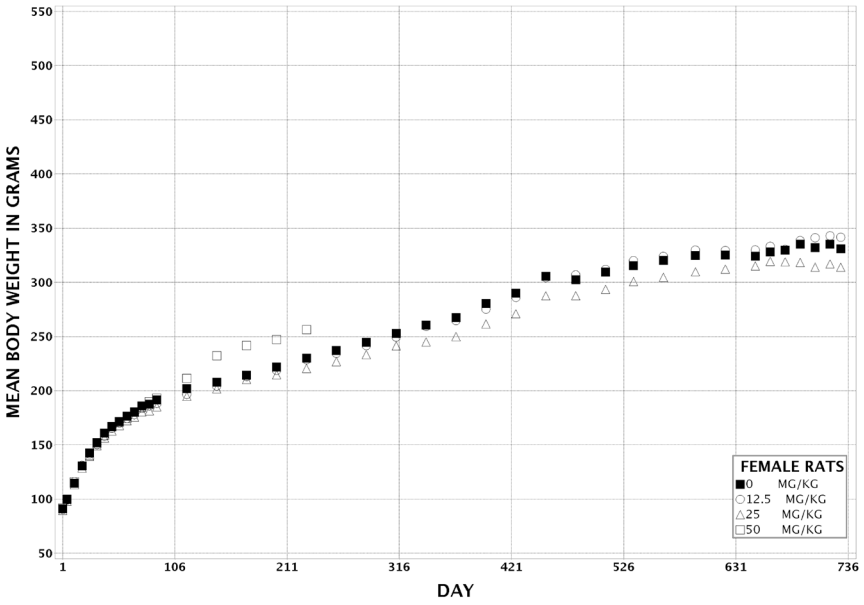


FIGURE 4
Growth Curves for Rats Administered α,β-Thujone by Gavage for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the preputial gland, adrenal gland, and testes and nonneoplastic lesions of the brain, pituitary gland, spleen, kidney, and lung. Due to the early mortality in 50 mg/kg rats, neoplasm data from these groups are not presented in this section. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Preputial Gland: Incidences of preputial gland carcinoma and adenoma or carcinoma (combined) in male rats occurred with positive trends, and the combined incidence in 25 mg/kg males was significantly greater than that in the vehicle control group (Tables 9, A1, and A2). The incidences of

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Preputial Gland in Male Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg
Number Examined Microscopically	49	49	50
Hyperplasia[b]	2 (2.5)[c]	0	1 (3.0)
Adenoma	2	1	4
Carcinoma[d]			
Overall rate[e]	1/49 (2%)	0/49 (0%)	5/50 (10%)
Adjusted rate[f]	2.5%	0.0%	13.9%
Terminal rate[g]	1/27 (4%)	0/24 (0%)	3/17 (18%)
First incidence (days)	729 (T)	—[i]	404
Poly-3 test[h]	P=0.033	P=0.492N	P=0.079
Adenoma or Carcinoma[j]			
Overall rate	3/49 (6%)	1/49 (2%)	9/50 (18%)
Adjusted rate	7.5%	2.4%	24.7%
Terminal rate	2/27 (7%)	0/24 (0%)	5/17 (29%)
First incidence (days)	615	599	404
Poly-3 test	P=0.018	P=0.290N	P=0.037

(T) Terminal sacrifice

[a] Data for the 50 mg/kg group are not presented due to 100% mortality; the incidences are included in Tables A1 and A4.

[b] Number of animals with lesion

[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

[d] Historical incidence for 2-year gavage studies with methylcellulose control groups (mean ± standard deviation): 1/99 (1.0% ± 1.4%), range 0%-2%; all routes: 19/1,295 (1.5% ± 1.7%), range 0%-4%

[e] Number of neoplasm-bearing animals/number of animals with preputial gland examined microscopically.

[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[g] Observed incidence at terminal kill

[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by **N**.

[i] Not applicable; no neoplasms in animal group

[j] Historical incidence for methylcellulose gavage studies: 6/99 (6.1% ± 0.1%), range 6%; all routes: 68/1,295 (5.2% ± 3.7%), range 0%-12%

carcinoma and adenoma or carcinoma (combined) in the 25 mg/kg group exceeded the historical control ranges for methylcellulose gavage studies and for all routes of study (Tables 9 and A3a). The increase in the incidences of preputial gland neoplasms was not dose related, and the incidences of hyperplasia (Tables 9 and A4) were not increased. Preputial gland carcinoma consisted of proliferation of glandular and/or squamous epithelial tissue that expanded the gland and invaded the surrounding connective tissue. Necrosis and inflammation were common in, but not diagnostic of, carcinoma. Adenoma lacked the local invasiveness that carcinoma displayed and tended to be smaller and better demarcated.

Adrenal Medulla: The incidence of benign pheochromocytoma was significantly increased in 25 mg/kg male rats compared to that in the vehicle control group and exceeded the historical control ranges for methylcellulose gavage studies and all study routes (Tables 10, A1, A2, and A3b). Incidences of hyperplasia were not dose related (Tables 10 and A4). Benign pheochromocytoma was a discrete lesion that in some cases was confined to the medulla; but in other cases, the lesion extended from the medulla into the cortex, causing compression of the surrounding parenchyma. The cells were often smaller within the pheochromocytoma than in the normal medulla and were arranged in nests and packets. Individual cells had oval nuclei with stippled chromatin and distinct nucleoli. Cytoplasm was granular and had a dull eosinophilic to slightly basophilic color. Malignant pheochromocytoma occurred in one 25 mg/kg male (Tables 10 and A1). The malignant pheochromocytoma was similar to benign pheochromocytoma, except the cells extended throughout the cortex and, in one area, extended through the capsule of the adrenal gland.

Testes: The incidence of interstitial cell adenoma of the testes was significantly greater in 25 mg/kg male rats than in the vehicle controls (vehicle control, 35/50; 12.5 mg/kg, 44/50; 25 mg/kg, 40/50); however, the incidence was within the historical control ranges for methylcellulose gavage studies (70% to 84%) and for all study routes (54% to 98%) (Tables A1, A2, and A3c). Although the increased incidence of interstitial cell adenoma in 12.5 mg/kg males was not statistically significant in the current study, the incidence exceeds the historical control range for methylcellulose gavage studies. Interstitial cell adenoma was typical of those seen in F344/N rats and was characterized by aggregates of interstitial cells that caused distortion of the normal architecture and compression and atrophy of the seminiferous tubules. The cells had abundant, finely vacuolated cytoplasm and round central nuclei with prominent nucleoli. In some adenomas, areas of smaller, spindle-shaped cells with oval, basophilic nuclei were present between large areas of interstitial cells. Interstitial cell adenomas were not considered related to α,β-thujone administration.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Medulla in Male Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg
Number Examined Microscopically	50	50	49
Hyperplasia[b]	18 (1.8)[c]	25 (1.9)	13 (2.2)
Bilateral Benign Pheochromocytoma	1	2	2
Benign Pheochromocytoma (includes bilateral)[d]			
Overall rate[e]	6/50 (12%)	8/50 (16%)	12/49 (24%)
Adjusted rate[f]	14.8%	18.5%	33.5%
Terminal rate[g]	5/27 (19%)	5/25 (20%)	6/17 (35%)
First incidence (days)	716	603	652
Poly-3 test[h]	P=0.038	P=0.435	P=0.045
Malignant Pheochromocytoma[i]	0	0	1
Benign or Malignant Pheochromocytoma[j]			
Overall rate	6/50 (12%)	8/50 (16%)	13/49 (27%)
Adjusted rate	14.8%	18.5%	36.3%
Terminal rate	5/27 (19%)	5/25 (20%)	7/17 (41%)
First incidence (days)	716	603	652
Poly-3 test	P=0.021	P=0.435	P=0.025

[a] Data for the 50 mg/kg group are not presented due to 100% mortality; the incidences are included in Tables A1 and A4.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year gavage studies with methylcellulose control groups (mean ± standard deviation): 13/100 (13.0% ± 1.4%), range 12%-14%; all routes: 183/1,295 (14.1% ± 4.5%), range 6%-22%
[e] Number of neoplasm-bearing animals/number of animals with adrenal gland examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[i] Historical incidence for methylcellulose gavage studies: 0/100; all routes: 24/1,295 (1.9% ± 2.4%), range 0%-8%
[j] Historical incidence for methylcellulose gavage studies: 14/100 (14.0% ± 2.8%), range 12%-16%; all routes: 208/1,295 (16.1% ± 5.5%), range 6%-26%

Brain: Most early deaths of 25 and 50 mg/kg rats occurred in animals that had previously been observed to have seizures. A review of the brain revealed the presence of pigment-laden macrophages in and around the choroid plexus in the third ventricle in female rats that were considered to be related to α,β-thujone administration. The pigment was consistent with hemosiderin. More in-depth pathology review of the brains of the vehicle control, 25, and 50 mg/kg rats revealed several additional lesions in dosed animals (Tables 11, A4, and B3). These lesions included necrosis in three 50 mg/kg males and in one male and three female 25 mg/kg rats. Neuronal necrosis was not recorded or described in the necrotic lesions of the brain; however, where there was brain necrosis of a significant degree, neuronal loss was present. This was especially true in animals in which the lesions were described as "cavitation and neuronal loss." This does not imply a specific targeting of the neurons, but rather a widespread area of necrosis affecting the neurons as well as other types of cells. Pigmentation occurred in one male and three female 12.5 mg/kg rats, five female 25 mg/kg rats, and three male and 19 female 50 mg/kg rats. Mineralization occurred in one male and one female 25 mg/kg rat and in one 50 mg/kg male. Several of the necrotic lesions were accompanied by gliosis; others were characterized by cavitations. Pigmentation, which was consistent with hemosiderin, and mineralization may be indicative of previous hemorrhage or necrosis.

Pituitary Gland: In female rats, increased incidences of atrophy of the pars distalis and dilatation of Rathke's cleft were observed in the 50 mg/kg and 25 or 50 mg/kg groups, respectively (Tables 11 and B3). There were a few occurrences of these lesions in treated male rats as well, along with a single occurrence of necrosis of the pars distalis in the 50 mg/kg group (Tables 11 and A4). Atrophy of the pars distalis was characterized by focal areas or, more often, a linear band of stromal collapse and pituitary cell dropout (Plates 5 and 6). The affected area of the pars distalis was that just overlying Rathke's pouch and the pars intermedia, immediately subjacent to the third ventricle of the brain. Within these areas, there were infiltrates of macrophages containing pigment that was consistent with hemosiderin. Dilatation of Rathke's cleft was characterized by a widening of the potential space between the pars distalis and pars intermedia; this dilated space was filled with eosinophilic proteinaceous material and contained minimal to moderate amounts of red blood cells. Occasionally, the space also contained pigment-laden macrophages, and infrequently, sterol clefts.

Spleen: There were significant increases in the incidences of pigmentation in 25 and 50 mg/kg males and 50 mg/kg females (Tables 11, A4, and B3). The pigment was golden brown and granular to globular and was located almost exclusively within macrophages in the spleen. A Prussian Blue stain was positive, indicating the pigment was consistent with hemosiderin.

Kidney: The incidences of mineralization of the kidney were significantly increased in all dosed groups of males (Tables 11 and A4). Mineralization was characterized by irregular, deeply basophilic concretions either located in or lining tubules in the corticomedullary region.

Lung: The incidence of alveolar epithelial hyperplasia in the lung of male rats in the 25 mg/kg group was significantly increased when compared to the vehicle control group (vehicle control, 11/5; 12.5 mg/kg, 17/50; 25 mg/kg, 19/50; 50 mg/kg, 3/50; Table A4). Alveolar epithelial hyperplasia was characterized by a focal area of increased numbers of type II pneumocytes. The biological relevance of the increase in incidence of this lesion is unknown.

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg[a]
Male				
Brain[b]	50	50	50	50
Mineralization[c]	0	0	1 (2.0)[d]	1 (2.0)
Necrosis	0	0	1 (4.0)	3* (2.7)
Pigmentation	0	1 (1.0)	0	3* (1.0)
Pituitary Gland	50	50	50	49
Pars Distalis, Atrophy	0	1 (3.0)	1 (4.0)	2 (3.0)
Pars Distalis, Necrosis	0	0	0	1 (4.0)
Rathke's Cleft, Dilatation	0	3 (2.0)	1 (1.0)	3* (1.3)
Spleen	50	50	49	48
Pigmentation	19 (2.0)	24 (1.5)	30** (2.1)	46** (2.2)
Kidney	48	48	44	49
Mineralization	17 (1.0)	33** (1.4)	41** (1.6)	38** (1.5)
Female				
Brain	50	50	50	50
Mineralization	1 (2.0)	0	1 (2.0)	0
Necrosis	0	0	3 (2.7)	0
Pigmentation	1 (1.0)	3 (1.0)	5 (1.2)	19** (1.3)
Pituitary Gland	50	49	49	48
Pars Distalis, Atrophy	0	0	2 (1.5)	12** (2.3)
Rathke's Cleft, Dilatation	7 (1.3)	1* (1.0)	13* (1.2)	26** (1.3)
Spleen	48	49	48	50
Pigmentation	39 (2.7)	40 (2.6)	39 (2.9)	45** (2.4)

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] There was 100% mortality in this dose group. See Table 6 for survival data.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

MICE
2-WEEK STUDY OF α-THUJONE

Four 100 mg/kg males and all five 100 mg/kg females died before the end of the study (Table 12). Final mean body weights and body weight gains of surviving dosed mice were similar to those of the vehicle controls. There were no biologically significant changes in relative or absolute organ weights (Table G4). Clinical findings included seizures and tremors in three 100 mg/kg males and hyperactivity in three male and one female 100 mg/kg mice. No gross or histologic findings were attributed to administration of α-thujone.

TABLE 12
Survival and Body Weights of Mice in the 2-Week Gavage Study of α-Thujone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	23.1 ± 0.6	27.0 ± 0.9	3.9 ± 0.5	
1	5/5	22.7 ± 0.6	26.2 ± 0.7	3.5 ± 0.3	97
3	5/5	23.0 ± 0.5	26.0 ± 0.8	3.0 ± 0.7	96
10	5/5	22.9 ± 0.5	26.9 ± 0.5	4.0 ± 0.3	100
30	5/5	23.4 ± 0.4	25.8 ± 0.7	2.4 ± 0.5	96
100	1/5[c]	23.1 ± 0.4	26.8	3.5	99
Female					
0	5/5	18.6 ± 0.4	20.7 ± 0.6	2.0 ± 0.3	
1	5/5	18.3 ± 0.2	20.6 ± 0.6	2.3 ± 0.5	99
3	5/5	18.8 ± 0.4	21.1 ± 0.3	2.3 ± 0.3	102
10	5/5	18.5 ± 0.6	21.0 ± 0.3	2.5 ± 0.4	101
30	5/5	18.1 ± 0.5	20.4 ± 0.4	2.3 ± 0.2	99
100	0/5[d]	18.5 ± 0.2	—	—	—

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the vehicle control group are not significant by Dunnett's test.
[b] Number of animals surviving at 18 days/number initially in group
[c] Days of death: 4, 4, 8, 15
[d] Days of death: 1, 1, 1, 4, 8

2-WEEK STUDY OF α,β-THUJONE

All 100 mg/kg male mice and two 100 mg/kg female mice died before the end of the study (Table 13). Final body weights of surviving dosed mice were similar to those of the vehicle controls; the mean body weight gains of 3 mg/kg males and 100 mg/kg females were significantly greater than those of the vehicle control groups. On day 9 of the study, all female mice in the 100 mg/kg group exhibited lethargy. There were no biologically significant differences in organ weights between dosed and vehicle control groups (Table G5). No seizures or tremors were observed in animals administered α,β-thujone. There were no gross or histologic findings associated with α,β-thujone administration.

Dose Selection Rationale: Because thujone occurs in nature as a mixture of α and β isomers and human exposure is to the mixture, the α,β-thujone mixture was selected for the 3-month gavage study rather than α-thujone. Based on the mortality of male and female mice administered 100 mg/kg and the lack of toxicity at 30 mg/kg, α,β-thujone doses selected for the 3-month gavage study in mice were 6.25, 12.5, 25, 50, and 75 mg/kg.

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TABLE 13
Survival and Body Weights of Mice in the 2-Week Gavage Study of α,β-Thujone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	23.1 ± 0.4	25.5 ± 0.4	2.4 ± 0.5	
1	5/5	23.2 ± 0.4	26.4 ± 0.5	3.2 ± 0.2	103
3	5/5	23.0 ± 0.4	26.8 ± 0.3	3.8 ± 0.1*	105
10	5/5	22.7 ± 0.2	26.2 ± 0.5	3.4 ± 0.4	103
30	5/5	23.7 ± 0.3	26.6 ± 0.4	2.8 ± 0.3	104
100	0/5[c]	23.0 ± 0.4	—	—	—
Female					
0	5/5	18.0 ± 0.4	20.4 ± 0.5	2.4 ± 0.4	
1	5/5	18.3 ± 0.5	21.5 ± 0.5	3.2 ± 0.2	105
3	5/5	18.7 ± 0.4	21.6 ± 0.4	2.9 ± 0.3	106
10	5/5	17.9 ± 0.2	20.7 ± 0.4	2.8 ± 0.2	101
30	5/5	18.3 ± 0.2	20.7 ± 0.3	2.4 ± 0.2	102
100	3/5[d]	17.9 ± 0.4	21.9 ± 0.6	3.7 ± 0.2*	107

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 18 days/number initially in group

[c] Days of death: 10, 11, 11, 15, 16

[d] Days of death: 11, 16

3-MONTH STUDY OF α,β-THUJONE

All mice receiving 75 mg/kg and nine male and seven female mice receiving 50 mg/kg died before the end of the study (Table 14). Final mean body weights and mean body weight gains of surviving dosed mice were similar to those of the vehicle controls (Table 14 and Figure 5). Early deaths of mice receiving 50 and 75 mg/kg occurred in animals that had previously been observed to have seizures; no female mice receiving 25 mg/kg died early. Seizures were observed in all mice receiving 50 or 75 mg/kg and in six of 10 female mice receiving 25 mg/kg. In male mice, seizures were observed during weeks 2 through 13 in mice receiving 50 mg/kg and during weeks 2 through 8 in mice receiving 75 mg/kg. In female mice, seizures occurred during weeks 8, 11, and 12 in mice receiving 25 mg/kg and during weeks 4 through 13 in mice receiving 50 or 75 mg/kg.

TABLE 14
Survival and Body Weights of Mice in the 3-Month Gavage Study of α,β-Thujone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	22.5 ± 0.3	35.1 ± 0.4	12.6 ± 0.4	
6.25	10/10	22.9 ± 0.3	36.9 ± 0.9	14.0 ± 0.7	105
12.5	10/10	22.7 ± 0.3	36.6 ± 0.8	13.9 ± 0.7	104
25	10/10	22.1 ± 0.4	35.4 ± 0.9	13.3 ± 0.8	101
50	1/10[c]	22.6 ± 0.4	29.8	7.3	85
75	0/10[d]	22.6 ± 0.3	—	—	—
Female					
0	10/10	19.2 ± 0.4	29.3 ± 1.0	10.2 ± 0.8	
6.25	10/10	19.1 ± 0.3	29.1 ± 0.7	10.0 ± 0.5	99
12.5	10/10	19.3 ± 0.2	28.7 ± 0.9	9.4 ± 1.0	98
25	10/10	19.3 ± 0.3	29.6 ± 0.9	10.3 ± 0.7	101
50	3/10[e]	18.7 ± 0.4	28.2 ± 0.9	9.8 ± 1.1	96
75	0/10[f]	18.6 ± 0.4	—	—	—

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the vehicle control group are not significant by Dunnett's test.
[b] Number of animals surviving at 3 months/number initially in group
[c] Weeks of death: 6, 8, 8, 8, 9, 11, 11, 13, 13
[d] Weeks of death: 5, 6, 6, 6, 6, 7, 7, 7, 8, 8
[e] Weeks of death: 9, 10, 11, 11, 11, 11, 12
[f] Weeks of death: 6, 6, 7, 7, 7, 7, 8, 8, 8, 8

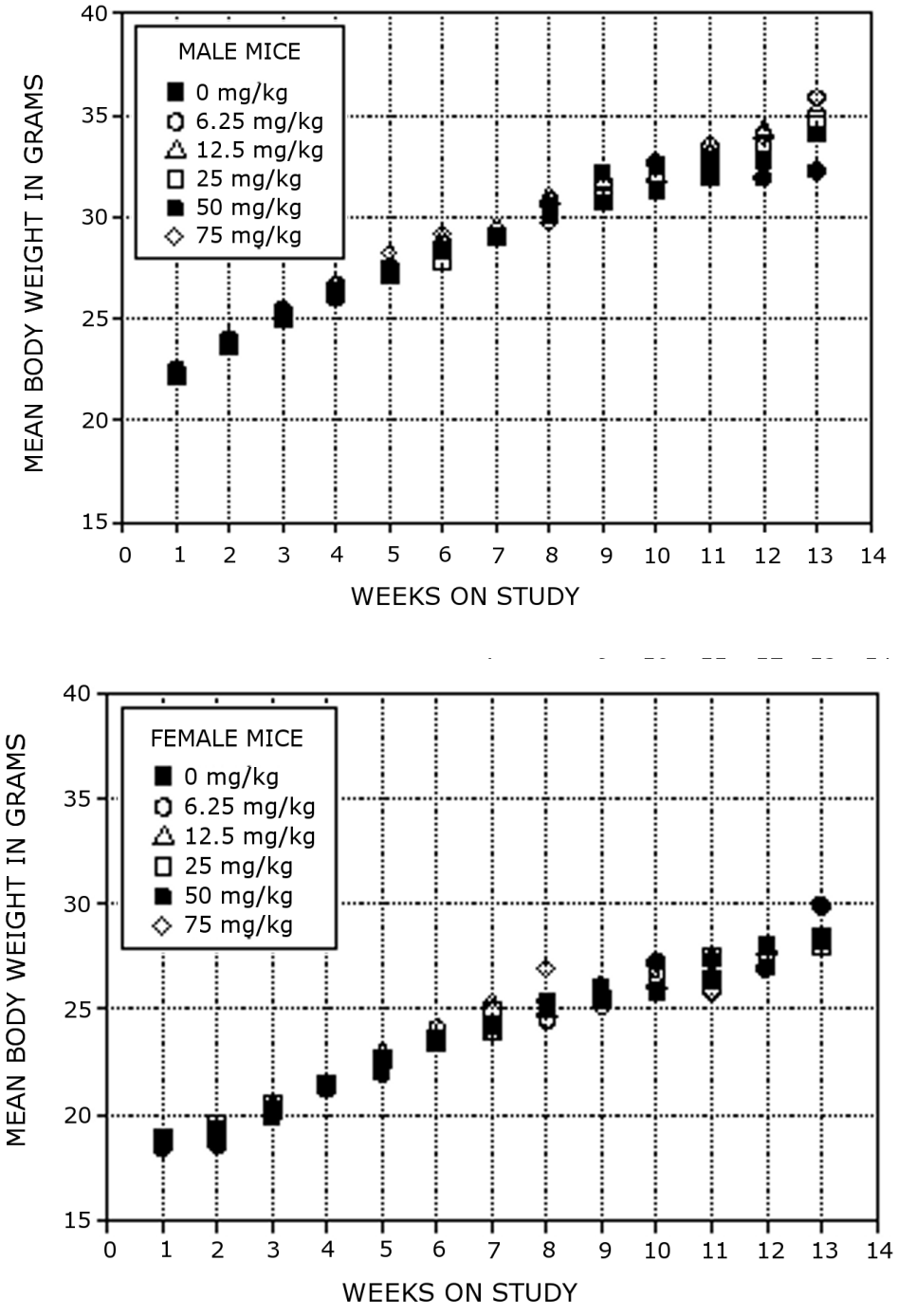

FIGURE 5
Growth Curves for Mice Administered α,β-Thujone by Gavage for 3 Months

Hematology data for the 3-month study are presented in Table F2. Differences in hematology parameters were not considered to be related to α,β-thujone administration.

Absolute and relative right kidney weights of 50 mg/kg females were significantly greater than those of the vehicle controls, but this finding was not considered to be biologically significant due to the mortality in this dose group (Table G6).

There were no significant differences in sperm parameters of male mice or the estrous cyclicity of female mice administered 12.5, 25, or 50 mg/kg α,β-thujone when compared to the vehicle controls (Tables H3 and H4).

The incidences of lung congestion in 75 mg/kg males (vehicle control, 0/10; 25 mg/kg, 0/10; 50 mg/kg, 1/10; 75 mg/kg, 5/10) and lung hemorrhage in 50 mg/kg males (0/10, 0/10, 5/10, 1/10) were significantly greater than those in the vehicle control groups. The incidence of lung congestion was also increased in 75 mg/kg females (0/10, 0/10, 2/10, 3/10).

Dose Selection Rationale: Based on decreased survival of 50 and 75 mg/kg mice in the 3-month study, α,β-thujone doses selected for the 2-year gavage study in mice were 0, 3, 6, 12, and 25 mg/kg. Although seizures were observed in females administered 25 mg/kg, they were not associated with deaths or histopathologic lesions and were not considered to adversely affect survival in a 2-year study. Administered doses of α,β thujone were substantially lower than target concentrations in all dose groups throughout the 3-month study as indicated by after dosing sample analysis of animal room samples (Appendix I). Since it was difficult to determine the exact dose mice received during the course of the 3-month study, an additional dose group was added to the 2-year study in the event that mortality occurred in the two highest dose groups after an appropriate dose formulation was achieved.

2-YEAR STUDY OF α,β-THUJONE

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 15 and in the Kaplan-Meier survival curves (Figure 6). Survival of male and female mice in the 25 mg/kg groups was significantly less than that of the vehicle controls. On day 316, the remaining eight female mice in this group were terminated. Early deaths occurred in mice that had previously been observed to have seizures. Survival of the remaining dosed groups was similar to that of the vehicle controls.

TABLE 15
Survival of Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male					
Animals initially in study	50	50	50	50	50
Accidental death[a]	0	1	0	0	0
Moribund	3	0	4	4	0
Natural deaths	7	7	5	9	36
Animals surviving to study termination	40	42	41	37	14[e]
Percent probability of survival at end of study[b]	80	86	82	74	28
Mean survival (days)[c]	701	704	719	682	405
Survival analysis[d]	P=0.336	P=0.526N	P=0.834N	P=0.624	P<0.001
Female					
Animals initially in study	50	50	50	50	50
Accidental deaths[a]	2	6	0	0	5
Other[a]	0	1	0	0	0
Moribund	4	4	2	3	8
Natural deaths	7	6	8	6	37
Animals surviving to study termination	37	33	40[e]	41	0
Percent probability of survival at end of study	77	77	80	82	0
Mean survival (days)	666	622	710	717	226
Survival analysis	P=0.500N	P=1.000	P=0.878N	P=0.640N	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. The 25 mg/kg group was excluded from the trend test due to high mortality. A negative trend or a lower mortality in a dose group is indicated by **N**.
[e] Includes one animal that died during the last week of the study

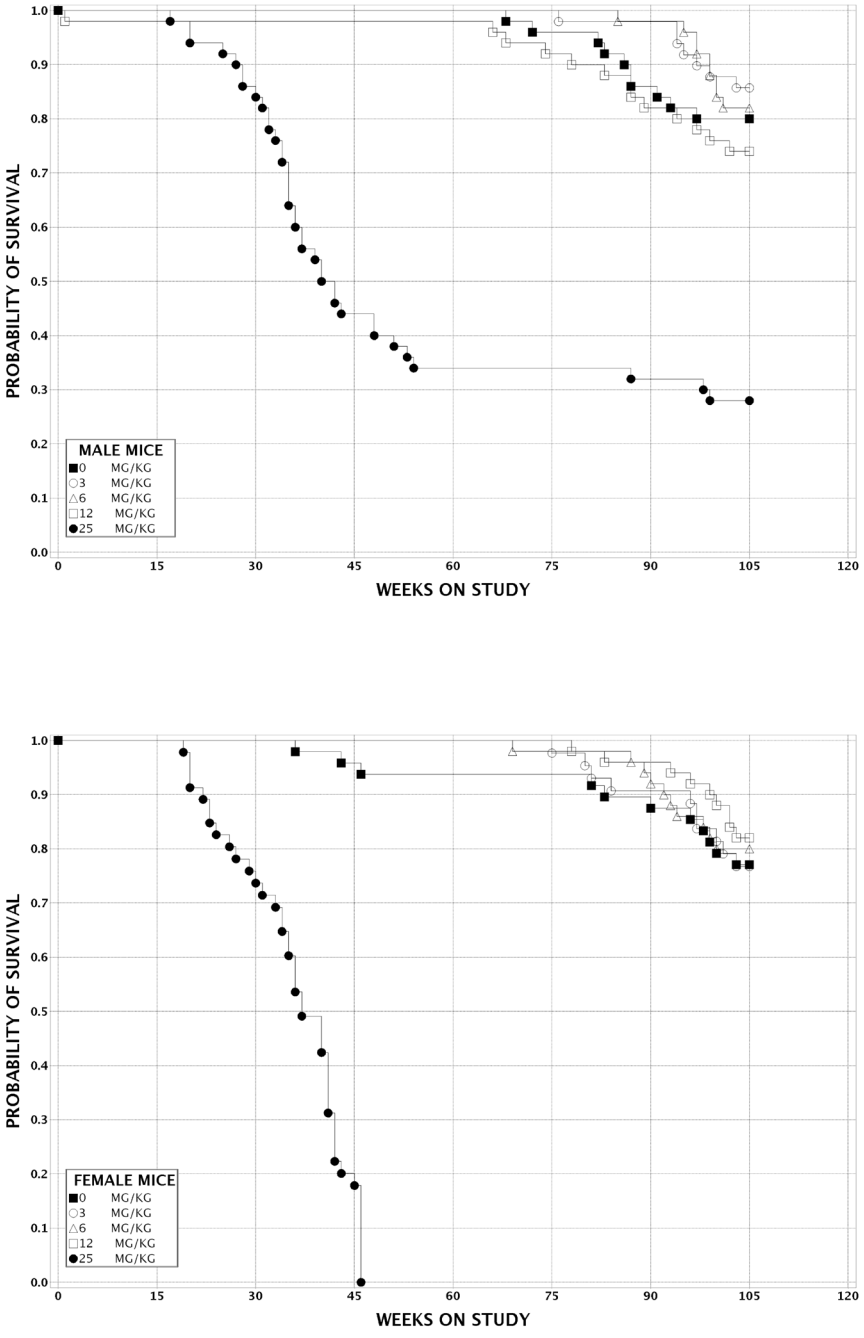

FIGURE 6
Kaplan-Meier Survival Curves for Mice Administered α,β-Thujone by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of all dosed groups of males and of females administered 12 mg/kg or less were within 10% of those in the vehicle control groups throughout the study (Tables 16 and 17; Figure 7). Mean body weights of 25 mg/kg females were less than those of the vehicle controls after week 29. Forty-four male and all female 25 mg/kg mice had seizures. The first appearance of seizures was on day 103 for male mice and day 1 for female mice. Seizures in 10 female mice occurred during the first week of dosing. Several of these were considered likely due to gavage errors or possibly due to toxicity from α,β-thujone absorbed across the lung lining. For animals that had seizures during the first week of dosing and were not removed as dosing accidents, the next seizures did not occur until day 79 to day 108. After the first week, the next day of appearance of seizures for female mice was day 73. Seizures in one vehicle control and one 3 mg/kg female were considered incidental. Seizures were not observed in vehicle control males or 3 mg/kg males or 6 or 12 mg/kg males or females.

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of α,β-Thujone

Day	Vehicle Control		3 mg/kg			6 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.5	50	22.7	101	50	22.4	100	50
4	22.7	50	22.6	100	49	22.4	99	50
11	24.0	50	24.3	101	49	24.0	100	50
18	25.3	50	25.3	100	49	25.2	100	50
25	26.5	50	26.6	100	49	26.2	99	50
32	27.6	50	28.0	101	49	27.5	100	50
39	28.7	50	28.8	100	49	28.6	100	50
46	29.5	50	29.7	101	49	29.8	101	50
53	30.4	50	30.3	100	49	30.5	100	50
60	31.6	50	31.7	100	49	31.7	100	50
67	32.8	50	32.7	100	49	32.6	100	50
74	34.0	50	34.0	100	49	33.5	99	50
81	34.2	50	34.6	101	49	34.1	100	50
88	35.3	50	35.1	99	49	35.0	99	50
116	38.3	50	38.1	100	49	37.6	98	50
144	41.0	50	40.5	99	49	40.0	98	50
172	43.7	50	42.6	98	49	42.7	98	50
200	45.9	50	45.1	98	49	44.9	98	50
228	47.1	50	47.0	100	49	46.2	98	50
256	48.5	50	48.9	101	49	47.8	99	50
284	49.1	50	50.0	102	49	48.6	99	50
312	49.1	50	49.9	102	49	48.9	100	50
340	49.8	50	50.6	102	49	49.2	99	50
368	50.4	50	51.1	102	49	50.1	100	50
396	51.1	50	52.0	102	49	50.7	99	50
424	51.2	50	51.9	101	49	50.8	99	50
452	51.4	50	52.0	101	49	51.7	101	50
480	52.7	49	53.0	101	49	52.5	100	50
508	52.1	48	53.0	102	49	52.6	101	50
536	52.0	48	52.9	102	48	53.3	103	50
564	52.2	48	53.0	102	48	53.5	103	50
592	51.9	46	51.7	100	48	52.2	101	49
620	53.4	43	52.0	98	48	51.9	97	49
648	53.9	42	51.3	95	48	51.6	96	49
676	53.0	41	50.6	96	44	50.2	95	47
704	53.5	40	50.2	94	43	51.1	96	41
Mean for weeks								
1-13	28.9		29.0	100		28.8	100	
14-52	45.8		45.9	100		45.1	98	
53-101	52.2		51.9	99		51.7	99	

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of α,β-Thujone

Day	12 mg/kg			25 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.6	100	50	22.6	101	50
4	22.6	100	49	22.7	100	50
11	24.2	101	49	24.2	101	50
18	25.3	100	49	25.4	101	50
25	26.5	100	49	26.5	100	50
32	27.7	101	49	27.5	100	50
39	28.6	99	49	28.6	99	50
46	29.7	101	49	29.3	99	50
53	30.5	100	49	30.1	99	50
60	31.6	100	49	31.0	98	50
67	32.6	99	49	31.3	96	50
74	33.7	99	49	32.5	96	50
81	34.4	101	49	32.9	96	50
88	35.0	99	49	33.5	95	50
116	37.8	99	49	35.8	94	50
144	40.2	98	49	37.8	92	47
172	43.3	99	49	40.6	93	46
200	45.3	99	49	43.2	94	43
228	47.2	100	49	45.2	96	38
256	48.7	100	49	46.6	96	28
284	49.8	101	49	48.2	98	25
312	50.0	102	49	49.0	100	22
340	50.6	102	49	49.7	100	20
368	51.4	102	49	51.3	102	18
396	52.2	102	49	52.6	103	17
424	51.8	101	49	52.5	103	17
452	51.9	101	49	52.4	102	17
480	53.1	101	47	54.0	103	17
508	52.9	102	47	53.6	103	17
536	53.2	102	46	53.5	103	17
564	53.4	102	45	54.2	104	17
592	52.3	101	44	52.9	102	17
620	54.1	101	41	52.4	98	16
648	53.9	100	41	52.6	98	16
676	53.1	100	40	50.8	96	16
704	53.7	100	38	51.8	97	14
Mean for weeks						
1-13	28.9	100		28.4	98	
14-52	45.9	100		44.0	96	
53-101	52.8	101		52.7	101	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control		3 mg/kg			6 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	50	18.9	100	50	18.8	100	50
5	18.9	50	18.4	97	45	18.6	98	50
12	19.1	50	18.9	99	44	19.1	100	50
19	20.2	50	20.3	101	44	20.1	100	50
26	21.1	50	20.9	99	44	21.1	100	50
33	22.2	50	22.0	99	44	22.2	100	50
40	23.0	50	22.8	99	44	23.0	100	50
47	23.7	50	23.5	99	44	23.5	99	50
54	24.8	50	24.2	97	44	24.3	98	50
61	25.7	50	25.0	97	44	25.3	99	50
68	26.0	50	25.5	98	44	25.8	99	50
75	26.3	50	25.8	98	44	26.1	99	50
82	27.0	50	26.6	99	44	26.5	98	50
89	27.8	50	27.1	97	44	27.0	97	50
117	30.6	49	30.3	99	44	29.8	97	50
145	33.0	48	33.2	101	44	31.9	97	50
173	36.9	48	36.3	98	44	36.5	99	50
201	38.4	48	38.4	100	44	37.3	97	50
229	41.5	48	40.6	98	44	40.0	97	50
257	44.2	47	43.6	99	44	43.6	99	50
285	46.3	47	45.7	99	44	45.7	99	50
313	47.7	46	46.5	97	44	46.9	98	50
341	50.6	45	48.7	96	44	49.3	97	50
369	51.9	45	50.8	98	44	51.2	99	50
397	56.3	45	54.9	98	44	55.3	98	50
425	56.7	45	55.2	97	44	56.2	99	50
453	59.8	45	57.8	97	44	59.7	100	50
481	59.5	45	58.8	99	44	61.0	103	50
509	63.5	45	60.8	96	44	62.7	99	49
537	63.9	45	62.7	98	43	63.4	99	49
565	64.7	44	63.8	99	42	64.2	99	49
593	63.5	43	64.2	101	40	61.9	97	49
621	63.4	43	64.7	102	40	62.3	98	47
649	63.0	42	63.4	101	40	61.8	98	44
677	62.1	41	64.3	104	37	59.7	96	43
705	61.4	38	64.5	105	35	61.1	100	40
Mean for weeks								
1-13	23.2		22.9	99		23.0	99	
14-52	41.0		40.4	99		40.1	98	
53-101	60.7		60.5	100		60.0	99	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of α,β-Thujone

Day	12 mg/kg			25 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.0	101	50	18.7	99	50
5	18.9	100	50	18.4	97	47
12	19.4	101	50	18.9	99	46
19	20.3	101	50	20.2	100	46
26	21.3	101	50	21.3	101	46
33	22.2	100	50	22.2	100	46
40	23.2	101	50	22.9	100	46
47	23.7	100	50	23.5	99	46
54	24.8	100	50	24.3	98	46
61	25.6	100	50	25.2	98	46
68	26.1	101	50	25.2	97	46
75	26.4	100	50	25.8	98	46
82	26.7	99	50	26.4	98	46
89	27.8	100	50	26.9	97	46
117	30.1	98	50	29.0	95	46
145	33.1	101	50	30.2	92	42
173	36.7	99	50	33.2	90	37
201	38.6	101	50	34.6	90	34
229	41.1	99	50	34.9	84	31
257	44.9	102	50	37.5	85	22
285	47.3	102	50	36.5	79	14
313	48.0	101	50	34.6	73	8
341	50.2	99	50			
369	52.0	100	50			
397	56.0	100	50			
425	56.8	100	50			
453	59.3	99	50			
481	60.5	102	50			
509	62.9	99	50			
537	63.1	99	50			
565	63.2	98	49			
593	63.7	100	48			
621	63.7	100	48			
649	63.1	100	47			
677	61.5	99	46			
705	60.6	99	44			
Mean for weeks						
1-13	23.2	100		22.9	99	
14-52	41.1	100		33.8	82	
53-101	60.5	100				

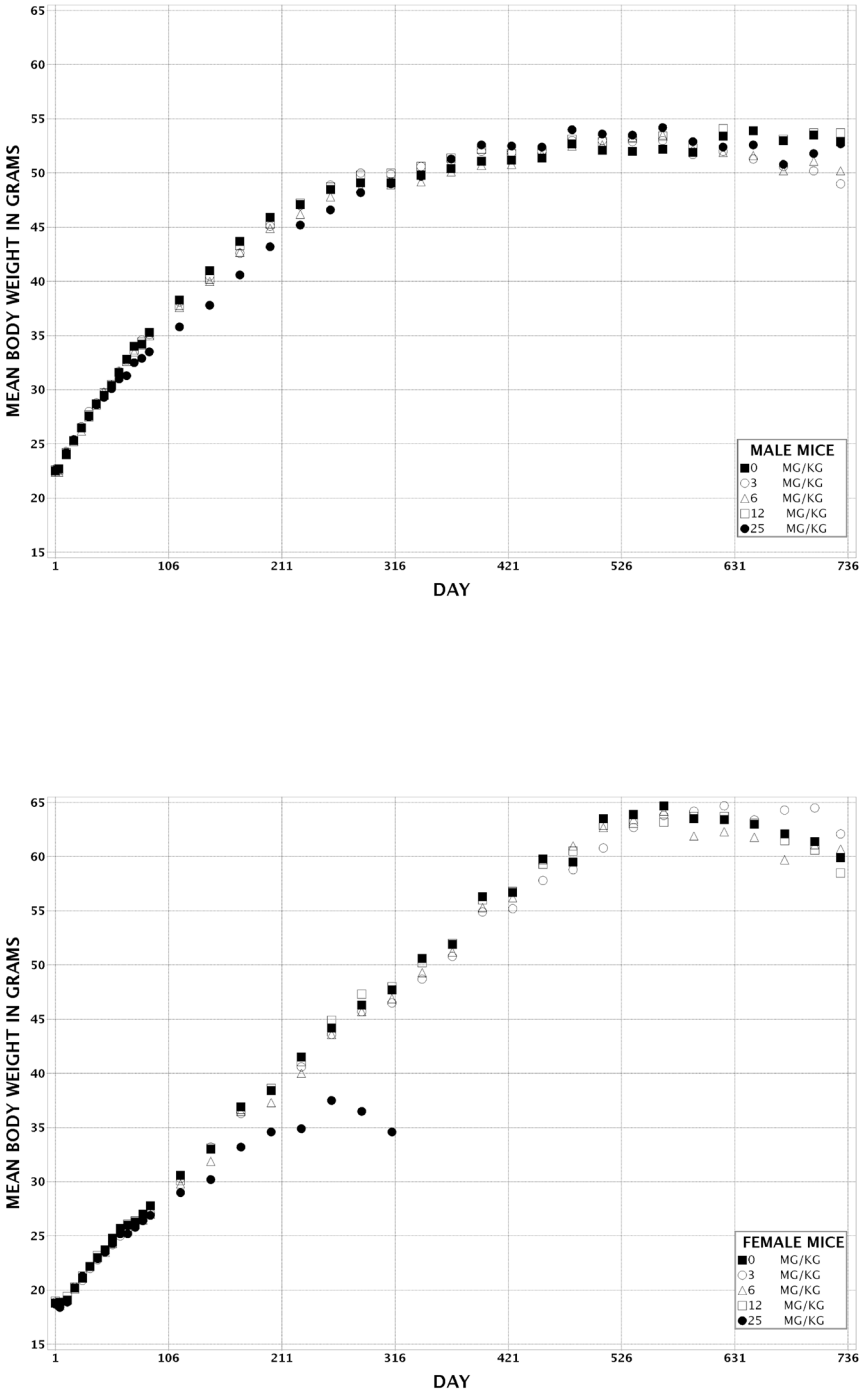

FIGURE 7
Growth Curves for Mice Administered α,β-Thujone by Gavage for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the small intestine. Due to the early mortality in 25 mg/kg mice, data from these groups are not presented in this section. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice.

Small Intestine: The incidence of carcinoma of the small intestine (duodenum, ileum, or jejunum) was increased in 6 mg/kg males, but the increase was not significant (vehicle control, 0/50; 3 mg/kg, 2/50; 6 mg/kg, 4/50; 12 mg/kg, 2/50; Tables C1 and C2) and did not exceed the historical control range for all routes (0% to 8%). The incidence of adenoma or carcinoma (combined) was significantly increased in 6 mg/kg males (0/50, 2/50, 5/50, 3/50) but did not exceed the historical control range for all routes (0% to 10%). Due to the lack of dose response, these neoplasms were not considered to be related to administration of α,β-thujone.

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

Single-dose toxicokinetic studies of α- and α,β-thujone were conducted in male and female F344/N rats and B6C3F1 mice following intravenous or oral gavage administration; these studies are presented in Appendix L. Intravenous doses of α-thujone and α,β-thujone, respectively, were 1.6 and 3.0 mg/kg for rats and 3.2 and 6.0 mg/kg for mice. Gavage doses of α-thujone and α,β-thujone, respectively, were 25 and 50 mg/kg for rats and 40 and 80 mg/kg for mice. Plasma concentration versus time data were analyzed using one- or two-compartment models, and brain concentration versus time data were analyzed using noncompartmental methods. Following intravenous administration of each formulation, α-thujone was rapidly distributed into the peripheral compartment in both species and sexes. Male rats showed extensive tissue distribution compared to female rats (Tables L2 and L6); this sex difference was not observed in mice (Tables L10 and L14). α-Thujone was distributed into the brain in both males and females following administration of each formulation, with females having higher brain:plasma ratios than males (Figures L3, L11, L19, and L27). Elimination of α-thujone was rapid in both species and sexes without showing any apparent dose dependence following administration of each formulation. The only apparent species difference was that mice had a shorter elimination half-life, faster clearance, and lower AUC_∞ values than rats.

Following a single gavage administration of α-thujone or α,β-thujone, absorption was rapid and independent of dose, species, or sex. There was no evidence that α-thujone absorption was saturated following administration of either formulation. α-Thujone was distributed into a peripheral compartment for both rats and mice. C_{max} and AUC_∞ values increased in a dose proportional manner, except in female rats following administration of α-thujone and in male and female mice following administration of α,β-thujone where the increase was more than proportional to dose, indicating possible saturation of elimination kinetics at the higher dose (Tables L4, L8, L12, and L16). As with intravenous administration, α-thujone was distributed into the brain in both males and females (Figures L8, L16, L24, and L32). The brain:plasma ratio was higher in females than males of both species, although the effect was not as pronounced in female mice as in female rats. Elimination of α-thujone was rapid in both rats and mice following gavage administration of both formulations. The only apparent species difference occurred in elimination where mice had a shorter elimination half-life, faster clearance, and lower AUC_∞ values than rats.

In male rats, the average oral bioavailability of α-thujone was 23.8% and 22.6% following administration of α-thujone and α,β-thujone, respectively. In female rats, the oral bioavailability following administration of α-thujone was considered to be best represented by the 25 mg/kg group (96.2%) since the 50 mg/kg group (177%) indicated potential saturation of elimination kinetics. This nonlinear behavior was not observed in female rats following administration of α,β-thujone; the average bioavailability estimated was 56.5%. Following administration of α-thujone in male mice, the average oral bioavailability was 13.6%. Female mice had an insufficient number of time points with measurable plasma concentrations at 40 mg/kg, therefore, the oral bioavailability was estimated as 13.1% at 80 mg/kg. Following administration of 40 and 80 mg/kg α,β-thujone, respectively, the oral bioavailability in male mice was 9.56% and 52.9% and in female mice was 9.77% and 26.8%. Based on these data, it can be concluded that the bioavailability of α-thujone was higher in female rats compared to male rats following administration of either formulation; there was no apparent sex difference in mice. Female rats and male and female mice showed a greater than proportional increase in bioavailability with increasing dose

following administration of α-thujone and α,β-thujone, respectively, which is likely due to saturation of elimination kinetics.

GENETIC TOXICOLOGY

Neither α,β-thujone (1 to 1,000 µg/plate; lot E58/L-2 used in the 2-year studies) nor α-thujone (10 to 10,000 µg/plate) was mutagenic in any of several bacterial tester strains (*Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535, as well as *Escherichia coli* strain WP2 *uvrA*/pKM101) when testing was conducted with or without exogenous metabolic activation provided by rat or hamster liver S9 mix (Tables E1, E2, and E3). *In vivo*, daily exposure by gavage to α,β-thujone (6.25 to 25 mg/kg) for 3 months did not result in an increase in micronucleated erythrocytes (normochromatic erythrocytes) in the peripheral blood of male mice (Table E4). However, female mice (6.25 to 50 mg/kg) had a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-month study; both a significant trend (P=0.006) and a significant elevation in frequency (P=0.0015) of micronucleated erythrocytes was observed in the 50 mg/kg group. No significant changes in the percentage of reticulocytes (polychromatic erythrocytes) among total erythrocytes was seen in either male or female mice at the end of the 3-month study, suggesting that α,β-thujone did not induce bone marrow toxicity.

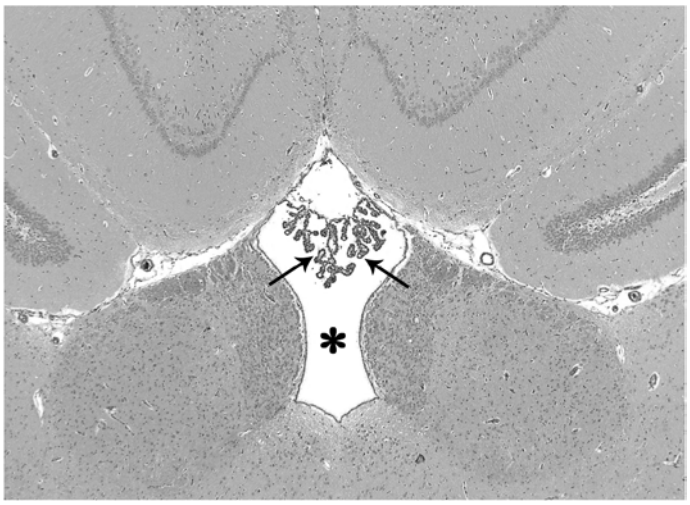

PLATE 1
The third ventricle (*) and choroid plexus (arrows) in the brain of a vehicle control female F344/N rat in the 3-month study of α,β-thujone. H&E

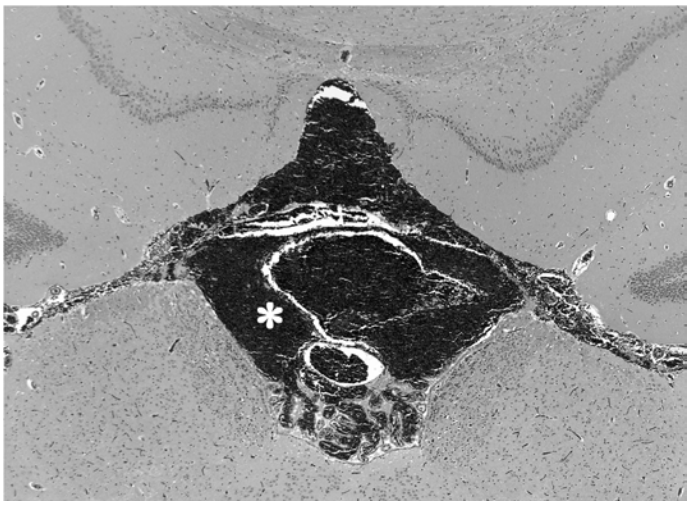

PLATE 2
The third ventricle (white *) in the brain of a female F344/N rat administered 100 mg α,β-thujone/kg body weight by gavage for 3 months. Note how the ventricle is filled with hemorrhage and congestion in the meningeal space. H&E

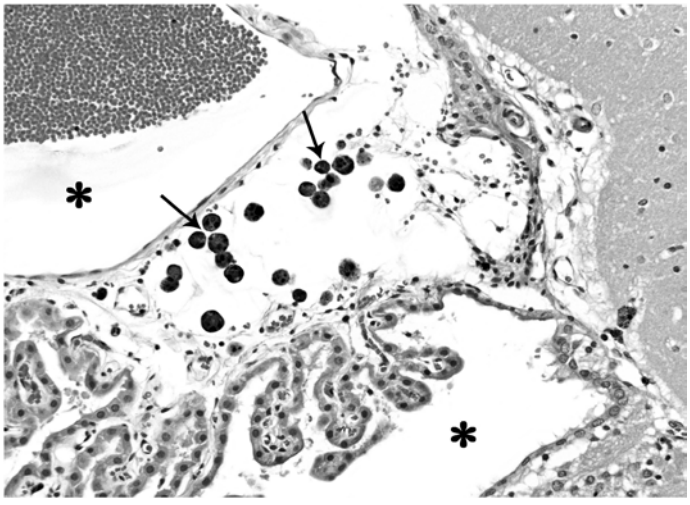

PLATE 3
Choroid plexus of the third ventricle (*) in the brain of a female F344/N rat administered 100 mg α,β-thujone/kg body weight by gavage for 3 months. Note the pigment (hemosiderin)-laden macrophages (arrows). H&E

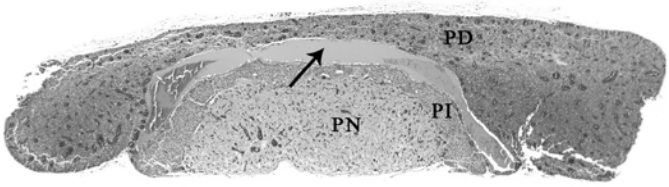

PLATE 4
A dilated Rathke's cleft (arrow) filled with eosinophilic proteinaceous material and blood in the pituitary gland of a female F344/N rat administered 100 mg α,β-thujone/kg body weight by gavage for 3 months. Pars nervosa (PN), pars intermedia (PI), pars distalis (PD). H&E

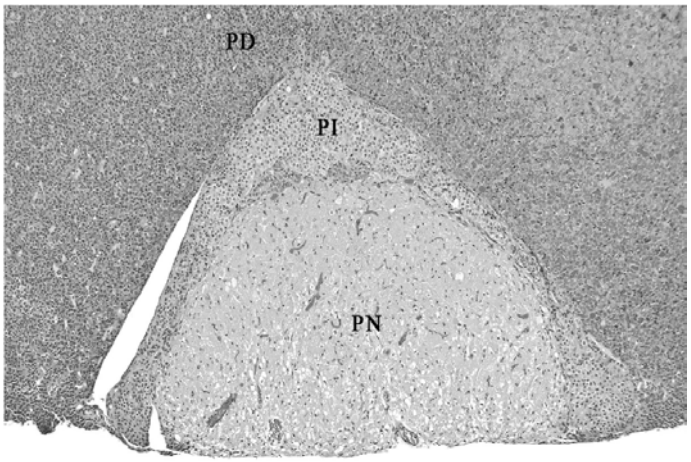

PLATE 5
Pituitary gland with no remarkable lesions from a vehicle control female F344/N rat in the 2-year gavage study of α,β-thujone. Pars nervosa (PN), pars intermedia (PI), pars distalis (PD). H&E

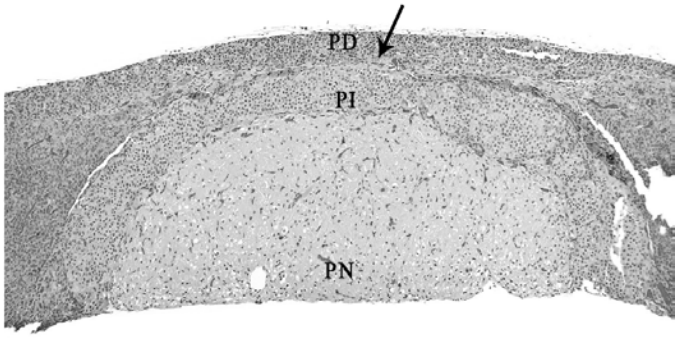

PLATE 6
Pituitary gland from a female F344/N rat administered 50 mg α,β-thujone/kg body weight by gavage for 2 years. Note the linear collapse of stroma (arrow) with accumulation of pigment-laden macrophages in the pars distalis (PD). Pars nervosa (PN), pars intermedia (PI). H&E

DISCUSSION AND CONCLUSIONS

α-Thujone was nominated by the National Cancer Institute (NCI) for study by the NTP based on the potential for widespread human exposure through herbs and essential oils used in the production of foods and beverages and medicinal and household products. Thujone is most frequently associated with the production of absinthe, a liqueur containing wormwood extract. Based on the documented toxicity of thujone, its presence in foods and beverages is regulated in many countries, including the United States. Although thujone itself is banned as a food additive in the United States, it appears in many other natural flavoring substances that are approved food additives and flavorings. In addition, various products containing thujone, including absinthe, can be purchased on the internet. At the time of its nomination, thujone was identified by NCI through a review of direct food additives given Generally Recognized as Safe status by the FDA (21 CFR, Part 182); 24 direct food additives in the FDA Priority-Based Assessment of Food Additives database contained thujone (NTP, 1997). A 2-year study of thujone was recommended due to the lack of chronic toxicity and carcinogenicity data.

Commercial preparations of essential oils containing thujone cause central nervous system effects including tonic and clonic convulsions/seizures in humans and in animals. In rodents, the seizures are often lethal (IPCS, 1981). In humans, thujone has been suggested as the neurotoxic cause of absinthism, a syndrome characterized by addiction, hyperexcitability, and hallucinations. The toxicity of thujone was responsible for the eventual ban of absinthe at the beginning of the twentieth century, although more recent studies have raised the possibility that other constituents of absinthe may also be responsible for its neurotoxic effects. Studies in experimental animals have demonstrated that α-thujone is more toxic than β-thujone or a racemic mixture of thujone (Rice and Wilson, 1976; Höld *et al.*, 2000). Studies on the mechanism of the neurotoxicity of α-thujone demonstrate that it is a γ-aminobutyric acid (GABA) type A receptor antagonist blocking GABA-mediated inhibition which has an excitatory effect on the brain (Höld *et al.*, 2000; Olsen, 2000; Dettling *et al.*, 2004). As such, seizures were a frequent clinical observation in the current NTP studies and are described in more detail in this Discussion.

Based on these observations, comparative 2-week toxicity studies of α-thujone and a mixture of α,β-thujone (71% and 12%, respectively) were conducted in F344/N rats and B6C3F1 mice. The doses for both species and compounds were 0, 1, 3, 10, 30, and 100 mg/kg. Convulsions/seizures followed by mortality were observed in only the 100 mg/kg groups administered α-thujone. Convulsion/seizures were observed in most of the high dose female rats and three male mice administered 100 mg/kg α-thujone; mortality was observed in three female rats, four male mice, and all female mice. Convulsions/seizures and tremors were not observed in animals administered the α,β-mixture and were not observed in any other dosed group. Mice were more sensitive than rats as indicated by the higher incidence of mortality following administration of both compounds. In general, there were no significant differences in final mean body weights, body weight gains, or organ weights. No gross or histologic findings were attributed to the administration of thujone. Since the toxicity results were similar between the compounds, with the exception of convulsions/seizures observed following α-thujone administration, the α,β-thujone mixture was selected for the 3-month gavage studies because thujone occurs in nature as a mixture of α and β isomers and human exposure is to the mixture.

In the 3-month rat study, the doses for males and females were 0, 12.5, 25, 50, 75, and 100 mg/kg. Seizures followed by mortality were observed at 75 and 100 mg/kg. Seizures were not observed until weeks 4 or 5 of dosing. The seizures typically occurred immediately after dosing with subsequent recovery by the animals, and mortality was observed days to weeks following the initial observation of seizures. Some rats dosed at 50 mg/kg experienced seizures, but none of these animals died as a result. One female rat administered 25 mg/kg also had seizures. In females, body weight gains were increased at doses of 50 mg/kg or greater. Hematology and clinical chemistry findings were not considered compound related. There was no evidence of α,β-thujone induced toxicity to the reproductive system of male rats; however, female rats in the highest dose group (50 mg/kg) were more likely to remain in extended diestrus than controls. Congestion and hemorrhage of the brain were associated with exposure to α,β-thujone. Hemorrhage

was only recorded in early death animals; however in female rats, pigment-laden macrophages were present in the brains of numerous treated rats, including some which were not early deaths. This pigment was consistent with previous hemorrhage, indicating that hemorrhage was not exclusively an agonal change. In addition, there were changes in the pituitary gland, including atrophy and dilatation of Rathke's cleft, that were associated with exposure to α,β-thujone. The exact pathogenesis of the hemorrhage in the brain or the lesions in the pituitary gland are not known, but possible mechanisms include a coagulopathy, damage secondary to necrosis, and vessel damage. Congestion, hemorrhage, and/or edema were observed in several other organs of rats that died before the end of the study, which suggests vessel damage or circulatory compromise. Mineralization of the kidney was significantly increased in dosed groups of female rats in the 3-month study and was the only lesion observed in animals at lower doses where seizures were not observed.

In the 3-month mouse study, the doses for males and females were 0, 6.25, 12.5, 25, 50, and 75 mg/kg; doses were lower than those for rats due to the increased mortality observed in mice in the 2-week study. Seizures followed by mortality were observed at the two highest doses. Seizures were observed as early as week 2 of dosing in male mice administered 50 or 75 mg/kg; however, the first incidences of death were not until weeks 5 or 6. Seizures were also observed in females receiving 25 mg/kg but were not associated with deaths. Seizures were observed at lower doses in female mice compared to rats. Body weights, organ weights, hematology findings, sperm parameters, and estrous cycles were not affected in males or females. The incidences of lung congestion were generally significantly increased in 75 mg/kg males and females. There were no gross or microscopic lesions that were correlated with the neurological signs in either sex.

In the 3-month studies in both species, administered doses of α,β-thujone were lower than target concentrations as indicated by after dosing sample analysis (Appendix I). In general, dose formulations administered to rats and mice were within 10% of the target concentrations, with larger differences observed with the lower concentrations. However, the animal room samples after dosing were substantially lower than the target concentrations at all doses. In the rat study, improvements in handling and storage resulted in progressively better recovery, and subsequent doses were closer to target concentrations. However, in the mouse study, animal room samples after dosing were substantially lower in all dose groups throughout the study. Since it was difficult to determine the exact dose mice received during the course of the 3-month study, an additional dose group was added to the 2-year study in the event that mortality occurred in the two highest dose groups after an appropriate dose formulation was achieved. A set of special experiments was undertaken prior to the beginning of the 2-year study that resulted in changes in the preparation of the dose formulations and subsequent resolution of this problem.

In the 2-year study in rats, there were no survivors in the high-dose groups (50 mg/kg) of males or females. All of the animals in the high-dose groups and most of the animals in the mid-dose (25 mg/kg) groups had seizures (43/50 males and 47/50 females). Reduced survival of rats and mice following seizures was not anticipated in the 2-year studies based on the respective 3-month study results. The doses for the 2-year studies were selected based on minimal incidences of seizures that occurred towards the end of the 3-month studies and did not result in mortality. Seizures typically occurred immediately after dosing, but not until the animals had been on study for some time. The seizures were transient, generally mild, and subsided quickly. Animals died days to months after the occurrence of seizures. The number of seizures observed per animal over the course of the study was highly variable following administration of the higher concentrations of α,β-thujone; fewer seizures per animal were observed following administration of the lower concentrations. In the 2-year study, both the incidences and the time of appearance of the first seizure were dose-related in male and female rats. Seizures first occurred on days 694, 612, 109, and 73 for male rats in the vehicle control, 12.5, 25, and 50 mg/kg groups, respectively. In female rats, seizures first occurred on days 304, 308, 47, and 21 for the vehicle control, 12.5, 25, and 50 mg/kg groups, respectively. The majority of seizures was classified as clonic, which was defined as the repetitive contraction and relaxation of muscle groups, distinct from tremors, and included myoclonic jerks. Most of the deaths in the 2-year study were natural deaths and not moribund sacrifices since the animals were not observed to be moribund following daily clinical examinations.

The occurrence of seizures in male and female rats was associated with the incidences of hemorrhage and congestion of the brain. During the initial and subsequent evaluations of the brain in the 2-year study of α,β-thujone in rats, evidence of previous hemorrhage was recorded in the form of pigment-laden macrophages. This pigment was consistent with hemosiderin. These macrophages were most commonly observed in the area of the third ventricle and the choroid plexus. The mechanism of the hemorrhage in animals exposed to α,β-thujone and whether it was due to a direct toxic effect on the vasculature (including

perturbations in blood pressure, vasodilation, or vasoconstriction) or secondary to seizures is unknown. More in-depth pathology review of the brains of the vehicle control, mid-dose, and high-dose rats from the 2-year rat study revealed several additional lesions in treated animals that may be associated with the administration of α,β-thujone.

In female rats, atrophy of the pars distalis of the pituitary gland and dilatation of Rathke's cleft were associated with administration of α,β-thujone in the 3-month and 2-year studies. There were a few occurrences of these lesions in dosed male rats as well. Atrophy of the pars distalis was most likely a result of previous necrosis, resulting in a decrease in pituitary cells and resultant stromal collapse. The suggestion that pars distalis atrophy is subsequent to necrosis is reinforced by the occurrence of marked necrosis of the pars distalis in one 2-year high-dose male and a couple of 3-month study rats. The relationship between dilatation of Rathke's pouch, atrophy of the pars distalis, and hemorrhage or pigmentation in the brain is not known, and the relationship of these lesions to the occurrence of seizures is not known.

Pigment-laden macrophages are not uncommon in the spleen of old rats, but the dose-related increase in the incidence of this lesion in male rats in the 2-year study, coupled with the early deaths in the high-dose group, indicate that this lesion was associated with α,β-thujone administration. Hemorrhages were seen in several tissues in treated rats, and multiple repeated hemorrhages, perhaps secondary to seizures, could account for an increase in hemosiderin in splenic macrophages.

Increased incidences of neoplasms of the preputial gland and adrenal gland were observed in male rats administered α,β-thujone for 2 years. The 50 mg/kg dose in the 2-year studies was considered to have exceeded the maximum tolerated dose for male and female rats, and the neoplasm incidences for these groups were not analyzed statistically. Although survival of 25 mg/kg males and females was significantly less than that of the respective vehicle control group, there was a sufficient number of animals surviving to study termination to determine treatment-related neoplastic effects. Incidences of preputial gland carcinoma and adenoma or carcinoma (combined) in male rats occurred with positive trends. The combined incidence in the 25 mg/kg males was signify-cantly greater than that in the vehicle control group and exceeded the historical control ranges for methylcellulose gavage studies and for all routes. Most of the preputial gland neoplasms in the 25 mg/kg group were carcinomas. Since the increase in the incidences of preputial gland neoplasms was not dose related and the incidences of hyperplasia were not increased, it was concluded that there was some evidence of carcinogenicity (rather than clear evidence) in the preputial gland. It is unlikely that the preputial gland neoplasms were related to the occurrence of seizures.

The incidences of benign pheochromocytoma of the adrenal medulla increased with increasing dose, and the incidence was significantly increased in 25 mg/kg male rats compared to that in the vehicle control group. The incidence in this group exceeded the historical control ranges for methylcellulose gavage studies and all study routes. However, the incidence was only one above the historical control range for all routes. One malignant pheochromocytoma was also observed in the 25 mg/kg group. The incidences of hyperplasia decreased with increasing dose in males, and there were no benign pheochromocytomas in dosed female rats. It was concluded that the increased incidence of pheo-chromocytoma in males may have been related to α,β-thujone administration. It is unlikely that the pheochromocytomas were related to the occurrence of seizures or stress associated with seizures since this neoplasm did not occur in female rats that also experienced seizures. In addition, one 25 mg/kg male rat with a benign pheochromocytoma was not observed to have seizures.

In the 2-year mouse study, the doses for males and females were 0, 3, 6, 12, and 25 mg/kg. Significant mortality was observed in the high dose males and females. The majority of these animals died following seizures. Due to the high incidence of mortality, the high-dose group of female mice was terminated on day 316 of the study. The 25 mg/kg dose in the 2-year studies was considered to have exceeded the maximum tolerated dose for male and female mice, and the neoplasm data were not statistically analyzed for this group. No histologic lesions were identified in the brains of the high dose male or female mice. Nevertheless, three dose groups remained without the occurrence of seizures. There was no significant effect of α,β-thujone on mortality or mean body weights in the remaining dose groups. In addition, there were no neoplasms or nonneoplastic lesions in mice associated with the administration of α,β-thujone.

In NTP single-dose toxicokinetic studies in rats and mice (Appendix L), α-thujone absorption was rapid following gavage administration of either α-thujone or α,β-thujone. The oral bioavailability was higher in female rats compared to male rats; there was no apparent sex difference in mice. α-Thujone was detected in the brain in high concentrations. The main sex difference was higher brain:plasma ratios in female

rats and female mice; the effect was not as pronounced in female mice as in female rats. These results may provide a partial explanation for the increased sensitivity of females compared to males to the neurotoxic effects of thujone. The only apparent species difference was in the faster elimination of α-thujone in mice compared to rats.

The α,β- thujone mixture used in the current studies contained 13% to 16% fenchone, a mono-terpene ketone that occurs in a number of essential oils including cedar leaf and fennel (Sell, 2006). The toxicity and carcinogenicity of this compound is poorly characterized, and as such, its contribution to the observed effects in the current studies is unknown.

In summary, acute and chronic toxicity was associated with the administration of α,β-thujone due to its pharmacological effect of inducing seizures as a $GABA_A$ receptor antagonist. Although mice appeared to be more sensitive to the toxicological effects of α,β-thujone, chronic toxicity and carcinogenicity were not observed at lower doses that did not induce seizures. In rats, it appears that the pharmacological/toxicological effects (i.e., seizures) of α,β-thujone and the development of neoplasms occurred at similar doses, likely through different mechanisms.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of α,β-thujone in male F344/N rats based on increased incidences of preputial gland neoplasms; increased incidences of benign pheochromocytoma of the adrenal medulla may have been related to administration of α,β-thujone in male F344/N rats administered 12.5 or 25 mg/kg. There was *no evidence of carcinogenic activity* of α,β-thujone in female F344/N rats administered 12.5 or 25 mg/kg. There was *no evidence of carcinogenic activity* of α,β-thujone in male or female B6C3F1 mice administered 3, 6, or 12 mg/kg.

Administration of α,β-thujone for 2 years resulted in increased incidences of seizures in F344/N rats and B6C3F1 mice and increased incidences of nonneoplastic lesions in the brain and spleen of male and female F344/N rats, the kidney of male F344/N rats, and the pituitary gland of female F344/N rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Peer Review Panel comments and the public discussion on this Technical Report appears on page 14.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF α,β-THUJONE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2		1	
Moribund	14	21	13	1
Natural deaths	7	4	19	49
Survivors				
Died last week of study	1			
Terminal sacrifice	26	25	17	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(48)	(48)	(40)	(39)
Intestine large, colon	(44)	(47)	(33)	(15)
Leiomyoma				1 (7%)
Intestine large, rectum	(46)	(48)	(41)	(42)
Intestine small, duodenum	(48)	(48)	(37)	(33)
Intestine small, ileum	(43)	(47)	(30)	(16)
Intestine small, jejunum	(43)	(47)	(33)	(25)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma		1 (2%)		
Ito cell tumor benign	1 (2%)			
Sarcoma, metastatic, skin	1 (2%)			
Mesentery	(11)	(10)	(11)	(4)
Oral mucosa	(0)	(1)	(0)	(1)
Squamous cell papilloma				1 (100%)
Pharyngeal, squamous cell carcinoma		1 (100%)		
Pancreas	(50)	(50)	(50)	(50)
Mixed tumor benign	1 (2%)		1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(49)
Stomach, glandular	(50)	(50)	(49)	(48)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign	2 (4%)			
Schwannoma malignant	1 (2%)			
Schwannoma malignant, metastatic, tissue NOS			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(49)	(49)
Pheochromocytoma benign	5 (10%)	6 (12%)	10 (20%)	
Pheochromocytoma malignant			1 (2%)	
Bilateral, pheochromocytoma benign	1 (2%)	2 (4%)	2 (4%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)	3 (6%)	
Carcinoma	2 (4%)	2 (4%)		
Parathyroid gland	(48)	(49)	(47)	(49)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	24 (48%)	20 (40%)	18 (36%)	4 (8%)
Pars distalis, adenoma, multiple		1 (2%)		
Thyroid gland	(46)	(48)	(42)	(45)
C-cell, adenoma	6 (13%)	1 (2%)	5 (12%)	1 (2%)
C-cell, carcinoma		1 (2%)		
Follicular cell, adenoma			2 (5%)	
General Body System				
Tissue NOS	(1)	(1)	(2)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (100%)			
Schwannoma malignant			1 (50%)	
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(1)	(0)	(1)
Preputial gland	(49)	(49)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Carcinoma	1 (2%)		5 (10%)	
Sarcoma, metastatic, skin	1 (2%)			
Prostate	(50)	(50)	(50)	(50)
Adenoma	2 (4%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Sarcoma, metastatic, kidney			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	25 (50%)	34 (68%)	30 (60%)	12 (24%)
Interstitial cell, adenoma	10 (20%)	10 (20%)	10 (20%)	4 (8%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(12)	(14)	(12)	(5)
Lymph node, mandibular	(2)	(0)	(0)	(3)
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Spleen	(50)	(50)	(49)	(48)
Thymus	(44)	(45)	(45)	(47)
Integumentary System				
Mammary gland	(48)	(47)	(49)	(42)
Adenoma			1 (2%)	
Carcinoma			1 (2%)	
Fibroadenoma	3 (6%)		3 (6%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	2 (4%)		
Basal cell carcinoma	1 (2%)	1 (2%)		1 (2%)
Hamartoma				1 (2%)
Keratoacanthoma	5 (10%)	3 (6%)	2 (4%)	1 (2%)
Sarcoma	2 (4%)			
Squamous cell carcinoma		1 (2%)		
Trichoepithelioma	2 (4%)			
Subcutaneous tissue, fibroma	11 (22%)	10 (20%)	5 (10%)	1 (2%)
Subcutaneous tissue, fibroma, multiple		2 (4%)	1 (2%)	
Subcutaneous tissue, fibrosarcoma	2 (4%)	1 (2%)	2 (4%)	
Subcutaneous tissue, fibrosarcoma, multiple	1 (2%)			
Subcutaneous tissue, lipoma		1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)	1 (2%)	
Schwannoma malignant, metastatic, tissue NOS			1 (2%)	
Maxilla, squamous cell carcinoma, metastatic, skin		1 (2%)		
Skeletal muscle	(2)	(4)	(2)	(0)
Chordoma, metastatic, uncertain primary site			1 (50%)	
Osteosarcoma, metastatic, bone	1 (50%)			
Schwannoma malignant, metastatic, tissue NOS			1 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)	
Granular cell tumor benign		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma			1 (2%)	
Alveolar/bronchiolar carcinoma, multiple	1 (2%)			
Basal cell carcinoma, metastatic, skin	1 (2%)			
Chordoma, metastatic, uncertain primary site			1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)		1 (2%)	
Sarcoma, metastatic, kidney			1 (2%)	
Schwannoma malignant, metastatic, tissue, NOS			1 (2%)	
Squamous cell carcinoma, metastatic, skin		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Special Senses System				
Eye	(49)	(50)	(46)	(48)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(1)	(1)	(0)
Carcinoma		1 (100%)	1 (100%)	
Urinary System				
Kidney	(48)	(48)	(44)	(49)
Sarcoma			1 (2%)	
Renal tubule, carcinoma		1 (2%)	1 (2%)	
Urinary bladder	(50)	(50)	(49)	(50)
Papilloma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	22 (44%)	18 (36%)	18 (36%)	
Lymphoma malignant		2 (4%)	1 (2%)	
Mesothelioma malignant	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	50	47	20
Total primary neoplasms	142	130	137	29
Total animals with benign neoplasms	44	47	44	18
Total benign neoplasms	104	99	99	27
Total animals with malignant neoplasms	31	31	32	2
Total malignant neoplasms	38	31	38	2
Total animals with metastatic neoplasms	5	1	4	1
Total metastatic neoplasms	11	2	9	1
Total animals with malignant neoplasms of uncertain primary site			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[b]	6/50 (12%)	8/50 (16%)	12/49 (24%)
Adjusted rate[c]	14.8%	18.5%	33.5%
Terminal rate[d]	5/27 (19%)	5/25 (20%)	6/17 (35%)
First incidence (days)	716	603	652
Poly-3 test[e]	P=0.038	P=0.435	P=0.045
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rate	6/50 (12%)	8/50 (16%)	13/49 (27%)
Adjusted rate	14.8%	18.5%	36.3%
Terminal rate	5/27 (19%)	5/25 (20%)	7/17 (41%)
First incidence (days)	716	603	652
Poly-3 test	P=0.021	P=0.435	P=0.025
Mammary Gland: Fibroadenoma			
Overall rate	3/50 (6%)	0/50 (0%)	3/50 (6%)[g]
Adjusted rate	7.3%	0.0%	8.5%
Terminal rate	2/27 (7%)	0/25 (0%)	1/17 (6%)
First incidence (days)	659	—[f]	615
Poly-3 test	P=0.590	P=0.112N	P=0.596
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma			
Overall rate	3/50 (6%)	0/50 (0%)	4/50 (8%)
Adjusted rate	7.3%	0.0%	11.3%
Terminal rate	2/27 (7%)	0/25 (0%)	2/17 (12%)
First incidence (days)	659	—	615
Poly-3 test	P=0.391	P=0.112N	P=0.422
Pancreatic Islets: Adenoma			
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.5%	4.7%	8.5%
Terminal rate	1/27 (4%)	2/25 (8%)	2/17 (12%)
First incidence (days)	729 (T)	729 (T)	677
Poly-3 test	P=0.190	P=0.515	P=0.255
Pancreatic Islets: Adenoma or Carcinoma			
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)
Adjusted rate	7.4%	9.5%	8.5%
Terminal rate	2/27 (7%)	4/25 (16%)	2/17 (12%)
First incidence (days)	674	729 (T)	677
Poly-3 test	P=0.505	P=0.520	P=0.592
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	24/50 (48%)	21/50 (42%)	18/50 (36%)
Adjusted rate	54.9%	48.4%	46.5%
Terminal rate	14/27 (52%)	15/25 (60%)	6/17 (35%)
First incidence (days)	356	610	313
Poly-3 test	P=0.250N	P=0.342N	P=0.290N
Preputial Gland: Adenoma			
Overall rate	2/49 (4%)	1/49 (2%)	4/50 (8%)
Adjusted rate	5.0%	2.4%	11.3%
Terminal rate	1/27 (4%)	0/24 (0%)	2/17 (12%)
First incidence (days)	615	599	608
Poly-3 test	P=0.221	P=0.485N	P=0.281

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg
Preputial Gland: Carcinoma			
Overall rate	1/49 (2%)	0/49 (0%)	5/50 (10%)
Adjusted rate	2.5%	0.0%	13.9%
Terminal rate	1/27 (4%)	0/24 (0%)	3/17 (18%)
First incidence (days)	729 (T)	—	404
Poly-3 test	P=0.033	P=0.492N	P=0.079
Preputial Gland: Adenoma or Carcinoma			
Overall rate	3/49 (6%)	1/49 (2%)	9/50 (18%)
Adjusted rate	7.5%	2.4%	24.7%
Terminal rate	2/27 (7%)	0/24 (0%)	5/17 (29%)
First incidence (days)	615	599	404
Poly-3 test	P=0.018	P=0.290N	P=0.037
Skin: Keratoacanthoma			
Overall rate	5/50 (10%)	3/50 (6%)	2/50 (4%)
Adjusted rate	12.3%	6.9%	5.7%
Terminal rate	5/27 (19%)	0/25 (0%)	2/17 (12%)
First incidence (days)	729 (T)	599	729 (T)
Poly-3 test	P=0.206N	P=0.319N	P=0.279N
Skin: Trichoepithelioma or Basal Cell Adenoma			
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.4%	4.7%	0.0%
Terminal rate	2/27 (7%)	2/25 (8%)	0/17 (0%)
First incidence (days)	702	729 (T)	—
Poly-3 test	P=0.110N	P=0.482N	P=0.147N
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma			
Overall rate	4/50 (8%)	3/50 (6%)	0/50 (0%)
Adjusted rate	9.8%	7.1%	0.0%
Terminal rate	3/27 (11%)	3/25 (12%)	0/17 (0%)
First incidence (days)	702	729 (T)	—
Poly-3 test	P=0.071N	P=0.479N	P=0.081N
Skin: Keratoacanthoma or Squamous Cell Carcinoma			
Overall rate	5/50 (10%)	4/50 (8%)	2/50 (4%)
Adjusted rate	12.3%	9.1%	5.7%
Terminal rate	5/27 (19%)	0/25 (0%)	2/17 (12%)
First incidence (days)	729 (T)	599	729 (T)
Poly-3 test	P=0.227N	P=0.452N	P=0.279N
Skin: Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma			
Overall rate	9/50 (18%)	7/50 (14%)	2/50 (4%)
Adjusted rate	22.1%	16.0%	5.7%
Terminal rate	8/27 (30%)	3/25 (12%)	2/17 (12%)
First incidence (days)	702	599	729 (T)
Poly-3 test	P=0.040N	P=0.330N	P=0.043N
Skin: Fibroma			
Overall rate	11/50 (22%)	12/50 (24%)	6/50 (12%)
Adjusted rate	26.3%	27.4%	16.8%
Terminal rate	7/27 (26%)	5/25 (20%)	4/17 (24%)
First incidence (days)	540	610	540
Poly-3 test	P=0.223N	P=0.554	P=0.228N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg
Skin: Fibrosarcoma			
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	7.2%	2.4%	5.7%
Terminal rate	2/27 (7%)	0/25 (0%)	1/17 (6%)
First incidence (days)	367	716	631
Poly-3 test	P=0.450N	P=0.297N	P=0.573
Skin: Sarcoma			
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.4%	0.0%	0.0%
Terminal rate	2/27 (7%)	0/25 (0%)	0/17 (0%)
First incidence (days)	677	—	—
Poly-3 test	P=0.041N	P=0.112N	P=0.148N
Skin: Fibrosarcoma or Sarcoma			
Overall rate	6/50 (12%)	1/50 (2%)	2/50 (4%)
Adjusted rate	14.4%	2.4%	5.7%
Terminal rate	4/27 (15%)	0/25 (0%)	1/17 (6%)
First incidence (days)	367	716	631
Poly-3 test	P=0.087N	P=0.052N	P=0.191N
Skin: Fibroma, Fibrosarcoma, or Sarcoma			
Overall rate	16/50 (32%)	13/50 (26%)	8/50 (16%)
Adjusted rate	37.4%	29.6%	22.1%
Terminal rate	10/27 (37%)	5/25 (20%)	5/17 (29%)
First incidence (days)	367	610	540
Poly-3 test	P=0.091N	P=0.295N	P=0.107N
Testes: Adenoma			
Overall rate	35/50 (70%)	44/50 (88%)	40/50 (80%)
Adjusted rate	79.6%	91.6%	93.1%
Terminal rate	24/27 (89%)	24/25 (96%)	17/17 (100%)
First incidence (days)	540	477	450
Poly-3 test	P=0.022	P=0.068	P=0.040
Thyroid Gland (C-Cell): Adenoma			
Overall rate	6/46 (13%)	1/48 (2%)	5/42 (12%)
Adjusted rate	15.9%	2.4%	15.4%
Terminal rate	6/26 (23%)	0/25 (0%)	2/17 (12%)
First incidence (days)	729 (T)	519	608
Poly-3 test	P=0.494N	P=0.040N	P=0.607N
Thyroid Gland (C-Cell): Adenoma or Carcinoma			
Overall rate	6/46 (13%)	2/48 (4%)	5/42 (12%)
Adjusted rate	15.9%	4.8%	15.4%
Terminal rate	6/26 (23%)	1/25 (4%)	2/17 (12%)
First incidence (days)	729 (T)	519	608
Poly-3 test	P=0.505N	P=0.102N	P=0.607N
Thyroid Gland (Follicular Cell): Adenoma			
Overall rate	0/46 (0%)	0/48 (0%)	2/42 (5%)
Adjusted rate	0.0%	0.0%	6.3%
Terminal rate	0/26 (0%)	0/25 (0%)	1/17 (6%)
First incidence (days)	—	—	608
Poly-3 test	P=0.083	—[h]	P=0.200

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg
All Organs: Mononuclear Cell Leukemia			
Overall rate	22/50 (44%)	18/50 (36%)	18/50 (36%)
Adjusted rate	49.9%	39.7%	46.4%
Terminal rate	10/27 (37%)	7/25 (28%)	5/17 (29%)
First incidence (days)	540	519	485
Poly-3 test	P=0.392N	P=0.220N	P=0.462N
All Organs: Benign Neoplasms			
Overall rate	44/50 (88%)	47/50 (94%)	44/50 (88%)
Adjusted rate	94.5%	97.1%	98.0%
Terminal rate	26/27 (96%)	25/25 (100%)	17/17 (100%)
First incidence (days)	356	477	313
Poly-3 test	P=0.219	P=0.445	P=0.336
All Organs: Malignant Neoplasms			
Overall rate	31/50 (62%)	31/50 (62%)	33/50 (66%)
Adjusted rate	68.3%	64.1%	76.6%
Terminal rate	16/27 (59%)	12/25 (48%)	11/17 (65%)
First incidence (days)	367	299	404
Poly-3 test	P=0.243	P=0.413N	P=0.257
All Organs: Benign or Malignant Neoplasms			
Overall rate	48/50 (96%)	50/50 (100%)	47/50 (94%)
Adjusted rate	99.7%	100.0%	99.5%
Terminal rate	27/27 (100%)	25/25 (100%)	17/17 (100%)
First incidence (days)	356	299	313
Poly-3 test	P=0.993N	P=1.000	P=1.000

(T) Terminal sacrifice

[a] No data presented for 50 mg/kg group due to 100% mortality.

[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group

[g] A single incidence of adenoma occurred in an animal that also had a fibroadenoma.

[h] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Preputial Gland Neoplasms in Control Male F344/N Rats[a]

Study (Study Start)	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Methylcellulose Gavage Studies		
Androstenedione (February 2003)	0/50	3/50
α,β-Thujone (March 2003)	1/49	3/49
Total (%)	1/99 (1.0%)	6/99 (6.1%)
Mean ± standard deviation	1.0% ± 1.4%	6.1% ± 0.1%
Range	0%-2%	6%
Overall Historical Incidence: All Routes		
Total (%)	19/1,295 (1.5%)	68/1,295 (5.3%)
Mean ± standard deviation	1.5% ± 1.7%	5.2% ± 3.7%
Range	0%-4%	0%-12%

[a] Data as of March 20, 2010

TABLE A3b
Historical Incidence of Pheochromocytoma of the Adrenal Medulla in Control Male F344/N Rats[a]

Study (Study Start)	Benign Pheochromocytoma	Malignant Pheochromocytoma	Benign or Malignant Pheochromocytoma
Historical Incidence: Methylcellulose Gavage Studies			
Androstenedione (February 2003)	7/50	0/50	8/50
α,β-Thujone (March 2003)	6/50	0/50	6/50
Total (%)	13/100 (13.0%)	0/100	14/100 (14.0%)
Mean ± standard deviation	13.0% ± 1.4%		14.0% ± 2.8%
Range	12%-14%		12%-16%
Overall Historical Incidence: All Routes			
Total (%)	183/1,295 (14.1%)	24/1,295 (1.9%)	208/1,295 (16.1%)
Mean ± standard deviation	14.1% ± 4.5%	1.9% ± 2.4%	16.1% ± 5.5%
Range	6%-22%	0%-8%	6%-26%

[a] Data as of March 20, 2010

TABLE A3c
Historical Incidence of Adenoma of the Testes in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Methylcellulose Gavage Studies	
Androstenedione (February 2003)	42/50
α,β-Thujone (June 2003)	35/50
Total (%)	77/100 (77.0%)
Mean ± standard deviation	77.0% ± 9.9%
Range	70%-84%
Overall Historical Incidence: All Routes	
Total (%)	1,053/1,298 (81.1%)
Mean ± standard deviation	81.1% ± 13.4%
Range	54%-98%

[a] Data as of March 20, 2010

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2		1	
Moribund	14	21	13	1
Natural deaths	7	4	19	49
Survivors				
Died last week of study	1			
Terminal sacrifice	26	25	17	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Hyperkeratosis				1 (2%)
Inflammation, acute	2 (4%)			
Perforation	1 (2%)			
Intestine large, cecum	(48)	(48)	(40)	(39)
Edema	1 (2%)			
Inflammation, acute	1 (2%)			
Necrosis, lymphoid				1 (3%)
Intestine large, colon	(44)	(47)	(33)	(15)
Edema	2 (5%)	1 (2%)	1 (3%)	
Hemorrhage			1 (3%)	
Necrosis			1 (3%)	
Intestine large, rectum	(46)	(48)	(41)	(42)
Inflammation, chronic				1 (2%)
Intestine small, duodenum	(48)	(48)	(37)	(33)
Necrosis, lymphoid				1 (3%)
Intestine small, ileum	(43)	(47)	(30)	(16)
Inflammation, acute			1 (3%)	1 (6%)
Inflammation, chronic active	1 (2%)			
Necrosis	1 (2%)	1 (2%)		
Intestine small, jejunum	(43)	(47)	(33)	(25)
Liver	(50)	(50)	(50)	(50)
Basophilic focus	29 (58%)	26 (52%)	25 (50%)	4 (8%)
Clear cell focus	19 (38%)	23 (46%)	11 (22%)	4 (8%)
Degeneration, cystic	13 (26%)	7 (14%)	4 (8%)	2 (4%)
Eosinophilic focus	6 (12%)	4 (8%)	4 (8%)	1 (2%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation		1 (2%)		
Hepatodiaphragmatic nodule	8 (16%)	3 (6%)	5 (10%)	3 (6%)
Infiltration cellular, mixed cell	21 (42%)	22 (44%)	18 (36%)	9 (18%)
Mixed cell focus	3 (6%)	6 (12%)	3 (6%)	2 (4%)
Necrosis, focal		1 (2%)		3 (6%)
Thrombosis		1 (2%)		
Bile duct, hyperplasia	41 (82%)	45 (90%)	40 (80%)	20 (40%)
Centrilobular, necrosis		1 (2%)	1 (2%)	5 (10%)
Hepatocyte, vacuolization cytoplasmic	7 (14%)	10 (20%)	4 (8%)	1 (2%)
Hepatocyte, vacuolization cytoplasmic, focal		2 (4%)	1 (2%)	1 (2%)
Kupffer cell, pigmentation		4 (8%)		
Mesentery	(11)	(10)	(11)	(4)
Accessory spleen		1 (10%)	1 (9%)	2 (50%)
Hemorrhage			1 (9%)	1 (25%)
Fat, necrosis	10 (91%)	9 (90%)	8 (73%)	1 (25%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Alimentary System (continued)				
Oral mucosa	(0)	(1)	(0)	(1)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	29 (58%)	35 (70%)	30 (60%)	18 (36%)
Cyst	21 (42%)	25 (50%)	15 (30%)	6 (12%)
Hemorrhage				1 (2%)
Necrosis		1 (2%)		1 (2%)
Acinus, hyperplasia, focal	8 (16%)	1 (2%)	6 (12%)	
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	7 (14%)	4 (8%)	9 (18%)	
Hyperplasia				1 (2%)
Inflammation, acute	1 (2%)		1 (2%)	
Inflammation, chronic		2 (4%)		
Necrosis				1 (2%)
Duct, hyperplasia	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(49)
Edema	2 (4%)	4 (8%)	1 (2%)	
Erosion	1 (2%)			
Inflammation, chronic active			1 (2%)	
Stromal hyperplasia				1 (2%)
Ulcer	1 (2%)	3 (6%)	2 (4%)	
Epithelium, hyperplasia	9 (18%)	12 (24%)	13 (26%)	3 (6%)
Stomach, glandular	(50)	(50)	(49)	(48)
Cyst		1 (2%)		
Edema		2 (4%)	1 (2%)	
Erosion	3 (6%)	6 (12%)	6 (12%)	1 (2%)
Hemorrhage				2 (4%)
Inflammation		1 (2%)		
Necrosis, lymphoid				2 (4%)
Ulcer	2 (4%)	5 (10%)	2 (4%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	44 (88%)	41 (82%)	47 (94%)	45 (90%)
Hemorrhage				2 (4%)
Hypertrophy		1 (2%)		
Thrombosis	3 (6%)	4 (8%)	2 (4%)	
Epicardium, inflammation		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Accessory adrenal cortical nodule	21 (42%)	19 (38%)	21 (43%)	4 (8%)
Degeneration, fatty	26 (52%)	28 (56%)	31 (63%)	34 (68%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)		
Hyperplasia, focal	17 (34%)	11 (22%)	9 (18%)	4 (8%)
Hypertrophy, focal	6 (12%)	2 (4%)		
Necrosis	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Bilateral, necrosis	1 (2%)			
Capsule, hyperplasia	1 (2%)		1 (2%)	
Adrenal medulla	(50)	(50)	(49)	(49)
Hyperplasia	18 (36%)	25 (50%)	13 (27%)	1 (2%)
Infiltration cellular, lymphocyte		2 (4%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)		3 (6%)	1 (2%)
Parathyroid gland	(48)	(49)	(47)	(49)
Hyperplasia, focal		1 (2%)	1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(49)
Pigmentation	7 (14%)	6 (12%)	9 (18%)	1 (2%)
Pars distalis, angiectasis	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Pars distalis, atrophy		1 (2%)	1 (2%)	2 (4%)
Pars distalis, cyst	6 (12%)	3 (6%)	4 (8%)	2 (4%)
Pars distalis, hemorrhage				2 (4%)
Pars distalis, hyperplasia, focal	16 (32%)	13 (26%)	3 (6%)	5 (10%)
Pars distalis, necrosis				1 (2%)
Pars distalis, pars intermedia, necrosis				1 (2%)
Pars intermedia, angiectasis		4 (8%)	1 (2%)	1 (2%)
Pars intermedia, cyst	4 (8%)		1 (2%)	
Pars intermedia, hyperplasia, focal	2 (4%)	1 (2%)		
Rathke's cleft, dilatation		3 (6%)	1 (2%)	3 (6%)
Thyroid gland	(46)	(48)	(42)	(45)
Degeneration, cystic	7 (15%)	6 (13%)	4 (10%)	7 (16%)
Ultimobranchial cyst	2 (4%)	2 (4%)	1 (2%)	2 (4%)
C-cell, hyperplasia	2 (4%)	7 (15%)	2 (5%)	
Follicular cell, hyperplasia, focal		2 (4%)	1 (2%)	1 (2%)
General Body System				
Tissue NOS	(1)	(1)	(2)	(0)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm		1 (2%)		
Penis	(0)	(1)	(0)	(1)
Inflammation, acute		1 (100%)		
Preputial gland	(49)	(49)	(50)	(50)
Cyst	1 (2%)			
Hyperplasia	2 (4%)		1 (2%)	
Inflammation, chronic	13 (27%)	19 (39%)	18 (36%)	5 (10%)
Necrosis		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)		1 (2%)	
Inflammation, chronic	32 (64%)	20 (40%)	23 (46%)	9 (18%)
Mineralization		1 (2%)		
Necrosis		1 (2%)	1 (2%)	
Epithelium, hyperplasia	23 (46%)	17 (34%)	18 (36%)	4 (8%)
Seminal vesicle	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Infiltration cellular				1 (2%)
Inflammation, acute		1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic		1 (2%)		
Necrosis		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	5 (10%)	2 (4%)	3 (6%)	4 (8%)
Interstitial cell, hyperplasia	9 (18%)	5 (10%)	6 (12%)	13 (26%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	7 (14%)	7 (14%)	12 (24%)	4 (8%)
Myelofibrosis	5 (10%)	4 (8%)		
Necrosis		1 (2%)		
Thrombosis		1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System (continued)				
Lymph node	(12)	(14)	(12)	(5)
Mediastinal, ectasia	1 (8%)			
Mediastinal, hemorrhage	2 (17%)			1 (20%)
Mediastinal, hyperplasia, lymphoid	1 (8%)	1 (7%)	3 (25%)	1 (20%)
Mediastinal, pigmentation			1 (8%)	
Pancreatic, hemorrhage	1 (8%)			1 (20%)
Pancreatic, hyperplasia, lymphoid		1 (7%)		3 (60%)
Pancreatic, necrosis, lymphoid				1 (20%)
Pancreatic, pigmentation	1 (8%)			1 (20%)
Lymph node, mandibular	(2)	(0)	(0)	(3)
Congestion				1 (33%)
Hyperplasia, lymphoid				1 (33%)
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Ectasia	1 (2%)	1 (2%)	1 (2%)	
Hemorrhage	4 (8%)	2 (4%)	10 (20%)	10 (20%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	4 (8%)	3 (6%)
Necrosis		1 (2%)	2 (4%)	1 (2%)
Spleen	(50)	(50)	(49)	(48)
Accessory spleen				1 (2%)
Angiectasis	1 (2%)			
Fibrosis	2 (4%)	3 (6%)	1 (2%)	
Hematopoietic cell proliferation	6 (12%)	7 (14%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)			
Infarct	1 (2%)	1 (2%)	1 (2%)	
Necrosis		1 (2%)	2 (4%)	3 (6%)
Pigmentation	19 (38%)	24 (48%)	30 (61%)	46 (96%)
Lymphoid follicle, atrophy		1 (2%)	2 (4%)	1 (2%)
Lymphoid follicle, hyperplasia	10 (20%)	12 (24%)	8 (16%)	3 (6%)
Thymus	(44)	(45)	(45)	(47)
Cyst		1 (2%)		
Ectopic parathyroid gland			1 (2%)	
Fibrosis	1 (2%)			
Integumentary System				
Mammary gland	(48)	(47)	(49)	(42)
Angiectasis		1 (2%)		
Fibrosis	1 (2%)			
Hyperplasia	2 (4%)	1 (2%)		
Duct, ectasia	28 (58%)	36 (77%)	34 (69%)	15 (36%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		2 (4%)	4 (8%)	
Edema				1 (2%)
Hemorrhage				1 (2%)
Hyperkeratosis	1 (2%)	3 (6%)	5 (10%)	1 (2%)
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic			4 (8%)	3 (6%)
Mineralization			1 (2%)	
Necrosis			1 (2%)	
Ulcer				2 (4%)
Ulcer, multiple				1 (2%)
Epidermis, hyperkeratosis	1 (2%)			
Epidermis, hyperplasia		1 (2%)	3 (6%)	
Epidermis, hyperplasia, focal	1 (2%)			
Hair follicle, cyst		1 (2%)		
Subcutaneous tissue, fibrosis			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Femur, osteopetrosis		2 (4%)	2 (4%)	
Skeletal muscle	(2)	(4)	(2)	(0)
Fibrosis		2 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	4 (8%)	11 (22%)	1 (2%)
Mineralization			1 (2%)	1 (2%)
Necrosis			1 (2%)	3 (6%)
Pigmentation		1 (2%)		3 (6%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte	31 (62%)	43 (86%)	33 (66%)	18 (36%)
Inflammation, chronic	1 (2%)	6 (12%)	2 (4%)	4 (8%)
Metaplasia, osseous	2 (4%)	5 (10%)	6 (12%)	2 (4%)
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia	11 (22%)	17 (34%)	19 (38%)	3 (6%)
Nose	(50)	(50)	(50)	(50)
Congestion				1 (2%)
Foreign body	11 (22%)	8 (16%)	9 (18%)	1 (2%)
Inflammation, chronic	13 (26%)	15 (30%)	13 (26%)	2 (4%)
Goblet cell, hyperplasia	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Respiratory epithelium, hyperplasia	13 (26%)	7 (14%)	10 (20%)	1 (2%)
Trachea	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)			
Special Senses System				
Eye	(49)	(50)	(46)	(48)
Cataract	6 (12%)	9 (18%)	10 (22%)	2 (4%)
Developmental malformation				1 (2%)
Edema	1 (2%)	1 (2%)		
Hemorrhage		1 (2%)		
Inflammation, chronic		2 (4%)		
Bilateral, cataract	1 (2%)	2 (4%)	2 (4%)	
Retina, degeneration	1 (2%)	2 (4%)		
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic	3 (6%)	4 (8%)	4 (8%)	5 (10%)
Pigmentation		1 (2%)		
Zymbal's gland	(0)	(1)	(1)	(0)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Urinary System				
Kidney	(48)	(48)	(44)	(49)
Casts granular	1 (2%)			
Casts protein	18 (38%)	30 (63%)	22 (50%)	11 (22%)
Cyst	1 (2%)	3 (6%)		
Hydronephrosis	1 (2%)	1 (2%)		
Infarct	3 (6%)	1 (2%)		
Inflammation, suppurative		2 (4%)		1 (2%)
Mineralization	17 (35%)	33 (69%)	41 (93%)	38 (78%)
Nephropathy	45 (94%)	44 (92%)	41 (93%)	46 (94%)
Artery, thrombosis	1 (2%)			
Papilla, necrosis	1 (2%)			
Renal tubule, accumulation, hyaline droplet	1 (2%)			
Renal tubule, necrosis				2 (4%)
Transitional epithelium, hyperplasia		1 (2%)	4 (9%)	2 (4%)
Urinary bladder	(50)	(50)	(49)	(50)
Atrophy		1 (2%)		
Hemorrhage				1 (2%)
Inflammation, acute		1 (2%)		
Inflammation, chronic		1 (2%)		1 (2%)
Necrosis		1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF α,β-THUJONE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	9	10	5
Natural deaths	9	8	21	45
Survivors				
Terminal sacrifice	35	33	19	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(41)	(44)	(39)	(47)
Intestine large, colon	(41)	(43)	(33)	(25)
Intestine large, rectum	(46)	(45)	(41)	(47)
Adenoma	1 (2%)			
Intestine small, duodenum	(44)	(45)	(40)	(47)
Intestine small, jejunum	(41)	(42)	(33)	(31)
Liver	(50)	(50)	(49)	(50)
Hepatocellular adenoma		1 (2%)		
Mesentery	(13)	(11)	(9)	(2)
Oral mucosa	(0)	(2)	(0)	(0)
Squamous cell papilloma		1 (50%)		
Pancreas	(49)	(49)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(48)	(47)	(48)	(50)
Tongue	(1)	(3)	(0)	(0)
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Schwannoma benign			2 (4%)	
Endocrine System				
Adrenal cortex	(47)	(49)	(46)	(50)
Adenoma	1 (2%)	2 (4%)		
Adrenal medulla	(48)	(50)	(48)	(50)
Pheochromocytoma benign	2 (4%)			
Pheochromocytoma complex	1 (2%)			
Pheochromocytoma malignant			1 (2%)	
Islets, pancreatic	(49)	(49)	(50)	(50)
Adenoma	1 (2%)			
Pituitary gland	(50)	(49)	(49)	(48)
Pars distalis, adenoma	25 (50%)	25 (51%)	15 (31%)	
Pars distalis, carcinoma	1 (2%)			
Pars distalis, ganglioneuroma	1 (2%)			
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(42)	(46)	(44)	(50)
Bilateral, C-cell, adenoma	1 (2%)			
C-cell, adenoma	3 (7%)	1 (2%)	1 (2%)	
C-cell, carcinoma	3 (7%)	1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
General Body System				
Tissue NOS	(1)	(1)	(0)	(0)
Schwannoma malignant		1 (100%)		
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	11 (22%)	9 (18%)	6 (12%)	
Carcinoma	2 (4%)	1 (2%)	3 (6%)	
Bilateral, adenoma	1 (2%)	1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign		1 (2%)		
Granulosa cell tumor malignant	1 (2%)			
Uterus	(50)	(50)	(49)	(50)
Granular cell tumor benign			1 (2%)	
Leiomyoma			1 (2%)	
Polyp stromal	17 (34%)	18 (36%)	12 (24%)	
Sarcoma		1 (2%)		
Sarcoma stromal			1 (2%)	
Vagina	(2)	(2)	(1)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(8)	(6)	(7)
Mediastinal, carcinoma, metastatic, thyroid gland	1 (14%)			
Lymph node, mandibular	(1)	(4)	(1)	(4)
Lymph node, mesenteric	(50)	(50)	(47)	(50)
Spleen	(48)	(49)	(48)	(50)
Thymus	(48)	(45)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Sarcoma, metastatic, uterus		1 (2%)		
Thymoma benign		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Carcinoma	2 (4%)	3 (6%)	1 (2%)	
Fibroadenoma	16 (32%)	18 (36%)	8 (16%)	
Fibroadenoma, multiple	10 (20%)	10 (20%)	7 (14%)	
Fibroma	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)		
Basal cell carcinoma	1 (2%)			
Hemangiosarcoma			1 (2%)	
Keratoacanthoma		1 (2%)		
Neural crest tumor		1 (2%)		
Subcutaneous tissue, fibroma	2 (4%)	1 (2%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)		1 (2%)	
Skeletal muscle	(0)	(0)	(1)	(0)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		3 (6%)		
Alveolar/bronchiolar carcinoma			1 (2%)	
Hemangiosarcoma, metastatic, skin			1 (2%)	
Sarcoma, metastatic, uterus		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(44)	(47)	(41)	(49)
Harderian gland	(50)	(50)	(49)	(50)
Carcinoma			1 (2%)	
Urinary System				
Kidney	(44)	(45)	(40)	(50)
Mesenchymal tumor malignant	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Leukemia mononuclear	10 (20%)	5 (10%)	5 (10%)	
Lymphoma malignant	1 (2%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	48	39	
Total primary neoplasms	121	109	70	
Total animals with benign neoplasms	46	46	35	
Total benign neoplasms	96	95	54	
Total animals with malignant neoplasms	20	12	14	
Total malignant neoplasms	25	13	16	
Total animals with metastatic neoplasms	1	2	2	
Total metastatic neoplasms	1	3	3	
Total animals with uncertain neoplasms-benign or malignant		1		
Total uncertain neoplasms		1		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma			
Overall rate[b]	3/48 (6%)	0/50 (0%)	1/48 (2%)
Adjusted rate[c]	7.0%	0.0%	3.2%
Terminal rate[d]	3/35 (9%)	0/33 (0%)	1/19 (5%)
First incidence (days)	729 (T)	—[f]	729 (T)
Poly-3 test[e]	P=0.214N	P=0.122N	P=0.420N
Clitoral Gland: Adenoma			
Overall rate	12/50 (24%)	10/50 (20%)	6/50 (12%)
Adjusted rate	27.3%	24.0%	17.2%
Terminal rate	10/35 (29%)	9/33 (27%)	3/19 (16%)
First incidence (days)	662	721	513
Poly-3 test	P=0.194N	P=0.459N	P=0.217N
Clitoral Gland: Carcinoma			
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.6%	2.4%	8.8%
Terminal rate	2/35 (6%)	1/33 (3%)	0/19 (0%)
First incidence (days)	729 (T)	729 (T)	500
Poly-3 test	P=0.350	P=0.515N	P=0.392
Clitoral Gland: Adenoma or Carcinoma			
Overall rate	14/50 (28%)	10/50 (20%)	9/50 (18%)
Adjusted rate	31.8%	24.0%	25.2%
Terminal rate	12/35 (34%)	9/33 (27%)	3/19 (16%)
First incidence (days)	662	721	500
Poly-3 test	P=0.278N	P=0.283N	P=0.344N
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	0.0%
Terminal rate	0/35 (0%)	3/33 (9%)	0/19 (0%)
First incidence (days)	—	729 (T)	—
Poly-3 test	P=0.507	P=0.111	—[g]
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	7.2%	2.9%
Terminal rate	0/35 (0%)	3/33 (9%)	0/19 (0%)
First incidence (days)	—	729 (T)	494
Poly-3 test	P=0.273	P=0.111	P=0.451
Mammary Gland: Fibroadenoma			
Overall rate	26/50 (52%)	28/50 (56%)	15/50 (30%)
Adjusted rate	59.1%	62.7%	42.9%
Terminal rate	24/35 (69%)	20/33 (61%)	9/19 (47%)
First incidence (days)	652	508	547
Poly-3 test	P=0.126N	P=0.445	P=0.107N
Mammary Gland: Carcinoma			
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.5%	7.1%	3.0%
Terminal rate	1/35 (3%)	2/33 (6%)	0/19 (0%)
First incidence (days)	579	508	681
Poly-3 test	P=0.549N	P=0.482	P=0.593N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg
Mammary Gland: Fibroma, Fibroadenoma, or Adenoma			
Overall rate	28/50 (56%)	29/50 (58%)	15/50 (30%)
Adjusted rate	63.2%	63.8%	42.9%
Terminal rate	25/35 (71%)	20/33 (61%)	9/19 (47%)
First incidence (days)	652	422	547
Poly-3 test	P=0.061N	P=0.566	P=0.050N
Mammary Gland: Adenoma or Carcinoma			
Overall rate	3/50 (6%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.8%	9.3%	3.0%
Terminal rate	1/35 (3%)	2/33 (6%)	0/19 (0%)
First incidence (days)	579	422	681
Poly-3 test	P=0.409N	P=0.486	P=0.411N
Mammary Gland: Fibroma, Fibroadenoma, Adenoma, or Carcinoma			
Overall rate	30/50 (60%)	30/50 (60%)	16/50 (32%)
Adjusted rate	67.0%	65.1%	45.5%
Terminal rate	26/35 (74%)	20/33 (61%)	9/19 (47%)
First incidence (days)	579	422	547
Poly-3 test	P=0.045N	P=0.511N	P=0.037N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	25/50 (50%)	25/49 (51%)	15/49 (31%)
Adjusted rate	53.6%	57.8%	43.3%
Terminal rate	17/35 (49%)	19/33 (58%)	8/19 (42%)
First incidence (days)	499	508	485
Poly-3 test	P=0.270N	P=0.425	P=0.245N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rate	26/50 (52%)	25/49 (51%)	15/49 (31%)
Adjusted rate	55.7%	57.8%	43.3%
Terminal rate	18/35 (51%)	19/33 (58%)	8/19 (42%)
First incidence (days)	499	508	485
Poly-3 test	P=0.203N	P=0.507	P=0.188N
Thyroid Gland (C-Cell): Adenoma			
Overall rate	4/42 (10%)	1/46 (2%)	1/44 (2%)
Adjusted rate	10.2%	2.5%	3.5%
Terminal rate	3/35 (9%)	1/33 (3%)	1/19 (5%)
First incidence (days)	689	729 (T)	729 (T)
Poly-3 test	P=0.146N	P=0.171N	P=0.291N
Thyroid Gland (C-Cell): Carcinoma			
Overall rate	3/42 (7%)	1/46 (2%)	0/44 (0%)
Adjusted rate	7.7%	2.5%	0.0%
Terminal rate	3/35 (9%)	1/33 (3%)	0/19 (0%)
First incidence (days)	729 (T)	729 (T)	—
Poly-3 test	P=0.095N	P=0.296N	P=0.186N
Thyroid Gland (C-Cell): Adenoma or Carcinoma			
Overall rate	7/42 (17%)	2/46 (4%)	1/44 (2%)
Adjusted rate	17.8%	5.0%	3.5%
Terminal rate	6/35 (17%)	2/33 (6%)	1/19 (5%)
First incidence (days)	689	729 (T)	729 (T)
Poly-3 test	P=0.025N	P=0.072N	P=0.081N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg
Uterus: Stromal Polyp			
Overall rate	17/50 (34%)	18/50 (36%)	12/50 (24%)
Adjusted rate	36.8%	40.8%	31.8%
Terminal rate	12/35 (34%)	14/33 (42%)	3/19 (16%)
First incidence (days)	462	412	338
Poly-3 test	P=0.399N	P=0.431	P=0.403N
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rate	17/50 (34%)	18/50 (36%)	13/50 (26%)
Adjusted rate	36.8%	40.8%	33.8%
Terminal rate	12/35 (34%)	14/33 (42%)	3/19 (16%)
First incidence (days)	462	412	338
Poly-3 test	P=0.464N	P=0.431	P=0.478N
All Organs: Mononuclear Cell Leukemia			
Overall rate	10/50 (20%)	5/50 (10%)	5/50 (10%)
Adjusted rate	22.3%	11.9%	14.8%
Terminal rate	6/35 (17%)	3/33 (9%)	2/19 (11%)
First incidence (days)	545	663	652
Poly-3 test	P=0.194N	P=0.158N	P=0.296N
All Organs: Benign Neoplasms			
Overall rate	46/50 (92%)	46/50 (92%)	35/50 (70%)
Adjusted rate	94.9%	95.4%	83.4%
Terminal rate	34/35 (97%)	31/33 (94%)	15/19 (79%)
First incidence (days)	462	412	338
Poly-3 test	P=0.036N	P=0.658	P=0.056N
All Organs: Malignant Neoplasms			
Overall rate	20/50 (40%)	12/50 (24%)	14/50 (28%)
Adjusted rate	42.6%	27.2%	37.5%
Terminal rate	12/35 (34%)	7/33 (21%)	3/19 (16%)
First incidence (days)	452	452	485
Poly-3 test	P=0.289N	P=0.091N	P=0.402N
All Organs: Benign or Malignant Neoplasms			
Overall rate	49/50 (98%)	48/50 (96%)	39/50 (78%)
Adjusted rate	98.0%	98.0%	89.4%
Terminal rate	34/35 (97%)	32/33 (97%)	16/19 (84%)
First incidence (days)	452	412	338
Poly-3 test	P=0.047N	P=0.755N	P=0.085N

(T) Terminal sacrifice

[a] No data presented for 50 mg/kg group due to 100% mortality.

[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group

[g] Value of statistic cannot be computed.

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	9	10	5
Natural deaths	9	8	21	45
Survivors				
Terminal sacrifice	35	33	19	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)			
Intestine large, cecum	(41)	(44)	(39)	(47)
Hemorrhage		1 (2%)		
Intestine large, colon	(41)	(43)	(33)	(25)
Intestine large, rectum	(46)	(45)	(41)	(47)
Hemorrhage				1 (2%)
Intestine small, duodenum	(44)	(45)	(40)	(47)
Necrosis			1 (3%)	
Necrosis, lymphoid				1 (2%)
Intestine small, jejunum	(41)	(42)	(33)	(31)
Necrosis, lymphoid				1 (3%)
Liver	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)		1 (2%)	
Basophilic focus	46 (92%)	46 (92%)	40 (82%)	12 (24%)
Clear cell focus	17 (34%)	21 (42%)	11 (22%)	
Eosinophilic focus	8 (16%)	2 (4%)	2 (4%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)	4 (8%)	
Hepatodiaphragmatic nodule	5 (10%)	8 (16%)	6 (12%)	4 (8%)
Infiltration cellular, histiocyte				1 (2%)
Infiltration cellular, mixed cell	30 (60%)	30 (60%)	32 (65%)	19 (38%)
Inflammation, chronic			1 (2%)	
Mixed cell focus	10 (20%)	4 (8%)	4 (8%)	
Necrosis, focal	1 (2%)	1 (2%)	5 (10%)	4 (8%)
Artery, inflammation		1 (2%)	1 (2%)	
Bile duct, hyperplasia	4 (8%)	2 (4%)	7 (14%)	
Capsule, fibrosis	1 (2%)			
Centrilobular, necrosis			3 (6%)	
Hepatocyte, vacuolization cytoplasmic	5 (10%)	4 (8%)	3 (6%)	
Kupffer cell, pigmentation		1 (2%)	1 (2%)	
Mesentery	(13)	(11)	(9)	(2)
Accessory spleen		1 (9%)	2 (22%)	1 (50%)
Necrosis				1 (50%)
Fat, necrosis	13 (100%)	10 (91%)	8 (89%)	1 (50%)
Oral mucosa	(0)	(2)	(0)	(0)
Inflammation, chronic active		1 (50%)		
Pancreas	(49)	(49)	(50)	(50)
Atrophy	25 (51%)	24 (49%)	23 (46%)	5 (10%)
Cyst	14 (29%)	11 (22%)	9 (18%)	1 (2%)
Acinus, hyperplasia, focal	2 (4%)	1 (2%)	2 (4%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	9 (18%)	10 (20%)	5 (10%)	2 (4%)
Hyperplasia				1 (2%)
Inflammation, chronic		1 (2%)		
Mineralization		2 (4%)		1 (2%)
Necrosis				1 (2%)
Vacuolization cytoplasmic		1 (2%)		1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	2 (4%)	1 (2%)		
Inflammation, chronic active			1 (2%)	
Ulcer	1 (2%)	2 (4%)		
Epithelium, hyperplasia	7 (14%)	8 (16%)	2 (4%)	
Epithelium, hyperplasia, atypical			1 (2%)	
Stomach, glandular	(48)	(47)	(48)	(50)
Cyst		1 (2%)		
Edema	1 (2%)			
Erosion	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Ulcer	1 (2%)		3 (6%)	1 (2%)
Tongue	(1)	(3)	(0)	(0)
Hyperplasia	1 (100%)	2 (67%)		
Ulcer		1 (33%)		
Tooth	(0)	(1)	(0)	(0)
Degeneration		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	40 (80%)	30 (60%)	30 (60%)	8 (16%)
Hemorrhage				1 (2%)
Artery, necrosis				1 (2%)
Endocrine System				
Adrenal cortex	(47)	(49)	(46)	(50)
Accessory adrenal cortical nodule	7 (15%)	12 (24%)	13 (28%)	9 (18%)
Degeneration, fatty	23 (49%)	18 (37%)	19 (41%)	9 (18%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	4 (9%)	
Hemorrhage			1 (2%)	3 (6%)
Hyperplasia, focal	22 (47%)	14 (29%)	9 (20%)	1 (2%)
Hypertrophy, focal	7 (15%)	6 (12%)	2 (4%)	1 (2%)
Inflammation, acute				1 (2%)
Necrosis	2 (4%)	1 (2%)	6 (13%)	4 (8%)
Adrenal medulla	(48)	(50)	(48)	(50)
Angiectasis				1 (2%)
Hyperplasia	7 (15%)	5 (10%)	3 (6%)	
Infiltration cellular, lymphoid		1 (2%)		
Islets, pancreatic	(49)	(49)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(49)	(48)
Pigmentation	5 (10%)	5 (10%)	9 (18%)	
Pars distalis, angiectasis	9 (18%)	4 (8%)	5 (10%)	2 (4%)
Pars distalis, atrophy			2 (4%)	12 (25%)
Pars distalis, cyst	18 (36%)	20 (41%)	17 (35%)	8 (17%)
Pars distalis, dilatation			1 (2%)	
Pars distalis, hyperplasia, focal	5 (10%)	13 (27%)	6 (12%)	1 (2%)
Pars intermedia, angiectasis	2 (4%)			
Pars intermedia, cyst	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Pars intermedia, hyperplasia	1 (2%)			
Rathke's cleft, dilatation	7 (14%)	1 (2%)	13 (27%)	26 (54%)
Thyroid gland	(42)	(46)	(44)	(50)
Degeneration, cystic	1 (2%)	2 (4%)	5 (11%)	
Ectopic thymus				1 (2%)
Ultimobranchial cyst	2 (5%)	1 (2%)	3 (7%)	2 (4%)
C-cell, hyperplasia	1 (2%)	11 (24%)	2 (5%)	
Follicular cell, hyperplasia, focal		1 (2%)	1 (2%)	
General Body System				
Tissue NOS	(1)	(1)	(0)	(0)
Fat, mediastinum, necrosis	1 (100%)			
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)		1 (2%)	
Hyperplasia	9 (18%)	7 (14%)	11 (22%)	
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic			1 (2%)	1 (2%)
Ovary	(50)	(50)	(50)	(50)
Cyst	3 (6%)	6 (12%)	2 (4%)	1 (2%)
Bilateral, cyst			1 (2%)	
Uterus	(50)	(50)	(49)	(50)
Cyst	1 (2%)			
Hyperplasia, cystic	22 (44%)	17 (34%)	19 (39%)	9 (18%)
Cervix, cyst			1 (2%)	
Fat, necrosis		1 (2%)		
Vagina	(2)	(2)	(1)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	6 (12%)	6 (12%)	
Infiltration cellular, histiocyte	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Myelofibrosis		1 (2%)		
Necrosis				1 (2%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System (continued)				
Lymph node	(7)	(8)	(6)	(7)
Deep cervical, hyperplasia		1 (13%)		
Deep cervical, pigmentation		1 (13%)		
Iliac, hemorrhage				1 (14%)
Iliac, hyperplasia, lymphoid				1 (14%)
Iliac, pigmentation				1 (14%)
Mediastinal, hemorrhage		2 (25%)	2 (33%)	2 (29%)
Mediastinal, hyperplasia, lymphoid		6 (75%)	3 (50%)	2 (29%)
Mediastinal, pigmentation		5 (63%)	1 (17%)	1 (14%)
Pancreatic, hemorrhage		1 (13%)	2 (33%)	1 (14%)
Pancreatic, hyperplasia, lymphoid			1 (17%)	4 (57%)
Pancreatic, pigmentation		1 (13%)	1 (17%)	1 (14%)
Lymph node, mandibular	(1)	(4)	(1)	(4)
Hyperplasia, lymphoid	1 (100%)	4 (100%)		2 (50%)
Pigmentation		1 (25%)		
Lymph node, mesenteric	(50)	(50)	(47)	(50)
Atrophy	1 (2%)	1 (2%)		
Ectasia	1 (2%)			
Hemorrhage	8 (16%)	9 (18%)	6 (13%)	3 (6%)
Hyperplasia, lymphoid	4 (8%)	5 (10%)	4 (9%)	5 (10%)
Necrosis			1 (2%)	1 (2%)
Pigmentation			1 (2%)	
Spleen	(48)	(49)	(48)	(50)
Ectopic spleen, multiple		1 (2%)		
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	21 (44%)	31 (63%)	21 (44%)	
Hemorrhage	1 (2%)			
Inflammation		1 (2%)		
Necrosis		1 (2%)	3 (6%)	4 (8%)
Pigmentation	39 (81%)	40 (82%)	39 (81%)	45 (90%)
Lymphoid follicle, atrophy	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Lymphoid follicle, hyperplasia	3 (6%)	3 (6%)	2 (4%)	8 (16%)
Lymphoid follicle, hyperplasia, focal			1 (2%)	
Thymus	(48)	(45)	(50)	(50)
Cyst		2 (4%)		
Ectopic parathyroid gland	1 (2%)			
Necrosis			1 (2%)	3 (6%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hyperplasia	19 (38%)	21 (42%)	10 (20%)	
Hyperplasia, focal	3 (6%)	1 (2%)		
Malformation			1 (2%)	
Duct, ectasia	47 (94%)	45 (90%)	39 (78%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)		1 (2%)	
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)			
Ulcer		1 (2%)		
Epidermis, hyperplasia	2 (4%)	1 (2%)		
Subcutaneous tissue, fibrosis	1 (2%)			

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Femur, osteopetrosis	1 (2%)	1 (2%)		
Skeletal muscle	(0)	(0)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	10 (20%)	6 (12%)	
Gliosis			1 (2%)	
Mineralization	1 (2%)		1 (2%)	
Necrosis			3 (6%)	
Pigmentation	1 (2%)	3 (6%)	5 (10%)	19 (38%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Hyperplasia		1 (2%)		
Infiltration cellular, histiocyte	46 (92%)	43 (86%)	36 (72%)	8 (16%)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic	10 (20%)	3 (6%)	5 (10%)	1 (2%)
Metaplasia, osseous		1 (2%)	2 (4%)	
Necrosis				1 (2%)
Alveolar epithelium, hyperplasia	23 (46%)	25 (50%)	17 (34%)	4 (8%)
Nose	(50)	(50)	(50)	(50)
Congestion				2 (4%)
Foreign body	7 (14%)	5 (10%)	3 (6%)	1 (2%)
Inflammation, chronic	11 (22%)	11 (22%)	9 (18%)	1 (2%)
Thrombosis	1 (2%)			
Goblet cell, hyperplasia	1 (2%)			
Nasolacrimal duct, cyst				1 (2%)
Respiratory epithelium, hyperplasia	5 (10%)	4 (8%)	2 (4%)	
Special Senses System				
Eye	(44)	(47)	(41)	(49)
Cataract		5 (11%)	2 (5%)	
Edema		1 (2%)	2 (5%)	
Hemorrhage	1 (2%)	1 (2%)		
Inflammation, acute		1 (2%)		
Inflammation, chronic	1 (2%)	1 (2%)		
Synechia		1 (2%)		
Bilateral, cataract			1 (2%)	
Bilateral, edema		1 (2%)		
Bilateral, retinal detachment		1 (2%)		
Retina, degeneration		1 (2%)	2 (5%)	1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Hemorrhage			4 (8%)	
Hyperplasia			1 (2%)	
Inflammation, chronic	9 (18%)	11 (22%)	9 (18%)	6 (12%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Urinary System				
Kidney	(44)	(45)	(40)	(50)
Casts protein	14 (32%)	8 (18%)	12 (30%)	
Cyst	1 (2%)	1 (2%)		
Infarct	2 (5%)	1 (2%)	1 (3%)	
Inflammation, suppurative	1 (2%)			
Inflammation, chronic		1 (2%)	3 (8%)	
Mineralization	16 (36%)	24 (53%)	17 (43%)	8 (16%)
Nephropathy	33 (75%)	36 (80%)	26 (65%)	4 (8%)
Renal tubule, accumulation, hyaline droplet	2 (5%)	5 (11%)	4 (10%)	
Renal tubule, necrosis			1 (3%)	
Transitional epithelium, hyperplasia	2 (5%)	1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Transitional epithelium, hyperplasia		1 (2%)		

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF α,β-THUJONE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death		1			
Moribund	3		4	4	
Natural deaths	7	7	5	9	36
Survivors					
Died last week of study					1
Terminal sacrifice	40	42	41	37	13
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Esophagus	(50)	(50)	(50)	(49)	(50)
Intestine small, duodenum	(48)	(46)	(50)	(46)	(48)
Adenoma			1 (2%)	1 (2%)	
Carcinoma			1 (2%)		
Intestine small, ileum	(50)	(48)	(48)	(47)	(49)
Carcinoma			1 (2%)	1 (2%)	
Intestine small, jejunum	(48)	(46)	(49)	(46)	(43)
Carcinoma		2 (4%)	2 (4%)	1 (2%)	
Leiomyosarcoma	1 (2%)				
Liver	(50)	(50)	(50)	(50)	(50)
Cholangioma				1 (2%)	
Hemangiosarcoma	2 (4%)	3 (6%)	2 (4%)	1 (2%)	
Hepatoblastoma	2 (4%)	3 (6%)	1 (2%)	2 (4%)	
Hepatoblastoma, multiple	1 (2%)				
Hepatocellular adenoma	10 (20%)	15 (30%)	16 (32%)	16 (32%)	6 (12%)
Hepatocellular adenoma, multiple	14 (28%)	8 (16%)	14 (28%)	12 (24%)	7 (14%)
Hepatocellular carcinoma	13 (26%)	18 (36%)	15 (30%)	13 (26%)	2 (4%)
Hepatocellular carcinoma, multiple	3 (6%)	8 (16%)	8 (16%)	3 (6%)	3 (6%)
Hepatocholangioma	1 (2%)				
Leiomyosarcoma, metastatic, stomach, glandular				1 (2%)	
Mast cell tumor malignant		2 (4%)			
Mesentery	(4)	(4)	(3)	(7)	(3)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (33%)		
Hemangioma				1 (14%)	
Hemangiosarcoma		1 (25%)			
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (25%)				
Leiomyosarcoma, metastatic, stomach, glandular				1 (14%)	
Sarcoma		1 (25%)			
Pancreas	(50)	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)	
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)				
Leiomyosarcoma, metastatic, stomach, glandular				1 (2%)	
Sarcoma, metastatic, mesentery		1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)	(50)
Squamous cell carcinoma				1 (2%)	
Squamous cell papilloma			1 (2%)	1 (2%)	1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Alimentary System (continued)					
Stomach, glandular	(50)	(50)	(50)	(49)	(50)
Leiomyosarcoma				1 (2%)	
Mast cell tumor malignant				1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)	
Tongue	(1)	(0)	(0)	(1)	(0)
Fibroma	1 (100%)				
Cardiovascular System					
Heart	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			2 (4%)		
Hepatoblastoma, metastatic, liver				1 (2%)	
Schwannoma malignant				1 (2%)	
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, stomach, glandular				1 (2%)	
Subcapsular, adenoma	2 (4%)	4 (8%)	2 (4%)	5 (10%)	
Adrenal medulla	(49)	(49)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)				
Bilateral, pheochromocytoma benign		1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Adenoma, multiple		1 (2%)			
Parathyroid gland	(43)	(48)	(47)	(46)	(48)
Pituitary gland	(48)	(50)	(48)	(49)	(49)
Pars distalis, adenoma		1 (2%)			
Thyroid gland	(49)	(50)	(50)	(49)	(50)
Follicular cell, adenoma	1 (2%)			1 (2%)	
Follicular cell, carcinoma	1 (2%)				
General Body System					
Tissue NOS	(1)	(1)	(1)	(1)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)		
Hemangiosarcoma		1 (100%)			
Hepatocellular carcinoma, metastatic, liver	1 (100%)				
Genital System					
Epididymis	(50)	(50)	(50)	(50)	(50)
Penis	(1)	(0)	(0)	(1)	(0)
Preputial gland	(49)	(49)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)			
Prostate	(49)	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, stomach, glandular				1 (2%)	
Sarcoma, metastatic, mesentery		1 (2%)			
Testes	(50)	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)	1 (2%)	1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)	
Mast cell tumor malignant		2 (4%)			
Lymph node	(3)	(0)	(1)	(1)	(0)
Hepatoblastoma, metastatic, liver				1 (100%)	
Mediastinal, hepatoblastoma, metastatic, liver				1 (100%)	
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung	1 (33%)				
Lymph node, mandibular	(47)	(49)	(46)	(47)	(44)
Mast cell tumor malignant		1 (2%)			
Lymph node, mesenteric	(49)	(48)	(47)	(47)	(50)
Leiomyosarcoma, metastatic, stomach, glandular				1 (2%)	
Plasma cell tumor malignant	1 (2%)				
Spleen	(50)	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)		3 (6%)		
Mast cell tumor malignant		2 (4%)			
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)	
Thymus	(44)	(43)	(44)	(40)	(49)
Hepatocellular carcinoma, metastatic, liver	1 (2%)				
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Keratoacanthoma					1 (2%)
Mast cell tumor malignant		1 (2%)			
Subcutaneous tissue, fibroma				1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)		1 (2%)	
Subcutaneous tissue, osteosarcoma, metastatic, uncertain primary site		1 (2%)			
Musculoskeletal System					
Bone	(50)	(50)	(50)	(49)	(50)
Osteoma	1 (2%)				
Skeletal muscle	(2)	(1)	(3)	(1)	(0)
Hemangiosarcoma	2 (100%)		1 (33%)		
Hepatoblastoma, metastatic, liver				1 (100%)	
Sarcoma		1 (100%)			
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	3 (6%)	8 (16%)	6 (12%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple		2 (4%)			
Alveolar/bronchiolar carcinoma	6 (12%)	4 (8%)	6 (12%)	3 (6%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple			3 (6%)	1 (2%)	2 (4%)
Hepatoblastoma, metastatic, liver				1 (2%)	
Hepatocellular carcinoma, metastatic, liver	4 (8%)	8 (16%)	9 (18%)	5 (10%)	1 (2%)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)				
Schwannoma malignant, metastatic, heart				1 (2%)	
Nose	(50)	(50)	(50)	(50)	(50)
Fibroma			1 (2%)		
Special Senses System					
Eye	(50)	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)	(49)
Adenoma	8 (16%)	8 (16%)	4 (8%)	1 (2%)	2 (4%)
Carcinoma	2 (4%)	2 (4%)	3 (6%)	1 (2%)	
Bilateral, adenoma		1 (2%)			
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		2 (4%)		
Hepatoblastoma, metastatic, liver				1 (2%)	
Renal tubule, adenoma			1 (2%)		
Urethra	(0)	(1)	(0)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)	(50)
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)			
Lymphoma malignant	4 (8%)	2 (4%)	7 (14%)	1 (2%)	
Total animals with primary neoplasms[c]	45	45	46	42	19
Total primary neoplasms	91	103	103	80	33
Total animals with benign neoplasms	32	32	36	35	16
Total benign neoplasms	50	46	50	47	25
Total animals with malignant neoplasms	30	33	38	27	7
Total malignant neoplasms	41	57	53	33	8
Total animals with metastatic neoplasms	6	10	11	9	1
Total metastatic neoplasms	11	11	15	21	1
Total animals with malignant neoplasms of uncertain primary site		1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[b]	2/50 (4%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate[c]	4.4%	8.5%	4.2%	11.3%
Terminal rate[d]	2/40 (5%)	3/42 (7%)	2/41 (5%)	3/37 (8%)
First incidence (days)	729 (T)	715	729 (T)	677
Poly-3 test[e]	P=0.195	P=0.355	P=0.672N	P=0.205
Harderian Gland: Adenoma				
Overall rate	8/50 (16%)	9/50 (18%)	4/50 (8%)	1/50 (2%)
Adjusted rate	17.6%	19.1%	8.3%	2.3%
Terminal rate	8/40 (20%)	9/42 (21%)	3/41 (7%)	1/37 (3%)
First incidence (days)	729 (T)	729 (T)	691	729 (T)
Poly-3 test	P=0.006N	P=0.534	P=0.148N	P=0.018N
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.4%	4.2%	6.2%	2.3%
Terminal rate	1/40 (3%)	2/42 (5%)	3/41 (7%)	1/37 (3%)
First incidence (days)	677	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.425N	P=0.682N	P=0.525	P=0.514N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	11/50 (22%)	7/50 (14%)	2/50 (4%)
Adjusted rate	21.9%	23.3%	14.5%	4.6%
Terminal rate	9/40 (23%)	11/42 (26%)	6/41 (15%)	2/37 (5%)
First incidence (days)	677	729 (T)	691	729 (T)
Poly-3 test	P=0.007N	P=0.534	P=0.252N	P=0.016N
Small Intestine (Duodenum, Ileum, or Jejunum): Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted rate	0.0%	4.2%	8.3%	4.6%
Terminal rate	0/40 (0%)	2/42 (5%)	3/41 (7%)	2/37 (5%)
First incidence (days)	—[f]	729 (T)	688	729 (T)
Poly-3 test	P=0.209	P=0.246	P=0.069	P=0.229
Small Intestine (Duodenum, Ileum, or Jejunum): Adenoma or Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted rate	0.0%	4.2%	10.3%	6.8%
Terminal rate	0/40 (0%)	2/42 (5%)	4/41 (10%)	3/37 (8%)
First incidence (days)	—	729 (T)	688	729 (T)
Poly-3 test	P=0.097	P=0.246	P=0.036	P=0.112
Liver: Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.4%	6.4%	4.2%	2.3%
Terminal rate	2/40 (5%)	3/42 (7%)	2/41 (5%)	1/37 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.326N	P=0.517	P=0.672N	P=0.512N
Liver: Hepatocellular Adenoma				
Overall rate	24/50 (48%)	23/50 (46%)	30/50 (60%)	28/50 (56%)
Adjusted rate	51.6%	48.1%	61.1%	61.7%
Terminal rate	22/40 (55%)	21/42 (50%)	26/41 (63%)	24/37 (65%)
First incidence (days)	476	529	589	545
Poly-3 test	P=0.116	P=0.445N	P=0.230	P=0.220

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
Liver: Hepatocellular Carcinoma				
Overall rate	16/50 (32%)	26/50 (52%)	23/50 (46%)	16/50 (32%)
Adjusted rate	32.9%	53.9%	46.8%	34.3%
Terminal rate	9/40 (23%)	21/42 (50%)	18/41 (44%)	9/37 (24%)
First incidence (days)	476	654	664	518
Poly-3 test	P=0.388N	P=0.028	P=0.115	P=0.530
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	33/50 (66%)	37/50 (74%)	40/50 (80%)	35/50 (70%)
Adjusted rate	67.9%	75.8%	80.2%	74.2%
Terminal rate	26/40 (65%)	31/42 (74%)	32/41 (78%)	27/37 (73%)
First incidence (days)	476	529	589	518
Poly-3 test	P=0.300	P=0.262	P=0.120	P=0.324
Liver: Hepatoblastoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.6%	6.3%	2.1%	4.6%
Terminal rate	3/40 (8%)	2/42 (5%)	0/41 (0%)	2/37 (5%)
First incidence (days)	729 (T)	663	698	729 (T)
Poly-3 test	P=0.352N	P=0.642N	P=0.283N	P=0.516N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	18/50 (36%)	27/50 (54%)	24/50 (48%)	18/50 (36%)
Adjusted rate	37.0%	56.0%	48.7%	38.6%
Terminal rate	11/40 (28%)	22/42 (52%)	18/41 (44%)	11/37 (30%)
First incidence (days)	476	654	664	518
Poly-3 test	P=0.411N	P=0.046	P=0.167	P=0.523
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	33/50 (66%)	38/50 (76%)	40/50 (80%)	36/50 (72%)
Adjusted rate	67.9%	77.8%	80.2%	76.3%
Terminal rate	26/40 (65%)	32/42 (76%)	32/41 (78%)	28/37 (76%)
First incidence (days)	476	529	589	518
Poly-3 test	P=0.240	P=0.190	P=0.120	P=0.243
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	7/50 (14%)	5/50 (10%)	8/50 (16%)	6/50 (12%)
Adjusted rate	15.4%	10.5%	16.6%	13.1%
Terminal rate	7/40 (18%)	4/42 (10%)	8/41 (20%)	3/37 (8%)
First incidence (days)	729 (T)	654	729 (T)	470
Poly-3 test	P=0.533N	P=0.349N	P=0.550	P=0.495N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	4/50 (8%)	9/50 (18%)	4/50 (8%)
Adjusted rate	13.1%	8.4%	18.5%	9.0%
Terminal rate	5/40 (13%)	3/42 (7%)	7/41 (17%)	3/37 (8%)
First incidence (days)	568	529	589	608
Poly-3 test	P=0.468N	P=0.346N	P=0.332	P=0.393N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	13/50 (26%)	9/50 (18%)	16/50 (32%)	10/50 (20%)
Adjusted rate	28.3%	18.7%	32.8%	21.7%
Terminal rate	12/40 (30%)	7/42 (17%)	14/41 (34%)	6/37 (16%)
First incidence (days)	568	529	589	470
Poly-3 test	P=0.422N	P=0.197N	P=0.401	P=0.311N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
Pancreatic Islets: Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	8.7%	4.2%	2.1%	2.3%
Terminal rate	3/40 (8%)	1/42 (2%)	1/41 (2%)	1/37 (3%)
First incidence (days)	498	715	729 (T)	729 (T)
Poly-3 test	P=0.117N	P=0.326N	P=0.166N	P=0.194N
Spleen: Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/49 (0%)
Adjusted rate	2.2%	0.0%	6.2%	0.0%
Terminal rate	1/40 (3%)	0/42 (0%)	3/41 (7%)	0/37 (0%)
First incidence (days)	729 (T)	—	729 (T)	—
Poly-3 test	P=0.528N	P=0.493N	P=0.327	P=0.510N
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	7/50 (14%)	4/50 (8%)	2/50 (4%)
Adjusted rate	8.8%	14.8%	8.3%	4.6%
Terminal rate	4/40 (10%)	5/42 (12%)	4/41 (10%)	1/37 (3%)
First incidence (days)	729 (T)	663	729 (T)	691
Poly-3 test	P=0.174N	P=0.287	P=0.610N	P=0.352N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	7/50 (14%)	4/50 (8%)	3/50 (6%)
Adjusted rate	8.8%	14.8%	8.3%	6.8%
Terminal rate	4/40 (10%)	5/42 (12%)	4/41 (10%)	2/37 (5%)
First incidence (days)	729 (T)	663	729 (T)	691
Poly-3 test	P=0.297N	P=0.287	P=0.610N	P=0.517N
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	2/50 (4%)	7/50 (14%)	1/50 (2%)
Adjusted rate	8.7%	4.2%	14.4%	2.3%
Terminal rate	3/40 (8%)	0/42 (0%)	5/41 (12%)	1/37 (3%)
First incidence (days)	498	529	696	729 (T)
Poly-3 test	P=0.295N	P=0.321N	P=0.291	P=0.194N
All Organs: Benign Neoplasms				
Overall rate	32/50 (64%)	32/50 (64%)	36/50 (72%)	35/50 (70%)
Adjusted rate	67.8%	66.5%	73.1%	73.1%
Terminal rate	29/40 (73%)	29/42 (69%)	31/41 (76%)	26/37 (70%)
First incidence (days)	476	529	589	456
Poly-3 test	P=0.267	P=0.533N	P=0.362	P=0.366
All Organs: Malignant Neoplasms				
Overall rate	30/50 (60%)	33/50 (66%)	38/50 (76%)	27/50 (54%)
Adjusted rate	60.0%	67.6%	76.0%	55.9%
Terminal rate	20/40 (50%)	27/42 (64%)	29/41 (71%)	17/37 (46%)
First incidence (days)	476	529	589	456
Poly-3 test	P=0.348N	P=0.284	P=0.066	P=0.418N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	45/50 (90%)	46/50 (92%)	42/50 (84%)
Adjusted rate	90.0%	92.1%	92.0%	85.7%
Terminal rate	35/40 (88%)	39/42 (93%)	37/41 (90%)	30/37 (81%)
First incidence (days)	476	529	589	456
Poly-3 test	P=0.250N	P=0.492	P=0.500	P=0.366N

(T) Terminal sacrifice
[a] No data presented for 25 mg/kg group due to high mortality.
[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, and spleen; for other tissues, denominator is number of animals necropsied.
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death		1			
Moribund	3		4	4	
Natural deaths	7	7	5	9	36
Survivors					
Died last week of study					1
Terminal sacrifice	40	42	41	37	13
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Esophagus	(50)	(50)	(50)	(49)	(50)
Inflammation, acute		1 (2%)			
Ulcer		1 (2%)			
Submucosa, edema		1 (2%)			
Intestine small, duodenum	(48)	(46)	(50)	(46)	(48)
Ulcer				1 (2%)	
Intestine small, ileum	(50)	(48)	(48)	(47)	(49)
Epithelium, hyperplasia		1 (2%)			
Intestine small, jejunum	(48)	(46)	(49)	(46)	(43)
Inflammation, chronic active				1 (2%)	
Liver	(50)	(50)	(50)	(50)	(50)
Basophilic focus	2 (4%)	1 (2%)		5 (10%)	2 (4%)
Clear cell focus	17 (34%)	14 (28%)	13 (26%)	21 (42%)	6 (12%)
Cyst		1 (2%)			
Eosinophilic focus	2 (4%)	5 (10%)	5 (10%)	5 (10%)	
Eosinophilic focus, multiple			1 (2%)		
Hematopoietic cell proliferation	1 (2%)			1 (2%)	
Hemorrhage				1 (2%)	
Inflammation, chronic					1 (2%)
Inflammation, chronic active	2 (4%)	1 (2%)	3 (6%)	3 (6%)	1 (2%)
Mixed cell focus	12 (24%)	7 (14%)	11 (22%)	7 (14%)	3 (6%)
Necrosis, focal	4 (8%)		5 (10%)	3 (6%)	3 (6%)
Necrosis, diffuse	1 (2%)			1 (2%)	1 (2%)
Tension lipidosis			1 (2%)		
Centrilobular, necrosis		2 (4%)	1 (2%)		
Hepatocyte, hypertrophy					1 (2%)
Hepatocyte, vacuolization cytoplasmic	23 (46%)	13 (26%)	20 (40%)	17 (34%)	5 (10%)
Kupffer cell, hyperplasia			1 (2%)		
Kupffer cell, pigmentation				1 (2%)	
Mesentery	(4)	(4)	(3)	(7)	(3)
Fat, necrosis	3 (75%)	3 (75%)	2 (67%)	6 (86%)	3 (100%)
Pancreas	(50)	(50)	(50)	(50)	(50)
Atrophy		3 (6%)	1 (2%)	2 (4%)	1 (2%)
Basophilic focus			2 (4%)		
Acinus, cytoplasmic alteration	2 (4%)	6 (12%)	3 (6%)	1 (2%)	
Acinus, hyperplasia, focal	1 (2%)		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	3 (6%)	10 (20%)	4 (8%)	3 (6%)	1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Alimentary System (continued)					
Stomach, forestomach	(50)	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)			
Inflammation, chronic	4 (8%)	5 (10%)	3 (6%)	3 (6%)	2 (4%)
Ulcer	1 (2%)	1 (2%)			
Epithelium, hyperplasia	7 (14%)	7 (14%)	8 (16%)	3 (6%)	5 (10%)
Stomach, glandular	(50)	(50)	(50)	(49)	(50)
Cyst	1 (2%)	3 (6%)	1 (2%)		
Erosion	2 (4%)	1 (2%)	1 (2%)	1 (2%)	
Epithelium, hyperplasia	1 (2%)		1 (2%)		1 (2%)
Epithelium, metaplasia, squamous				1 (2%)	
Tongue	(1)	(0)	(0)	(1)	(0)
Cardiovascular System					
Heart	(50)	(50)	(50)	(50)	(50)
Cardiomyopathy		2 (4%)		1 (2%)	
Inflammation, chronic active				1 (2%)	
Mineralization	1 (2%)				
Thrombosis	1 (2%)				
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	4 (8%)	5 (10%)	7 (14%)	2 (4%)	4 (8%)
Cyst					1 (2%)
Degeneration, fatty		1 (2%)			1 (2%)
Hyperplasia, focal	5 (10%)	7 (14%)	5 (10%)	2 (4%)	1 (2%)
Hypertrophy, focal	16 (32%)	20 (40%)	20 (40%)	14 (28%)	2 (4%)
Subcapsular, hyperplasia	11 (22%)	7 (14%)	4 (8%)	7 (14%)	4 (8%)
Adrenal medulla	(49)	(49)	(50)	(50)	(50)
Hyperplasia		2 (4%)	1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)	(50)
Atrophy				1 (2%)	
Hyperplasia	12 (24%)	8 (16%)	12 (24%)	10 (20%)	3 (6%)
Parathyroid gland	(43)	(48)	(47)	(46)	(48)
Cyst				4 (9%)	
Pituitary gland	(48)	(50)	(48)	(49)	(49)
Pars distalis, cyst	4 (8%)	3 (6%)	2 (4%)	2 (4%)	
Pars distalis, hyperplasia, focal	1 (2%)	2 (4%)	1 (2%)		
Rathke's cleft, dilatation					2 (4%)
Thyroid gland	(49)	(50)	(50)	(49)	(50)
Follicle, cyst				1 (2%)	
Follicle, degeneration, focal	13 (27%)	10 (20%)	11 (22%)	16 (33%)	6 (12%)
Follicular cell, hyperplasia		1 (2%)			
General Body System					
Tissue NOS	(1)	(1)	(1)	(1)	(0)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Genital System					
Epididymis	(50)	(50)	(50)	(50)	(50)
Granuloma sperm		2 (4%)			
Inflammation, chronic				1 (2%)	
Spermatocele		1 (2%)			
Penis	(1)	(0)	(0)	(1)	(0)
Cyst				1 (100%)	
Preputial gland	(49)	(49)	(50)	(50)	(50)
Cyst	12 (24%)	15 (31%)	7 (14%)	17 (34%)	3 (6%)
Inflammation, chronic	13 (27%)	11 (22%)	10 (20%)	10 (20%)	5 (10%)
Prostate	(49)	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte			1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)			
Germinal epithelium, atrophy		4 (8%)	2 (4%)	1 (2%)	
Interstitial cell, hyperplasia			1 (2%)		
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Hyperplasia	23 (46%)	25 (50%)	23 (46%)	21 (42%)	7 (14%)
Lymph node	(3)	(0)	(1)	(1)	(0)
Lymph node, mandibular	(47)	(49)	(46)	(47)	(44)
Atrophy	1 (2%)	1 (2%)	1 (2%)		
Hemorrhage				1 (2%)	
Hyperplasia, lymphoid	5 (11%)	1 (2%)	9 (20%)	5 (11%)	1 (2%)
Infiltration cellular, plasma cell	1 (2%)				
Pigmentation		2 (4%)		1 (2%)	
Lymph node, mesenteric	(49)	(48)	(47)	(47)	(50)
Angiectasis	1 (2%)	2 (4%)	3 (6%)		
Atrophy	1 (2%)	1 (2%)	1 (2%)	3 (6%)	
Ectasia		1 (2%)		1 (2%)	
Hematopoietic cell proliferation	2 (4%)	2 (4%)		2 (4%)	
Hemorrhage	6 (12%)	4 (8%)	6 (13%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	5 (10%)	4 (9%)	5 (11%)	3 (6%)
Infiltration cellular, plasma cell				1 (2%)	
Pigmentation		1 (2%)			
Spleen	(50)	(50)	(50)	(49)	(50)
Accessory spleen		1 (2%)			
Angiectasis		1 (2%)			
Hematopoietic cell proliferation	20 (40%)	26 (52%)	24 (48%)	25 (51%)	9 (18%)
Pigmentation					1 (2%)
Lymphoid follicle, atrophy	2 (4%)	1 (2%)	3 (6%)	1 (2%)	
Lymphoid follicle, hyperplasia	1 (2%)	5 (10%)	5 (10%)	3 (6%)	1 (2%)
Thymus	(44)	(43)	(44)	(40)	(49)
Atrophy	3 (7%)	8 (19%)	4 (9%)	6 (15%)	2 (4%)
Cyst	1 (2%)	2 (5%)	1 (2%)	4 (10%)	
Hyperplasia, lymphoid	1 (2%)	1 (2%)		1 (3%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		2 (4%)			
Edema		1 (2%)	1 (2%)	2 (4%)	
Fibrosis			1 (2%)		
Inflammation, chronic			1 (2%)		
Ulcer		2 (4%)		2 (4%)	
Epidermis, hyperplasia			2 (4%)	1 (2%)	
Musculoskeletal System					
Bone	(50)	(50)	(50)	(49)	(50)
Hyperostosis	1 (2%)	1 (2%)			
Cranium, osteopetrosis	1 (2%)	1 (2%)		2 (4%)	
Skeletal muscle	(2)	(1)	(3)	(1)	(0)
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Necrosis				1 (2%)	
Pigmentation					1 (2%)
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(50)
Foreign body			1 (2%)		1 (2%)
Hemorrhage	2 (4%)	1 (2%)	1 (2%)		5 (10%)
Infiltration cellular, histiocyte		1 (2%)	1 (2%)	1 (2%)	
Inflammation, chronic active		1 (2%)			
Metaplasia, osseous		1 (2%)	1 (2%)		1 (2%)
Thrombosis				2 (4%)	
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)	5 (10%)	4 (8%)	
Nose	(50)	(50)	(50)	(50)	(50)
Foreign body		2 (4%)	1 (2%)	2 (4%)	
Inflammation, chronic	5 (10%)	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Respiratory epithelium, hyperplasia	1 (2%)				
Special Senses System					
Eye	(50)	(50)	(50)	(50)	(50)
Atrophy	1 (2%)				
Cataract		1 (2%)			
Inflammation, chronic		3 (6%)	2 (4%)		1 (2%)
Cornea, hyperplasia		2 (4%)	1 (2%)		
Cornea, ulcer					1 (2%)
Harderian gland	(50)	(50)	(50)	(50)	(49)
Atrophy				1 (2%)	
Hyperplasia, focal	1 (2%)	4 (8%)		2 (4%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Cyst	11 (22%)	15 (30%)	9 (18%)	9 (18%)	1 (2%)
Hydronephrosis		1 (2%)			1 (2%)
Infarct	2 (4%)	3 (6%)	6 (12%)	5 (10%)	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)	6 (12%)	5 (10%)	5 (10%)	1 (2%)
Metaplasia, osseous	2 (4%)		5 (10%)	6 (12%)	1 (2%)
Necrosis			1 (2%)		
Nephropathy	40 (80%)	41 (82%)	38 (76%)	38 (76%)	16 (32%)
Artery, inflammation, chronic active				1 (2%)	
Papilla, necrosis					1 (2%)
Renal tubule, dilatation, focal					1 (2%)
Renal tubule, hyperplasia					1 (2%)
Renal tubule, pigmentation	1 (2%)	3 (6%)		1 (2%)	
Urethra	(0)	(1)	(0)	(0)	(0)
Hemorrhage		1 (100%)			
Inflammation, acute		1 (100%)			
Urinary bladder	(50)	(50)	(50)	(50)	(50)
Dilatation		1 (2%)		1 (2%)	
Infiltration cellular, lymphocyte	2 (4%)				
Transitional epithelium, hyperplasia					1 (2%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF α,β-THUJONE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental deaths	2	6			5
Moribund	4	4	2	3	8
Natural deaths	7	6	8	6	37
Survivors					
Died last week of study			1		
Terminal sacrifice	37	33	39	41	
Other		1			
Animals examined microscopically	50	49	50	50	50
Alimentary System					
Esophagus	(50)	(48)	(49)	(50)	(50)
Gallbladder	(43)	(39)	(43)	(46)	(46)
Intestine large, cecum	(48)	(46)	(46)	(49)	(49)
Intestine small, duodenum	(46)	(44)	(45)	(46)	(48)
Adenoma				1 (2%)	
Leiomyosarcoma		1 (2%)			
Intestine small, ileum	(48)	(47)	(45)	(49)	(48)
Intestine small, jejunum	(47)	(47)	(45)	(48)	(47)
Leiomyosarcoma		1 (2%)		1 (2%)	
Liver	(50)	(49)	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)		
Hepatocellular adenoma	10 (20%)	3 (6%)	9 (18%)	5 (10%)	
Hepatocellular adenoma, multiple	3 (6%)	1 (2%)	2 (4%)	3 (6%)	
Hepatocellular carcinoma	2 (4%)	7 (14%)	3 (6%)		
Hepatocellular carcinoma, multiple	1 (2%)				
Plasma cell tumor malignant	1 (2%)				
Mesentery	(10)	(6)	(5)	(6)	(0)
Hemangioma			1 (20%)		
Hemangiosarcoma			1 (20%)		
Leiomyosarcoma, metastatic, intestine small, jejunum				1 (17%)	
Schwannoma malignant, metastatic, skin	1 (10%)				
Pancreas	(48)	(49)	(48)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)			
Salivary glands	(49)	(48)	(49)	(50)	(50)
Stomach, forestomach	(50)	(49)	(48)	(50)	(50)
Hemangioma			1 (2%)		
Stomach, glandular	(48)	(49)	(47)	(48)	(50)
Tongue	(0)	(0)	(0)	(0)	(1)
Cardiovascular System					
Heart	(50)	(49)	(50)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Endocrine System					
Adrenal cortex	(50)	(49)	(49)	(50)	(50)
Adenoma	1 (2%)				
Plasma cell tumor malignant	1 (2%)				
Adrenal medulla	(50)	(47)	(46)	(48)	(49)
Pheochromocytoma benign		1 (2%)	1 (2%)		
Islets, pancreatic	(48)	(49)	(48)	(50)	(50)
Adenoma	1 (2%)	1 (2%)			
Parathyroid gland	(45)	(42)	(46)	(47)	(49)
Pituitary gland	(49)	(48)	(48)	(49)	(47)
Pars distalis, adenoma	2 (4%)	3 (6%)	3 (6%)		
Pars intermedia, adenoma			1 (2%)		
Pars intermedia, carcinoma				1 (2%)	
Thyroid gland	(50)	(48)	(48)	(49)	(49)
Follicular cell, adenoma			1 (2%)		
General Body System					
Tissue NOS	(1)	(0)	(0)	(0)	(0)
Plasma cell tumor malignant	1 (100%)				
Genital System					
Clitoral gland	(48)	(46)	(48)	(49)	(47)
Ovary	(48)	(47)	(49)	(48)	(50)
Choriocarcinoma	1 (2%)				
Cystadenoma	2 (4%)		4 (8%)	3 (6%)	
Granulosa cell tumor benign		1 (2%)			
Hemangiosarcoma			1 (2%)		
Luteoma			2 (4%)	1 (2%)	
Plasma cell tumor malignant	1 (2%)				
Uterus	(50)	(49)	(50)	(50)	(50)
Leiomyosarcoma		1 (2%)		1 (2%)	
Polyp stromal	1 (2%)	1 (2%)	1 (2%)		
Sarcoma stromal		1 (2%)			
Hematopoietic System					
Bone marrow	(50)	(49)	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)		
Mast cell tumor benign				1 (2%)	
Plasma cell tumor malignant	1 (2%)				
Lymph node	(6)	(3)	(3)	(1)	(0)
Plasma cell tumor malignant	1 (17%)				
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung		1 (33%)			
Mediastinal, plasma cell tumor malignant	1 (17%)				
Lymph node, mandibular	(48)	(46)	(45)	(48)	(49)
Plasma cell tumor malignant	1 (2%)				
Lymph node, mesenteric	(46)	(49)	(46)	(48)	(47)
Plasma cell tumor malignant	1 (2%)				
Spleen	(50)	(49)	(48)	(50)	(50)
Hemangiosarcoma	1 (2%)		2 (4%)		
Plasma cell tumor malignant	1 (2%)				
Thymus	(46)	(45)	(46)	(48)	(49)
Fibrosarcoma, metastatic, skin			1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Integumentary System					
Mammary gland	(50)	(49)	(50)	(50)	(50)
Adenoma				1 (2%)	
Carcinoma	1 (2%)	1 (2%)			
Skin	(50)	(49)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum		1 (2%)			
Subcutaneous tissue, fibrosarcoma	2 (4%)	4 (8%)	1 (2%)		
Subcutaneous tissue, hemangiosarcoma	1 (2%)		1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			1 (2%)	
Musculoskeletal System					
Bone	(50)	(49)	(50)	(50)	(50)
Skeletal muscle	(3)	(2)	(0)	(0)	(0)
Rhabdomyosarcoma	1 (33%)				
Sarcoma		2 (100%)			
Schwannoma malignant, metastatic, skin	1 (33%)				
Nervous System					
Brain	(50)	(49)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland				1 (2%)	
Cranial nerve, schwannoma malignant	1 (2%)				
Respiratory System					
Lung	(50)	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	4 (8%)	2 (4%)	2 (4%)	
Alveolar/bronchiolar adenoma, multiple	1 (2%)				
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)	2 (4%)	1 (2%)	
Alveolar/bronchiolar carcinoma, multiple			1 (2%)		
Carcinoma, metastatic, Harderian gland			1 (2%)	1 (2%)	
Carcinoma, metastatic, uncertain primary site				1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)		1 (2%)		
Plasma cell tumor malignant	1 (2%)				
Nose	(50)	(49)	(50)	(50)	(50)
Pleura	(0)	(0)	(0)	(0)	(4)
Trachea	(50)	(49)	(50)	(50)	(50)
Special Senses System					
Eye	(50)	(49)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland			1 (2%)	1 (2%)	
Harderian gland	(50)	(49)	(50)	(49)	(50)
Adenoma	5 (10%)	1 (2%)	5 (10%)	9 (18%)	1 (2%)
Carcinoma	1 (2%)	2 (4%)	4 (8%)	3 (6%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Urinary System					
Kidney	(50)	(49)	(49)	(50)	(50)
Plasma cell tumor malignant	1 (2%)				
Urinary bladder	(50)	(49)	(49)	(50)	(50)
Plasma cell tumor malignant	1 (2%)				
Systemic Lesions					
Multiple organs[b]	(50)	(49)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	2 (4%)		4 (8%)	
Lymphoma malignant	6 (12%)	9 (18%)	13 (26%)	13 (26%)	
Mesothelioma malignant			1 (2%)		
Total animals with primary neoplasms[c]	35	31	36	34	1
Total primary neoplasms	66	51	66	52	1
Total animals with benign neoplasms	22	14	23	19	1
Total benign neoplasms	28	16	33	26	1
Total animals with malignant neoplasms	19	25	25	24	
Total malignant neoplasms	38	35	33	26	
Total animals with metastatic neoplasms	2	3	2	4	
Total metastatic neoplasms	3	3	4	5	
Total animals with malignant neoplasms of uncertain primary site				1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
Harderian Gland: Adenoma				
Overall rate[b]	5/50 (10%)	1/49 (2%)	5/50 (10%)	9/50 (18%)
Adjusted rate[c]	11.6%	2.5%	10.6%	18.6%
Terminal rate[d]	5/37 (14%)	1/33 (3%)	3/40 (8%)	7/41 (17%)
First incidence (days)	729 (T)	729 (T)	638	647
Poly-3 test[e]	P=0.067	P=0.119N	P=0.572N	P=0.264
Harderian Gland: Carcinoma				
Overall rate	1/50 (2%)	2/49 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.3%	5.0%	8.5%	6.3%
Terminal rate	1/37 (3%)	1/33 (3%)	3/40 (8%)	3/41 (7%)
First incidence (days)	729 (T)	669	698	729 (T)
Poly-3 test	P=0.291	P=0.475	P=0.205	P=0.345
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	3/49 (6%)	9/50 (18%)	12/50 (24%)
Adjusted rate	13.9%	7.5%	19.0%	24.8%
Terminal rate	6/37 (16%)	2/33 (6%)	6/40 (15%)	10/41 (24%)
First incidence (days)	729 (T)	669	638	647
Poly-3 test	P=0.041	P=0.277N	P=0.358	P=0.150
Liver: Hepatocellular Adenoma				
Overall rate	13/50 (26%)	4/49 (8%)	11/49 (22%)	8/50 (16%)
Adjusted rate	30.1%	9.9%	23.8%	16.7%
Terminal rate	12/37 (32%)	3/33 (9%)	10/40 (25%)	8/41 (20%)
First incidence (days)	700	524	691	729 (T)
Poly-3 test	P=0.196N	P=0.019N	P=0.332N	P=0.102N
Liver: Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	7/49 (14%)	3/49 (6%)	0/50 (0%)
Adjusted rate	6.9%	17.2%	6.5%	0.0%
Terminal rate	2/37 (5%)	5/33 (15%)	3/40 (8%)	0/41 (0%)
First incidence (days)	683	524	729 (T)	—[f]
Poly-3 test	P=0.028N	P=0.132	P=0.632N	P=0.102N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	15/50 (30%)	10/49 (20%)	13/49 (27%)	8/50 (16%)
Adjusted rate	34.6%	24.6%	28.1%	16.7%
Terminal rate	13/37 (35%)	8/33 (24%)	12/40 (30%)	8/41 (20%)
First incidence (days)	683	524	691	729 (T)
Poly-3 test	P=0.042N	P=0.221N	P=0.333N	P=0.040N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	4/49 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	7.0%	10.0%	4.3%	4.2%
Terminal rate	3/37 (8%)	3/33 (9%)	2/40 (5%)	1/41 (2%)
First incidence (days)	729 (T)	677	729 (T)	694
Poly-3 test	P=0.265N	P=0.461	P=0.463N	P=0.451N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	3/49 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.3%	7.5%	6.4%	2.1%
Terminal rate	1/37 (3%)	2/33 (6%)	1/40 (3%)	1/41 (2%)
First incidence (days)	729 (T)	677	682	729 (T)
Poly-3 test	P=0.434N	P=0.280	P=0.338	P=0.736N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	6/49 (12%)	5/50 (10%)	3/50 (6%)
Adjusted rate	9.3%	15.0%	10.6%	6.2%
Terminal rate	4/37 (11%)	5/33 (15%)	3/40 (8%)	2/41 (5%)
First incidence (days)	729 (T)	677	682	694
Poly-3 test	P=0.254N	P=0.324	P=0.555	P=0.441N
Ovary: Cystadenoma				
Overall rate	2/48 (4%)	0/47 (0%)	4/49 (8%)	3/48 (6%)
Adjusted rate	4.9%	0.0%	8.7%	6.5%
Terminal rate	2/36 (6%)	0/32 (0%)	4/40 (10%)	3/40 (8%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.293	P=0.250N	P=0.391	P=0.550
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/49 (4%)	3/48 (6%)	3/48 (6%)	0/49 (0%)
Adjusted rate	4.6%	7.7%	6.7%	0.0%
Terminal rate	1/37 (3%)	3/32 (9%)	3/39 (8%)	0/40 (0%)
First incidence (days)	669	729 (T)	729 (T)	—
Poly-3 test	P=0.133N	P=0.451	P=0.519	P=0.220N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	2/50 (4%)	4/49 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.6%	9.8%	2.1%	0.0%
Terminal rate	0/37 (0%)	2/33 (6%)	0/40 (0%)	0/41 (0%)
First incidence (days)	628	566	617	—
Poly-3 test	P=0.070N	P=0.305	P=0.473N	P=0.217N
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	3/50 (6%)	5/49 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.9%	12.3%	4.2%	2.1%
Terminal rate	1/37 (3%)	2/33 (6%)	0/40 (0%)	1/41 (2%)
First incidence (days)	628	566	483	729 (T)
Poly-3 test	P=0.106N	P=0.319	P=0.458N	P=0.273N
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	0/49 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	8.5%	0.0%
Terminal rate	0/37 (0%)	0/33 (0%)	3/40 (8%)	0/41 (0%)
First incidence (days)	715	—	609	—
Poly-3 test	P=0.455N	P=0.516N	P=0.207	P=0.479N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	0/49 (0%)	6/50 (12%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	12.7%	0.0%
Terminal rate	0/37 (0%)	0/33 (0%)	5/40 (13%)	0/41 (0%)
First incidence (days)	715	—	609	—
Poly-3 test	P=0.482N	P=0.516N	P=0.072	P=0.479N
All Organs: Histiocytic Sarcoma				
Overall rate	2/50 (4%)	2/49 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	4.6%	5.0%	0.0%	8.3%
Terminal rate	1/37 (3%)	1/33 (3%)	0/40 (0%)	1/41 (2%)
First incidence (days)	715	694	—	710
Poly-3 test	P=0.269	P=0.667	P=0.220N	P=0.389

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	9/49 (18%)	13/50 (26%)	13/50 (26%)
Adjusted rate	13.9%	22.5%	26.8%	26.8%
Terminal rate	6/37 (16%)	8/33 (24%)	8/40 (20%)	11/41 (27%)
First incidence (days)	729 (T)	719	609	647
Poly-3 test	P=0.106	P=0.232	P=0.103	P=0.103
All Organs: Benign Neoplasms				
Overall rate	22/50 (44%)	14/49 (29%)	23/50 (46%)	19/50 (38%)
Adjusted rate	50.7%	34.4%	48.7%	39.1%
Terminal rate	20/37 (54%)	12/33 (36%)	21/40 (53%)	16/41 (39%)
First incidence (days)	669	524	638	647
Poly-3 test	P=0.271N	P=0.096N	P=0.510N	P=0.183N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	25/49 (51%)	25/50 (50%)	25/50 (50%)
Adjusted rate	41.8%	58.9%	50.0%	50.8%
Terminal rate	12/37 (32%)	16/33 (49%)	15/40 (38%)	18/41 (44%)
First incidence (days)	246	524	483	579
Poly-3 test	P=0.368	P=0.081	P=0.277	P=0.254
All Organs: Benign or Malignant Neoplasms				
Overall rate	35/50 (70%)	31/49 (63%)	36/50 (72%)	34/50 (68%)
Adjusted rate	76.7%	73.1%	72.0%	69.0%
Terminal rate	27/37 (73%)	22/33 (67%)	26/40 (65%)	27/41 (66%)
First incidence (days)	246	524	483	579
Poly-3 test	P=0.241N	P=0.443N	P=0.388N	P=0.273N

(T) Terminal sacrifice

[a] No data presented for 25 mg/kg group due to 100% mortality.

[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasms in animal group

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of α,β-Thujone[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental deaths	2	6			5
Moribund	4	4	2	3	8
Natural deaths	7	6	8	6	37
Survivors					
Died last week of study			1		
Terminal sacrifice	37	33	39	41	
Other		1			
Animals examined microscopically	50	49	50	50	50
Alimentary System					
Esophagus	(50)	(48)	(49)	(50)	(50)
Inflammation, acute		6 (13%)			4 (8%)
Ulcer		4 (8%)			2 (4%)
Submucosa, edema			1 (2%)		1 (2%)
Gallbladder	(43)	(39)	(43)	(46)	(46)
Inflammation, acute	1 (2%)				
Pigmentation				2 (4%)	
Epithelium, cytoplasmic alteration	1 (2%)				
Intestine large, cecum	(48)	(46)	(46)	(49)	(49)
Edema				2 (4%)	
Intestine small, duodenum	(46)	(44)	(45)	(46)	(48)
Diverticulum				1 (2%)	
Intestine small, ileum	(48)	(47)	(45)	(49)	(48)
Epithelium, hyperplasia				1 (2%)	
Intestine small, jejunum	(47)	(47)	(45)	(48)	(47)
Hyperplasia, lymphoid	1 (2%)			1 (2%)	
Epithelium, hyperplasia	1 (2%)				
Liver	(50)	(49)	(49)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)		
Basophilic focus	1 (2%)	2 (4%)		4 (8%)	
Clear cell focus	3 (6%)	2 (4%)	4 (8%)	2 (4%)	
Cyst	1 (2%)				
Eosinophilic focus	3 (6%)	3 (6%)	3 (6%)	4 (8%)	
Hematopoietic cell proliferation	3 (6%)	3 (6%)	3 (6%)	2 (4%)	
Infiltration cellular, lymphocyte	2 (4%)	1 (2%)		4 (8%)	
Inflammation, chronic active	4 (8%)	3 (6%)	2 (4%)	7 (14%)	
Mixed cell focus	1 (2%)	5 (10%)		5 (10%)	
Necrosis, focal	2 (4%)	6 (12%)	3 (6%)		2 (4%)
Necrosis, diffuse		1 (2%)		1 (2%)	
Tension lipidosis	1 (2%)	1 (2%)	2 (4%)	1 (2%)	
Centrilobular, necrosis		1 (2%)	2 (4%)		
Hepatocyte, hyperplasia, focal			1 (2%)		
Hepatocyte, vacuolization cytoplasmic	7 (14%)	3 (6%)	2 (4%)	2 (4%)	
Kupffer cell, hyperplasia	2 (4%)	1 (2%)	1 (2%)	1 (2%)	
Mesentery	(10)	(6)	(5)	(6)	(0)
Necrosis	1 (10%)				
Fat, necrosis	8 (80%)	6 (100%)	3 (60%)	6 (100%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Alimentary System (continued)					
Pancreas	(48)	(49)	(48)	(50)	(50)
Atrophy	2 (4%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Basophilic focus	1 (2%)	1 (2%)	1 (2%)		
Cyst	1 (2%)		2 (4%)	3 (6%)	
Infiltration cellular, lymphocyte	3 (6%)		1 (2%)	1 (2%)	
Acinus, cytoplasmic alteration	2 (4%)		1 (2%)		
Salivary glands	(49)	(48)	(49)	(50)	(50)
Infiltration cellular, lymphocyte	11 (22%)	13 (27%)	19 (39%)	13 (26%)	
Stomach, forestomach	(50)	(49)	(48)	(50)	(50)
Diverticulum		1 (2%)	1 (2%)		
Erosion				1 (2%)	
Inflammation, chronic			1 (2%)		
Epithelium, hyperplasia			1 (2%)	2 (4%)	1 (2%)
Stomach, glandular	(48)	(49)	(47)	(48)	(50)
Cyst	2 (4%)	1 (2%)	2 (4%)	2 (4%)	
Epithelium, cytoplasmic alteration				1 (2%)	
Epithelium, hyperplasia	1 (2%)			1 (2%)	
Tongue	(0)	(0)	(0)	(0)	(1)
Cyst					1 (100%)
Cardiovascular System					
Heart	(50)	(49)	(50)	(50)	(50)
Cardiomyopathy		1 (2%)			
Inflammation, acute		2 (4%)			
Mineralization	1 (2%)	2 (4%)	1 (2%)	1 (2%)	
Endocrine System					
Adrenal cortex	(50)	(49)	(49)	(50)	(50)
Accessory adrenal cortical nodule	7 (14%)	5 (10%)	6 (12%)	9 (18%)	3 (6%)
Amyloid deposition				1 (2%)	
Degeneration, fatty	1 (2%)			1 (2%)	
Hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)		
Hypertrophy, focal			1 (2%)	2 (4%)	
Capsule, hyperplasia	1 (2%)	5 (10%)		4 (8%)	
Subcapsular, cyst					1 (2%)
Subcapsular, hyperplasia, focal				1 (2%)	
Adrenal medulla	(50)	(47)	(46)	(48)	(49)
Hyperplasia	1 (2%)	1 (2%)		1 (2%)	
Islets, pancreatic	(48)	(49)	(48)	(50)	(50)
Cyst		1 (2%)			
Hyperplasia	1 (2%)		2 (4%)	4 (8%)	
Parathyroid gland	(45)	(42)	(46)	(47)	(49)
Cyst	2 (4%)		1 (2%)		4 (8%)
Hyperplasia			1 (2%)		
Pituitary gland	(49)	(48)	(48)	(49)	(47)
Pars distalis, cyst	3 (6%)	2 (4%)		3 (6%)	
Pars distalis, cytoplasmic alteration, focal	1 (2%)				
Pars distalis, hemorrhage				1 (2%)	
Pars distalis, hyperplasia, focal	9 (18%)	4 (8%)	7 (15%)	4 (8%)	
Rathke's cleft, dilatation					1 (2%)
Thyroid gland	(50)	(48)	(48)	(49)	(49)
Artery, inflammation, chronic active		1 (2%)			
Follicle, cyst	1 (2%)	2 (4%)			
Follicle, degeneration, focal	28 (56%)	15 (31%)	28 (58%)	23 (47%)	2 (4%)
Follicular cell, hyperplasia				3 (6%)	

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
General Body System					
Tissue NOS	(1)	(0)	(0)	(0)	(0)
Genital System					
Clitoral gland	(48)	(46)	(48)	(49)	(47)
Ovary	(48)	(47)	(49)	(48)	(50)
Amyloid deposition				1 (2%)	
Angiectasis	1 (2%)	1 (2%)	2 (4%)	4 (8%)	
Cyst	15 (31%)	14 (30%)	14 (29%)	14 (29%)	2 (4%)
Hemorrhage	7 (15%)	5 (11%)	13 (27%)	10 (21%)	
Inflammation, granulomatous		1 (2%)			
Mineralization		1 (2%)			
Pigmentation			2 (4%)		
Thrombosis	1 (2%)		1 (2%)		
Granulosa cell, hyperplasia		1 (2%)			
Uterus	(50)	(49)	(50)	(50)	(50)
Amyloid deposition				1 (2%)	
Angiectasis	1 (2%)	1 (2%)	2 (4%)	4 (8%)	
Infiltration cellular, histiocyte	1 (2%)				
Inflammation, suppurative	1 (2%)				
Inflammation, acute		1 (2%)		1 (2%)	
Necrosis				1 (2%)	
Pigmentation	1 (2%)				
Thrombosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Endometrium, hyperplasia, cystic	39 (78%)	26 (53%)	34 (68%)	35 (70%)	15 (30%)
Myometrium, hypertrophy		1 (2%)			
Hematopoietic System					
Bone marrow	(50)	(49)	(49)	(50)	(50)
Angiectasis		1 (2%)			
Hyperplasia	19 (38%)	20 (41%)	13 (27%)	16 (32%)	5 (10%)
Myelofibrosis		1 (2%)			
Lymph node	(6)	(3)	(3)	(1)	(0)
Bronchial, atrophy		1 (33%)			
Bronchial, pigmentation		1 (33%)			
Iliac, ectasia	1 (17%)				
Iliac, hematopoietic cell proliferation	1 (17%)				
Mediastinal, atrophy		1 (33%)			
Mediastinal, ectasia	1 (17%)				
Mediastinal, pigmentation		1 (33%)			
Lymph node, mandibular	(48)	(46)	(45)	(48)	(49)
Atrophy	1 (2%)				
Hematopoietic cell proliferation	2 (4%)		1 (2%)		1 (2%)
Hyperplasia, lymphoid	9 (19%)	6 (13%)	7 (16%)	2 (4%)	3 (6%)
Infiltration cellular, histiocyte				1 (2%)	
Pigmentation	4 (8%)	3 (7%)	4 (9%)	3 (6%)	
Lymph node, mesenteric	(46)	(49)	(46)	(48)	(47)
Atrophy		2 (4%)	1 (2%)	1 (2%)	1 (2%)
Ectasia	1 (2%)			2 (4%)	
Hematopoietic cell proliferation			1 (2%)	1 (2%)	
Hemorrhage	2 (4%)	4 (8%)	1 (2%)	4 (8%)	
Hyperplasia, lymphoid	6 (13%)	6 (12%)	2 (4%)	7 (15%)	
Infiltration cellular, histiocyte				1 (2%)	
Necrosis		1 (2%)			
Pigmentation	1 (2%)			1 (2%)	

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Hematopoietic System (continued)					
Spleen	(50)	(49)	(48)	(50)	(50)
Amyloid deposition	1 (2%)				
Atrophy				1 (2%)	
Hematopoietic cell proliferation	33 (66%)	39 (80%)	36 (75%)	37 (74%)	5 (10%)
Pigmentation	1 (2%)			1 (2%)	1 (2%)
Lymphoid follicle, atrophy				1 (2%)	
Lymphoid follicle, hyperplasia	12 (24%)	14 (29%)	12 (25%)	17 (34%)	
Thymus	(46)	(45)	(46)	(48)	(49)
Atrophy	4 (9%)	8 (18%)	5 (11%)	6 (13%)	3 (6%)
Cyst		1 (2%)			
Hemorrhage	1 (2%)	1 (2%)			
Hyperplasia, lymphoid	6 (13%)	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Inflammation, chronic active		3 (7%)			3 (6%)
Integumentary System					
Mammary gland	(50)	(49)	(50)	(50)	(50)
Hyperplasia	5 (10%)	5 (10%)	3 (6%)	6 (12%)	
Skin	(50)	(49)	(50)	(50)	(50)
Cyst epithelial inclusion				1 (2%)	
Edema		1 (2%)		1 (2%)	
Hemorrhage					1 (2%)
Inflammation, chronic active		1 (2%)			
Ulcer	2 (4%)	1 (2%)			
Subcutaneous tissue, inflammation, chronic active		1 (2%)			
Musculoskeletal System					
Bone	(50)	(49)	(50)	(50)	(50)
Fibrosis	1 (2%)	2 (4%)	1 (2%)	3 (6%)	
Fracture	1 (2%)		2 (4%)		
Femur, hyperostosis	1 (2%)				
Skeletal muscle	(3)	(2)	(0)	(0)	(0)
Nervous System					
Brain	(50)	(49)	(50)	(50)	(50)
Compression	1 (2%)			1 (2%)	
Cyst epithelial inclusion	1 (2%)				
Hemorrhage				1 (2%)	1 (2%)
Necrosis	1 (2%)			1 (2%)	
Artery, meninges, inflammation, chronic active		1 (2%)			

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of α,β-Thujone

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Respiratory System					
Lung	(50)	(49)	(50)	(50)	(50)
Fibrosis		1 (2%)			
Foreign body		8 (16%)			5 (10%)
Hemorrhage	2 (4%)		1 (2%)	7 (14%)	6 (12%)
Infiltration cellular, histiocyte			1 (2%)		
Infiltration cellular, lymphocyte	2 (4%)		2 (4%)	4 (8%)	
Inflammation, suppurative		1 (2%)			
Inflammation, granulomatous		1 (2%)			
Inflammation, acute		4 (8%)	1 (2%)		
Inflammation, chronic	1 (2%)				1 (2%)
Inflammation, chronic active		2 (4%)			1 (2%)
Metaplasia, osseous			1 (2%)		
Thrombosis	1 (2%)			1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)		
Arteriole, hypertrophy					1 (2%)
Nose	(50)	(49)	(50)	(50)	(50)
Foreign body	1 (2%)	4 (8%)			
Inflammation, chronic	2 (4%)	4 (8%)	2 (4%)		
Pleura	(0)	(0)	(0)	(0)	(4)
Inflammation, acute					4 (100%)
Trachea	(50)	(49)	(50)	(50)	(50)
Inflammation, chronic active					1 (2%)
Special Senses System					
Eye	(50)	(49)	(50)	(50)	(50)
Atrophy		1 (2%)		1 (2%)	
Cataract			2 (4%)		
Inflammation, chronic	1 (2%)	2 (4%)	2 (4%)	2 (4%)	
Cornea, hyperplasia		1 (2%)	2 (4%)	1 (2%)	
Harderian gland	(50)	(49)	(50)	(49)	(50)
Cyst			1 (2%)		
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, lymphocyte			1 (2%)		
Urinary System					
Kidney	(50)	(49)	(49)	(50)	(50)
Amyloid deposition				1 (2%)	
Cyst	3 (6%)	3 (6%)		1 (2%)	
Glomerulosclerosis		1 (2%)			
Infarct	2 (4%)	5 (10%)	6 (12%)	1 (2%)	
Infiltration cellular, lymphocyte	3 (6%)	9 (18%)	5 (10%)	5 (10%)	
Inflammation, chronic	1 (2%)				
Metaplasia, osseous	1 (2%)	1 (2%)	2 (4%)	3 (6%)	
Mineralization				1 (2%)	
Nephropathy	11 (22%)	11 (22%)	5 (10%)	7 (14%)	3 (6%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	2 (4%)	1 (2%)	2 (4%)	
Renal tubule, cytoplasmic alteration			1 (2%)		
Renal tubule, pigmentation	1 (2%)		2 (4%)		
Urinary bladder	(50)	(49)	(49)	(50)	(50)
Infiltration cellular, lymphocyte	2 (4%)	1 (2%)	2 (4%)	4 (8%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

One independent assay for bacterial mutagenicity was conducted with α,β-thujone, and two independent assays were conducted with α-thujone. Assays on α,β-thujone and α-thujone performed at BioReliance Corporation (Rockville, MD) followed protocols reported by Zeiger *et al.* (1992) and used *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver). The assay on α,β-thujone was conducted with the same lot (E58/L-2) used in the 2-year studies. An assay on α-thujone performed at SITEK Research Laboratories (Rockville, MD) used a slightly modified protocol (activation only with rat liver S9) and used *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. In all assays, the test article was sent to the laboratory as a coded aliquot and incubated with the bacterial tester strains for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of test article. For α,β-thujone, the high dose was limited by toxicity to 1,000 µg/plate; for α-thujone, concentrations up to 10,000 µg/plate were tested. All trials were repeated at the same or higher S9 concentration.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs, or mature erythrocytes) in up to five animals in the 0, 6.25, 12.5, 25, and 50 (females) mg/kg groups. In addition, the percentage of polychromatic erythrocytes (PCEs, or reticulocytes) among 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

Neither α,β-thujone (1 to 1,000 µg/plate; lot E58/L-2 used in the 2-year studies) nor α-thujone (10 to 10,000 µg/plate) was mutagenic in any of several bacterial tester strains (*S. typhimurium* strains TA97, TA98, TA100, and TA1535 and *E. coli* strain WP2 *uvrA*/pKM101) when testing was conducted with or without exogenous metabolic activation provided by rat or hamster liver S9 mix (Tables E1, E2, and E3). *In vivo*, daily exposure by gavage to α,β-thujone (6.25 to 25 mg/kg) for 3 months did not result in an increase in micronucleated erythrocytes (NCEs) in the peripheral blood of male mice (Table E4). However, female mice (6.25 to 50 mg/kg) had a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-month study; both a significant trend (P=0.006) and a significant elevation in frequency (P=0.0015) of micronucleated erythrocytes was observed in the female 50 mg/kg group. No significant changes in the percentage of reticulocytes (PCEs) among total erythrocytes was seen in either male or female mice at the end of the 3-month study, suggesting that α,β-thujone did not induce bone marrow toxicity.

TABLE E1
Mutagenicity of α,β-Thujone (lot E58/L-2) in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA100	0	179 ± 6	173 ± 16	145 ± 3	182 ± 3	143 ± 0[b]	146 ± 5
	1	193 ± 5					
	3.3	167 ± 15	185 ± 8	158 ± 16	215 ± 11	174 ± 7	155 ± 1
	10	166 ± 14	157 ± 5	176 ± 7	213 ± 9	167 ± 9	166 ± 12
	33	142 ± 17	128 ± 5	180 ± 6	187 ± 38	155 ± 5	166 ± 7
	50	163 ± 8					
	100		154 ± 3	161 ± 5	177 ± 5	153 ± 9	169 ± 9
	200				165 ± 7.0		136 ± 6.0
	333		Toxic	108 ± 2.0[c]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		622 ± 12	488 ± 3	454 ± 21	1,430 ± 69	665 ± 40	626 ± 8
TA1535	0	10 ± 4	13 ± 2	14 ± 3	11 ± 1	19 ± 2	11 ± 2
	1	9 ± 1					
	3.3	9 ± 2	18 ± 3		13 ± 2		11 ± 1
	10	10 ± 2	17 ± 3	16 ± 2	14 ± 1	17 ± 1	12 ± 1
	33	11 ± 3	12 ± 2	17 ± 2	11 ± 2	14 ± 2	14 ± 2
	100	11 ± 0	12 ± 1[c]	14 ± 3	9 ± 1	20 ± 1	13 ± 1
	333		0 ± 0[c]	16 ± 3[c]	10 ± 1	14 ± 3[c]	12 ± 2
	1,000			0 ± 0[c]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		137 ± 4	257 ± 17	116 ± 9	161 ± 23	102 ± 3	66 ± 5
TA97	0	123 ± 2	158 ± 11	144 ± 2	126 ± 8	147 ± 9	156 ± 9
	1	127 ± 10					
	3.3	118 ± 6	163 ± 4		115 ± 11		159 ± 6
	10	114 ± 10	188 ± 3	169 ± 20	132 ± 11	154 ± 13	169 ± 7
	33	116 ± 6	172 ± 13	160 ± 9	139 ± 11	138 ± 20	169 ± 8
	100	134 ± 8	191 ± 10	177 ± 6	129 ± 10	135 ± 8	157 ± 11
	333		Toxic	128 ± 4[c]	131 ± 10[c]	114 ± 3[c]	141 ± 13
	1,000			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		287 ± 22	883 ± 14	1,389 ± 48	921 ± 91	1,006 ± 65	413 ± 38

TABLE E1
Mutagenicity of α,β-Thujone (lot E58/L-2) in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA98	0	16 ± 1	20 ± 2	26 ± 4	15 ± 2	23 ± 4	15 ± 1
	1	13 ± 1					
	3.3	14 ± 1	16 ± 1		15 ± 1		17 ± 1
	10	16 ± 1	19 ± 1	30 ± 3	17 ± 2	24 ± 2	19 ± 1
	33	16 ± 0	13 ± 1	26 ± 3	15 ± 1	20 ± 2	16 ± 1
	50	15 ± 0					
	100		20 ± 5	23 ± 1	16 ± 2	25 ± 2	14 ± 1
	200				17 ± 1		16 ± 1
	333		0 ± 0[c]	23 ± 4[c]		21 ± 1	
	1,000			0 ± 0[c]		0 ± 0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		157 ± 41	98 ± 10	925 ± 94	1,068 ± 40	321 ± 29	370 ± 60

[a] Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al*. (1992). 0 μg/plate was the solvent control.
[b] Contamination
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Mutagenicity of α-Thujone in Bacterial Tester Strains[a]

Strain	Dose (μg/plate)	Without S9	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	48 ± 3	59 ± 15		67 ± 8	57 ± 4
	10	51 ± 4	31 ± 4			
	50	53 ± 2	31 ± 4		75 ± 7	54 ± 3
	100	40 ± 2	51 ± 2		66 ± 3	61 ± 5
	250	35 ± 1	39 ± 4			
	500	16 ± 3	13 ± 3		51 ± 8	53 ± 6
	1,000				25 ± 6	28 ± 4
	1,500				2 ± 2	
	2,000					Toxic
Trial summary		Negative	Negative		Negative	Negative
Positive control[b]		574 ± 1	570 ± 13		1,121 ± 88	544 ± 7
TA98	0	28 ± 1	13 ± 1	15 ± 1	22 ± 4	18 ± 4
	10	26 ± 1	13 ± 1	22 ± 2		
	50	23 ± 2	13 ± 2	24 ± 2	26 ± 4	23 ± 3
	100	27 ± 2	18 ± 4	28 ± 5	20 ± 4	18 ± 3
	250	27 ± 2	4 ± 1	7 ± 1[c]		
	350		1 ± 1	7 ± 2[c]		
	500	Toxic		5 ± 2[c]	25 ± 5	31 ± 8
	1,000				21 ± 4	26 ± 2
	1,500					6 ± 2
	2,000				Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		468 ± 17	572 ± 8	471 ± 24	1,008 ± 32	1,261 ± 30
***Escherichia coli* WP2 *uvrA*/pKM101**						
	0	164 ± 15	139 ± 2		160 ± 13	208 ± 7
	10	174 ± 27	143 ± 8			
	50	144 ± 2	161 ± 6		210 ± 32	228 ± 13
	100	142 ± 13	175 ± 15		139 ± 12	212 ± 3
	250	168 ± 17	174 ± 7			
	500	72 ± 9	131 ± 4		137 ± 5	183 ± 11
	1,000				95 ± 10	173 ± 12
	1,500				79 ± 11	
	2,000					Toxic
Trial summary		Negative	Negative		Negative	Negative
Positive control		854 ± 66	1,529 ± 121		821 ± 28	1,204 ± 24

[a] Study was performed at SITEK Research Laboratories. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 μg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

[c] Precipitate on plate

TABLE E3
Mutagenicity of α-Thujone in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA100	0	92 ± 2	203 ± 12	137 ± 8	124 ± 8	160 ± 32	103 ± 2
	10	90 ± 3	191 ± 6	138 ± 5	108 ± 7	170 ± 14	128 ± 10
	33	80 ± 6	202 ± 14	146 ± 15	114 ± 3	182 ± 15	125 ± 6
	100	100 ± 12	186 ± 1[b]	141 ± 7	112 ± 8	185 ± 1	126 ± 5
	333	42 ± 12[c]	123 ± 6	112 ± 4	101 ± 0	163 ± 12	116 ± 6
	500	56 ± 5[c]			68 ± 17[c]		57 ± 11[c]
	1,000		Toxic	5 ±3[c]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		375 ± 6	465 ± 33	282 ± 6	596 ± 10	361 ± 10	699 ± 27
TA1535	0	8 ± 1	10 ± 1	10 ± 2	8 ± 1	12 ± 3	15 ± 4
	10	12 ± 2	10 ± 3	11 ± 2	13 ± 2	8 ± 1	14 ± 2
	33	10 ± 2	9 ± 1	10 ± 1	7 ± 4	9 ± 1	9 ± 1
	100	10 ± 0	9 ± 1	11 ± 3	13 ± 0	11 ± 2	15 ± 2
	333	11 ± 1[c]	6 ± 1	7 ± 1	16 ± 3	10 ± 2	12 ± 1
	1,000	0 ± 0[c]	Toxic	Toxic	Toxic	Toxic	1 ± 1[c]
	3,333	Toxic			Toxic		Toxic
	10,000	0 ± 0[e]			0 ± 0[e]		0 ± 0[e]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		226 ± 13	169 ± 29	34 ± 1	64 ± 1	176 ± 17	158 ± 20
TA97	0	122 ± 16	130 ± 3	179 ± 8	236 ± 13	171 ± 7	232 ± 4
	10	124 ± 5	154 ± 0	199 ± 17	288 ± 23	179 ± 10	283 ± 20
	33	118 ± 7	148 ± 9	176 ± 8	236 ± 22	169 ± 6	200 ± 6
	100	118 ± 7	142 ± 11	164 ± 10	291 ± 7	181 ± 7	197 ± 11
	333	151 ± 10	89 ± 8[c]	155 ± 13	242 ± 13	115 ± 20	222 ± 34[c]
	500	97 ± 12[c]			152 ± 29[c]		129 ± 5[c]
	1,000		Toxic	15 ± 3[c]		13 ± 4[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		705 ± 65	435 ± 44	548 ± 24	1,322 ± 90	1,652 ± 103	790 ± 97
TA98	0	21 ± 2	15 ± 2	14 ± 1	20 ± 1	20 ± 3	21 ± 2
	10	15 ± 1	12 ± 3	22 ± 4	23 ± 1	22 ± 3	17 ± 2
	33	20 ± 3	9 ± 2	16 ± 2	19 ± 3	14 ± 3	18 ± 1
	100	13 ± 0	12 ± 1	22 ± 3	20 ± 3	19 ± 1	18 ± 1
	333	5 ± 1[c]	8 ± 1	21 ± 2	16 ± 1	12 ± 2	18 ± 1
	500	Toxic			4 ± 1[c]	2 ± 1[c]	6 ± 3[c]
	1,000		Toxic	Toxic			
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		69 ± 4	67 ± 5	219 ± 7	507 ± 39	156 ± 8	199 ± 10

[a] Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Contamination
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] Slight toxicity and precipitate on plate

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of α,β-Thujone by Gavage for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
0.5% Methylcellulose[d]	0	5	1.90 ± 0.19		2.160 ± 0.11
α,β-Thujone	6.25	5	2.70 ± 0.60	0.1188	2.260 ± 0.07
	12.5	5	2.30 ± 0.34	0.2683	2.300 ± 0.10
	25	5	2.70 ± 0.25	0.1188	2.180 ± 0.07
			P=0.185[e]		
Female					
0.5% Methylcellulose	0	5	0.70 ± 0.20		2.240 ± 0.17
α,β-Thujone	6.25	5	1.70 ± 0.12	0.0206	2.120 ± 0.20
	12.5	5	1.70 ± 0.30	0.0206	2.300 ± 0.10
	25	5	1.90 ± 0.19	0.0093	2.500 ± 0.14
	50	3	2.50 ± 0.29	0.0015	2.433 ± 0.12
			P=0.006		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.008 for male mice and P≤0.006 for female mice.
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Male						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	8	2
Hematology						
Hematocrit (spun) (%)						
Day 4	43.5 ± 0.5	43.1 ± 0.5	43.0 ± 0.7	42.7 ± 0.6	43.3 ± 0.8	42.9 ± 0.6
Day 25	46.6 ± 0.6	45.4 ± 0.4	45.0 ± 0.4	45.3 ± 0.3	45.0 ± 0.3	45.8 ± 0.5
Week 14	44.9 ± 0.4	43.4 ± 0.5	43.5 ± 0.4	42.4 ± 0.7*	43.1 ± 0.3	43.7 ± 3.7
Hematocrit (auto) (%)						
Day 4	43.6 ± 0.6	42.9 ± 0.6	43.2 ± 0.7	42.8 ± 0.6	43.4 ± 0.9	43.1 ± 0.6
Day 25	46.8 ± 0.6	45.9 ± 0.5	46.0 ± 0.4	45.8 ± 0.4	45.9 ± 0.4	46.4 ± 0.5
Week 14	45.7 ± 0.6	44.3 ± 0.5	44.4 ± 0.4	43.2 ± 0.7	43.7 ± 0.5	44.0 ± 3.6
Hemoglobin (g/dL)						
Day 4	14.4 ± 0.2	14.3 ± 0.2	14.3 ± 0.2	14.2 ± 0.2	14.4 ± 0.3	14.3 ± 0.2
Day 25	15.5 ± 0.2	15.2 ± 0.2	15.3 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.4 ± 0.1
Week 14	15.2 ± 0.2	14.7 ± 0.1	14.7 ± 0.1	14.3 ± 0.2	14.3 ± 0.1	14.6 ± 1.1
Erythrocytes (10^6/μL)						
Day 4	7.61 ± 0.10	7.46 ± 0.10	7.55 ± 0.14	7.47 ± 0.10	7.56 ± 0.12	7.53 ± 0.12
Day 25	8.17 ± 0.10	8.04 ± 0.08	8.06 ± 0.07	8.05 ± 0.06	8.03 ± 0.07	8.14 ± 0.10
Week 14	8.82 ± 0.09	8.53 ± 0.09	8.58 ± 0.07	8.36 ± 0.14	8.39 ± 0.07	8.41 ± 0.66
Reticulocytes (10^5/μL)						
Day 4	5.20 ± 0.24	5.27 ± 0.14	5.13 ± 0.13	5.07 ± 0.17	5.19 ± 0.22	5.04 ± 0.33
Day 25	2.52 ± 0.09	2.83 ± 0.11	2.91 ± 0.10*	2.74 ± 0.11	2.74 ± 0.09	2.80 ± 0.15
Week 14	2.16 ± 0.04	2.42 ± 0.05*	2.34 ± 0.06	2.05 ± 0.10	2.12 ± 0.16	2.75 ± 0.62
Reticulocytes (%)						
Day 4	6.82 ± 0.31	7.09 ± 0.22	6.83 ± 0.25	6.78 ± 0.18	6.87 ± 0.29	6.73 ± 0.47
Day 25	3.07 ± 0.13	3.51 ± 0.14	3.63 ± 0.13*	3.41 ± 0.13	3.41 ± 0.12	3.45 ± 0.20
Week 14	2.45 ± 0.07	2.84 ± 0.06*	2.73 ± 0.07	2.47 ± 0.12	2.53 ± 0.19	3.30 ± 1.00
Nucleated erythrocytes/100 leukocytes						
Day 4	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.40 ± 0.2
Day 25	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.0
Week 14	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	1.00 ± 1.0
Mean cell volume (fL)						
Day 4	57.3 ± 0.2	57.5 ± 0.1	57.2 ± 0.3	57.4 ± 0.2	57.4 ± 0.4	57.2 ± 0.4
Day 25	57.2 ± 0.3	57.2 ± 0.4	57.0 ± 0.2	56.9 ± 0.3	57.2 ± 0.4	57.0 ± 0.4
Week 14	51.8 ± 0.2	51.9 ± 0.3	51.7 ± 0.1	51.7 ± 0.1	52.1 ± 0.1	52.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.0 ± 0.1	19.2 ± 0.1	18.9 ± 0.2	19.1 ± 0.1	19.1 ± 0.1	19.0 ± 0.1
Day 25	19.0 ± 0.1	18.9 ± 0.1	19.1 ± 0.2	18.9 ± 0.1	19.0 ± 0.2	19.0 ± 0.2
Week 14	17.2 ± 0.1	17.3 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	17.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.2 ± 0.1	33.3 ± 0.1	33.1 ± 0.3	33.2 ± 0.1	33.3 ± 0.1	33.3 ± 0.1
Day 25	33.2 ± 0.3	33.1 ± 0.2	33.4 ± 0.2	33.2 ± 0.2	33.2 ± 0.1	33.3 ± 0.3
Week 14	33.3 ± 0.1	33.2 ± 0.1	33.2 ± 0.1	33.1 ± 0.1	32.8 ± 0.2	33.3 ± 0.2
Platelets (10^3/μL)						
Day 4	980.0 ± 20.8	973.7 ± 19.1	928.8 ± 30.2	1,033.1 ± 21.5	936.9 ± 20.6	962.2 ± 26.0
Day 25	936.0 ± 16.6	911.2 ± 25.0	905.8 ± 21.9	900.3 ± 23.9	906.5 ± 12.5	914.3 ± 30.4
Week 14	667.0 ± 9.9	683.4 ± 13.3	729.0 ± 12.1*	713.8 ± 28.5	656.8 ± 16.6	882.0 ± 151.0

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Male (continued)						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	8	2
Hematology (continued)						
Leukocytes (10^3/μL)						
Day 4	9.03 ± 0.27	8.26 ± 0.14	8.70 ± 0.31	8.75 ± 0.34	8.70 ± 0.26	8.80 ± 0.37
Day 25	10.58 ± 0.22	10.55 ± 0.31	10.41 ± 0.30	10.80 ± 0.20	10.48 ± 0.34	10.94 ± 0.30
Week 14	10.11 ± 0.30	10.43 ± 0.36	11.31 ± 0.39	12.17 ± 0.38	11.31 ± 0.37	10.31 ± 0.25
Segmented neutrophils (10^3/μL)						
Day 4	0.85 ± 0.03	0.78 ± 0.03	0.81 ± 0.02	0.82 ± 0.04	0.83 ± 0.02	0.80 ± 0.03
Day 25	0.98 ± 0.07	1.10 ± 0.03	1.03 ± 0.04	1.15 ± 0.05	1.18 ± 0.04**	1.03 ± 0.02
Week 14	1.39 ± 0.04	1.45 ± 0.03	1.44 ± 0.03	1.82 ± 0.24	1.81 ± 0.15	1.62 ± 0.32
Lymphocytes (10^3/μL)						
Day 4	7.93 ± 0.25	7.24 ± 0.12	7.65 ± 0.30	7.68 ± 0.30	7.62 ± 0.24	7.73 ± 0.36
Day 25	9.31 ± 0.24	9.15 ± 0.29	9.07 ± 0.27	9.32 ± 0.16	8.96 ± 0.33	9.59 ± 0.27
Week 14	8.34 ± 0.27	8.64 ± 0.33	9.48 ± 0.37	9.87 ± 0.26*	9.15 ± 0.36	8.34 ± 0.13
Monocytes (10^3/μL)						
Day 4	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.15 ± 0.01
Day 25	0.15 ± 0.02	0.16 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.02
Week 14	0.20 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.23 ± 0.02	0.17 ± 0.02	0.20 ± 0.04
Basophils (10^3/μL)						
Day 4	0.029 ± 0.003	0.031 ± 0.004	0.032 ± 0.002	0.036 ± 0.005	0.036 ± 0.004	0.036 ± 0.004
Day 25	0.030 ± 0.003	0.027 ± 0.002	0.031 ± 0.004	0.035 ± 0.003	0.032 ± 0.004	0.027 ± 0.002
Week 14	0.041 ± 0.006	0.041 ± 0.003	0.053 ± 0.008	0.064 ± 0.010	0.046 ± 0.011	0.035 ± 0.005
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Day 25	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.07 ± 0.01*	0.07 ± 0.00**	0.06 ± 0.00*
Week 14	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
Large unstained cells (10^3/μL)						
Day 4	0.050 ± 0.003	0.049 ± 0.005	0.046 ± 0.003	0.050 ± 0.005	0.045 ± 0.003	0.054 ± 0.004
Day 25	0.057 ± 0.005	0.058 ± 0.006	0.059 ± 0.008	0.060 ± 0.007	0.065 ± 0.008	0.063 ± 0.005
Week 14	0.064 ± 0.006	0.060 ± 0.005	0.078 ± 0.010	0.089 ± 0.011	0.068 ± 0.013	0.050 ± 0.010
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	15.9 ± 0.4	15.4 ± 0.6	15.8 ± 0.3	16.1 ± 0.4	15.9 ± 0.5	16.1 ± 0.7
Day 25	21.5 ± 0.4	17.7 ± 0.5**	19.7 ± 0.6	18.6 ± 0.6**	19.6 ± 0.8	19.1 ± 0.5
Week 14	14.6 ± 0.5	16.3 ± 0.9	15.6 ± 0.5	16.1 ± 0.7	13.3 ± 0.7	15.9 ± 1.1
Creatinine (mg/dL)						
Day 4	0.50 ± 0.00	0.49 ± 0.01	0.51 ± 0.01	0.49 ± 0.01	0.48 ± 0.01	0.50 ± 0.00
Day 25	0.60 ± 0.01	0.61 ± 0.01	0.60 ± 0.00	0.59 ± 0.01	0.61 ± 0.01	0.62 ± 0.01
Week 14	0.61 ± 0.01	0.66 ± 0.02*	0.71 ± 0.03**	0.73 ± 0.04**	0.71 ± 0.01**	0.70 ± 0.00*
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.9 ± 0.1
Day 25	6.3 ± 0.1	6.2 ± 0.1	6.4 ± 0.0	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1
Week 14	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.3 ± 0.2	6.5 ± 0.2
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.2 ± 0.1
Day 25	4.3 ± 0.0	4.2 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0
Week 14	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.5 ± 0.1

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Male (continued)						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	8	2
Clinical Chemistry (continued)						
Alanine aminotransferase (IU/L)						
Day 4	53 ± 2	56 ± 2	57 ± 2	54 ± 1	57 ± 2	58 ± 3
Day 25	62 ± 1	55 ± 1**	60 ± 2	56 ± 1*	61 ± 2	60 ± 1
Week 14	109 ± 8	71 ± 4**	61 ± 3**	56 ± 6**	50 ± 4**	33 ± 13**
Alkaline phosphatase (IU/L)						
Day 4	640 ± 14	622 ± 6	622 ± 10	616 ± 7	607 ± 10	587 ± 15*
Day 25	400 ± 6	390 ± 5	399 ± 6	388 ± 6	377 ± 5	383 ± 6
Week 14	220 ± 5	232 ± 5	228 ± 5	221 ± 9	214 ± 17	240 ± 13
Creatine kinase (IU/L)						
Day 4	589 ± 32	541 ± 33	525 ± 39	540 ± 31	597 ± 41	503 ± 24
Day 25	243 ± 25	276 ± 22	249 ± 21	252 ± 28	285 ± 24	240 ± 9
Week 14	204 ± 21	192 ± 17	182 ± 21	160 ± 14	157 ± 13	161 ± 48
Sorbitol dehydrogenase (IU/L)						
Day 4	5 ± 1	6 ± 1	8 ± 1[b]	7 ± 1	4 ± 1	7 ± 1
Day 25	12 ± 1	12 ± 1	14 ± 1	13 ± 1	11 ± 1	11 ± 1
Week 14	30 ± 2	21 ± 1**	19 ± 1**	15 ± 2**	15 ± 2**	12 ± 2**
Bile acids (µmol/L)						
Day 4	25.7 ± 1.7	23.7 ± 1.2	20.9 ± 0.7	25.9 ± 1.6	22.6 ± 0.8	22.4 ± 1.2
Day 25	25.3 ± 2.3	23.3 ± 1.6	25.2 ± 1.6	23.7 ± 1.4	25.8 ± 1.6	23.9 ± 1.5
Week 14	24.4 ± 3.8	19.8 ± 2.6	20.7 ± 2.3	27.3 ± 3.0	18.6 ± 2.1	18.6 ± 1.5
Female						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	9	8
Week 14	10	10	10	10	2	1[c]
Hematology						
Hematocrit (spun) (%)						
Day 4	43.9 ± 0.4	43.7 ± 0.8	44.3 ± 0.5	44.2 ± 0.6	44.2 ± 0.9	44.0 ± 0.6
Day 25	45.2 ± 0.5	45.5 ± 0.5	45.5 ± 0.5	45.7 ± 0.8	44.8 ± 0.6	45.1 ± 0.4
Week 14	44.1 ± 0.3	43.5 ± 0.5	44.7 ± 0.2	43.4 ± 0.6	42.3 ± 1.8	41.5
Hematocrit (auto) (%)						
Day 4	44.3 ± 0.5	44.3 ± 0.7	44.6 ± 0.5	44.8 ± 0.7	44.8 ± 0.8	44.0 ± 0.6
Day 25	46.0 ± 0.5	47.0 ± 0.7	46.8 ± 0.5	46.7 ± 0.7	45.8 ± 0.5	46.3 ± 0.6
Week 14	43.3 ± 0.4	43.0 ± 0.5	43.6 ± 0.3	42.4 ± 0.6	40.5 ± 1.5	41.1
Hemoglobin (g/dL)						
Day 4	15.1 ± 0.1	15.0 ± 0.2	15.1 ± 0.2	15.2 ± 0.2	15.2 ± 0.2	15.0 ± 0.2
Day 25	15.5 ± 0.2	15.8 ± 0.2	15.7 ± 0.2	15.7 ± 0.2	15.5 ± 0.2	15.6 ± 0.1
Week 14	14.4 ± 0.1	14.4 ± 0.2	14.5 ± 0.1	14.1 ± 0.2	13.7 ± 0.4	13.7
Erythrocytes (10^6/µL)						
Day 4	7.81 ± 0.08	7.83 ± 0.12	7.91 ± 0.09	7.91 ± 0.11	7.94 ± 0.13	7.81 ± 0.11
Day 25	8.14 ± 0.09	8.33 ± 0.10	8.23 ± 0.09	8.22 ± 0.11	8.10 ± 0.08	8.18 ± 0.11
Week 14	8.48 ± 0.07	8.42 ± 0.12	8.53 ± 0.05	8.18 ± 0.11	8.00 ± 0.22	8.02

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Female (continued)						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	9	8
Week 14	10	10	10	10	2	1
Hematology (continued)						
Reticulocytes (10^5/μL)						
Day 4	4.05 ± 0.29	4.24 ± 0.14	4.40 ± 0.17	4.57 ± 0.25	4.39 ± 0.24	4.35 ± 0.15
Day 25	1.93 ± 0.05	1.90 ± 0.03	1.99 ± 0.05	1.78 ± 0.05	1.93 ± 0.05	1.87 ± 0.08
Week 14	1.90 ± 0.08	1.82 ± 0.12	1.74 ± 0.07	1.99 ± 0.21	1.99 ± 0.01	1.83
Reticulocytes (%)						
Day 4	5.19 ± 0.37	5.43 ± 0.21	5.57 ± 0.22	5.78 ± 0.30	5.54 ± 0.29	5.60 ± 0.25
Day 25	2.39 ± 0.08	2.28 ± 0.05	2.42 ± 0.06	2.18 ± 0.07	2.38 ± 0.07	2.29 ± 0.08
Week 14	2.24 ± 0.10	2.18 ± 0.17	2.05 ± 0.09	2.45 ± 0.27	2.50 ± 0.10	2.30
Nucleated erythrocytes/100 leukocytes						
Day 4	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.2
Day 25	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Week 14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)						
Day 4	56.7 ± 0.2	56.6 ± 0.3	56.4 ± 0.2	56.7 ± 0.2	56.4 ± 0.3	56.3 ± 0.2
Day 25	56.6 ± 0.4	56.4 ± 0.3	56.9 ± 0.3	56.8 ± 0.2	56.5 ± 0.3	56.6 ± 0.3
Week 14	51.1 ± 0.2	51.1 ± 0.3	51.1 ± 0.1	51.9 ± 0.2	50.7 ± 0.5	51.2
Mean cell hemoglobin (pg)						
Day 4	19.3 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1
Day 25	19.0 ± 0.1	19.0 ± 0.1	19.0 ± 0.1	19.1 ± 0.1	19.1 ± 0.1	19.1 ± 0.2
Week 14	17.0 ± 0.1	17.1 ± 0.1	17.0 ± 0.0	17.3 ± 0.1	17.1 ± 0.1	17.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.1 ± 0.2	33.9 ± 0.2	33.9 ± 0.1	34.0 ± 0.1	34.0 ± 0.2	34.1 ± 0.1
Day 25	33.7 ± 0.1	33.6 ± 0.2	33.5 ± 0.2	33.6 ± 0.2	33.8 ± 0.2	33.7 ± 0.2
Week 14	33.3 ± 0.2	33.4 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.7 ± 0.4	33.5
Platelets (10^3/μL)						
Day 4	934.9 ± 31.7	935.0 ± 21.7	1,004.4 ± 25.0	1,009.0 ± 27.8	947.2 ± 35.3	1,055.1 ± 25.4**
Day 25	897.5 ± 25.4	881.8 ± 18.6	894.3 ± 21.2	933.4 ± 20.7	890.1 ± 24.1	876.9 ± 27.7
Week 14	683.6 ± 20.8	675.6 ± 30.8	713.3 ± 17.1	741.6 ± 23.1	672.5 ± 21.5	635.0
Leukocytes (10^3/μL)						
Day 4	9.03 ± 0.46	8.17 ± 0.29	8.58 ± 0.44	8.79 ± 0.30	8.62 ± 0.54	8.05 ± 0.46
Day 25	8.91 ± 0.46	9.72 ± 0.57	9.42 ± 0.41	9.14 ± 0.44	9.19 ± 0.65	9.71 ± 0.45
Week 14	8.28 ± 0.37	8.56 ± 0.69	8.21 ± 0.43	8.75 ± 0.34	10.78 ± 0.45	7.63
Segmented neutrophils (10^3/μL)						
Day 4	0.87 ± 0.06	0.89 ± 0.04	0.89 ± 0.04	0.94 ± 0.04	0.98 ± 0.08	0.88 ± 0.03
Day 25	0.94 ± 0.05	1.03 ± 0.07	1.03 ± 0.07	1.00 ± 0.05	1.04 ± 0.04	1.02 ± 0.07
Week 14	1.13 ± 0.04	1.19 ± 0.07	1.13 ± 0.08	1.16 ± 0.08	1.13 ± 0.03	1.20
Lymphocytes (10^3/μL)						
Day 4	7.90 ± 0.43	7.06 ± 0.26	7.43 ± 0.39	7.59 ± 0.29	7.38 ± 0.49	6.91 ± 0.44
Day 25	7.65 ± 0.40	8.37 ± 0.52	8.08 ± 0.33	7.87 ± 0.40	7.86 ± 0.61	8.40 ± 0.40
Week 14	6.85 ± 0.31	7.05 ± 0.61	6.79 ± 0.37	7.27 ± 0.36	9.32 ± 0.46	6.15
Monocytes (10^3/μL)						
Day 4	0.13 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.16 ± 0.02
Day 25	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.13 ± 0.01	0.15 ± 0.01	0.15 ± 0.02
Week 14	0.16 ± 0.02	0.13 ± 0.01	0.15 ± 0.02	0.18 ± 0.02	0.15 ± 0.01	0.10
Basophils (10^3/μL)						
Day 4	0.036 ± 0.004	0.029 ± 0.004	0.036 ± 0.004	0.040 ± 0.003	0.035 ± 0.004	0.034 ± 0.004
Day 25	0.026 ± 0.004	0.028 ± 0.002	0.023 ± 0.002	0.027 ± 0.003	0.024 ± 0.003	0.026 ± 0.003
Week 14	0.028 ± 0.006	0.042 ± 0.009	0.037 ± 0.010	0.027 ± 0.004	0.045 ± 0.005	0.070

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Female (continued)						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	9	8
Week 14	10	10	10	10	2	1
Hematology (continued)						
Eosinophils (10^3/μL)						
Day 4	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
Day 25	0.08 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Week 14	0.06 ± 0.00	0.10 ± 0.03	0.06 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.06
Large unstained cells (10^3/μL)						
Day 4	0.040 ± 0.004	0.033 ± 0.003	0.044 ± 0.006	0.038 ± 0.004	0.036 ± 0.002	0.031 ± 0.004
Day 25	0.056 ± 0.007	0.059 ± 0.008	0.050 ± 0.006	0.043 ± 0.003	0.050 ± 0.007	0.043 ± 0.006
Week 14	0.055 ± 0.009	0.053 ± 0.010	0.049 ± 0.007	0.053 ± 0.005	0.085 ± 0.015	0.060
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	15.3 ± 0.7	15.5 ± 0.5	15.4 ± 0.6	15.0 ± 0.5	15.8 ± 0.5	15.8 ± 0.6
Day 25	18.6 ± 0.4	17.6 ± 0.5	18.4 ± 0.3	17.1 ± 0.3	17.8 ± 0.5	18.0 ± 0.6
Week 14	16.7 ± 0.4	16.3 ± 0.2	15.9 ± 0.6	17.9 ± 0.7	16.2 ± 0.7	16.4
Creatinine (mg/dL)						
Day 4	0.52 ± 0.03	0.54 ± 0.02	0.54 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	0.56 ± 0.02
Day 25	0.55 ± 0.02	0.53 ± 0.02	0.59 ± 0.02	0.56 ± 0.02	0.58 ± 0.01	0.54 ± 0.02
Week 14	0.62 ± 0.01	0.59 ± 0.01	0.60 ± 0.01	0.62 ± 0.02	0.65 ± 0.05	0.60
Total protein (g/dL)						
Day 4	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1
Day 25	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
Week 14	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.2
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.1	4.4 ± 0.0	4.4 ± 0.0	4.3 ± 0.0
Day 25	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.4 ± 0.1
Week 14	4.6 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.5 ± 0.0	4.4 ± 0.1	4.4
Alanine aminotransferase (IU/L)						
Day 4	48 ± 1	52 ± 2*	52 ± 1*	51 ± 1*	54 ± 1**	52 ± 1**
Day 25	44 ± 1	39 ± 2	43 ± 1	42 ± 1	45 ± 1	46 ± 2
Week 14	74 ± 7	67 ± 7	79 ± 10	58 ± 3	48 ± 3	37
Alkaline phosphatase (IU/L)						
Day 4	554 ± 10	547 ± 13	555 ± 9	552 ± 13	556 ± 14	513 ± 11
Day 25	320 ± 8	312 ± 8	327 ± 6	303 ± 8	311 ± 6	307 ± 6
Week 14	192 ± 7	185 ± 8	177 ± 6	196 ± 7	201 ± 2	209
Creatine kinase (IU/L)						
Day 4	618 ± 56	566 ± 74	594 ± 63	581 ± 37	616 ± 63	595 ± 58
Day 25	340 ± 28	333 ± 38	291 ± 21	302 ± 29	321 ± 49	286 ± 32
Week 14	221 ± 20	240 ± 22	235 ± 26	229 ± 24	227 ± 6	242
Sorbitol dehydrogenase (IU/L)						
Day 4	7 ± 1	8 ± 1	5 ± 1	6 ± 1	8 ± 2	7 ± 2
Day 25	9 ± 1	11 ± 1	12 ± 1	9 ± 1	12 ± 1	11 ± 1
Week 14	20 ± 2	18 ± 1	21 ± 3	16 ± 1	16 ± 5	14

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of α,β-Thujone

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Female (continued)						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	9	8
Week 14	10	10	10	10	2	1
Clinical Chemistry (continued)						
Bile acids (µmol/L)						
Day 4	21.2 ± 1.1	21.5 ± 1.6	24.7 ± 0.9	21.6 ± 1.4	23.4 ± 1.8	24.2 ± 1.2
Day 25	19.4 ± 1.6	19.0 ± 1.9	20.6 ± 1.2	22.2 ± 1.8	21.7 ± 1.5	24.3 ± 1.2
Week 14	26.6 ± 2.8	29.6 ± 2.3	38.1 ± 3.1	35.3 ± 4.6	53.6 ± 17.9	15.8

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] No standard error was calculated; less than two measurements were available.

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of α,β-thujone[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg
Male					
n	9	10	10	10	1[b]
Hematocrit (spun) (%)	47.8 ± 0.4[c]	47.7 ± 0.5	47.8 ± 0.5	48.4 ± 0.7	47.0
Hematocrit (auto) (%)	48.6 ± 0.6	48.3 ± 0.5	48.7 ± 0.6	49.6 ± 0.9	47.9
Hemoglobin (g/dL)	16.6 ± 0.2	16.4 ± 0.2	16.5 ± 0.2	16.8 ± 0.3	16.2
Erythrocytes (10^6/μL)	10.70 ± 0.13	10.57 ± 0.11	10.72 ± 0.15	10.89 ± 0.21	10.41
Reticulocytes (10^5/μL)	2.87 ± 0.08	2.83 ± 0.07	2.95 ± 0.07	2.93 ± 0.06	2.80
Reticulocytes (%)	2.69 ± 0.08	2.68 ± 0.06	2.75 ± 0.09	2.68 ± 0.04	2.70
Nucleated erythrocytes/ 100 leukocytes	0.0 ± 0.0[c]	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0
Mean cell volume (fL)	45.4 ± 0.1	45.7 ± 0.2	45.4 ± 0.1	45.5 ± 0.3	46.1
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.5 ± 0.0	15.4 ± 0.1	15.4 ± 0.1	15.6
Mean cell hemoglobin concentration (g/dL)	34.2 ± 0.1	34.1 ± 0.1	33.9 ± 0.1	33.9 ± 0.1	33.8
Platelets (10^3/μL)	1,105.3 ± 56.6	1,169.7 ± 44.0	1,068.3 ± 73.7	1,093.7 ± 59.8	1,307.0
Leukocytes (10^3/μL)	6.04 ± 0.20	6.12 ± 0.29	5.83 ± 0.26	5.35 ± 0.36	4.95
Segmented neutrophils (10^3/μL)	0.68 ± 0.04	0.84 ± 0.05	0.75 ± 0.06	0.67 ± 0.05	0.67
Lymphocytes (10^3/μL)	5.06 ± 0.18	4.97 ± 0.26	4.80 ± 0.21	4.40 ± 0.32	4.12
Monocytes (10^3/μL)	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.05
Basophils (10^3/μL)	0.026 ± 0.003	0.021 ± 0.003	0.023 ± 0.004	0.020 ± 0.004	0.020
Eosinophils (10^3/μL)	0.15 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.08
Large unstained cells (10^3/μL)	0.021 ± 0.003	0.023 ± 0.003	0.021 ± 0.002	0.019 ± 0.004	0.010

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of α,β-thujone

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg
Female					
n	10	10	10	10	3
Hematocrit (spun) (%)	46.2 ± 0.7	47.8 ± 0.5	47.0 ± 0.3	46.8 ± 0.3	50.3 ± 1.3*
Hematocrit (auto) (%)	46.4 ± 0.7	47.5 ± 0.7	46.9 ± 0.4	46.4 ± 0.3	51.3 ± 1.9*
Hemoglobin (g/dL)	15.9 ± 0.3	16.2 ± 0.2	16.0 ± 0.1	15.8 ± 0.1	17.3 ± 0.6*
Erythrocytes (10^6/μL)	10.34 ± 0.18	10.49 ± 0.16	10.44 ± 0.08	10.31 ± 0.08	11.46 ± 0.45*
Reticulocytes (10^5/μL)	3.04 ± 0.25	2.99 ± 0.19	3.02 ± 0.20	3.10 ± 0.12	3.96 ± 0.22
Reticulocytes (%)	2.96 ± 0.25	2.85 ± 0.19	2.89 ± 0.20	3.02 ± 0.13	3.47 ± 0.09
Nucleated erythrocytes/ 100 leukocytes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	44.9 ± 0.2	45.3 ± 0.2	45.0 ± 0.2	45.1 ± 0.2	44.8 ± 0.2
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.4 ± 0.1	15.4 ± 0.1	15.4 ± 0.1	15.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.3 ± 0.1	34.1 ± 0.1	34.2 ± 0.1	34.1 ± 0.1	33.8 ± 0.2
Platelets (10^3/μL)	989.9 ± 54.4	1,037.4 ± 74.9	921.9 ± 68.2	1,000.7 ± 40.6	1,111.7 ± 140.8
Leukocytes (10^3/μL)	4.79 ± 0.27	4.72 ± 0.24	4.84 ± 0.27	5.02 ± 0.21	4.58 ± 0.09
Segmented neutrophils (10^3/μL)	0.51 ± 0.06	0.53 ± 0.05	0.58 ± 0.05	0.53 ± 0.04	0.59 ± 0.12
Lymphocytes (10^3/μL)	4.03 ± 0.22	3.94 ± 0.20	4.01 ± 0.25	4.24 ± 0.17	3.80 ± 0.19
Monocytes (10^3/μL)	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.02
Basophils (10^3/μL)	0.015 ± 0.002	0.015 ± 0.003	0.018 ± 0.003	0.020 ± 0.001	0.013 ± 0.003
Eosinophils (10^3/μL)	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.11 ± 0.02
Large unstained cells (10^3/μL)	0.019 ± 0.003	0.017 ± 0.002	0.016 ± 0.002	0.021 ± 0.002	0.013 ± 0.003

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test

[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. No data were available for male or female 75 mg/kg mice due to 100% mortality in those dose groups.

[b] No standard error was calculated; less than two measurements were available.

[c] n=10

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of α-Thujone[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	177 ± 4	177 ± 4	160 ± 3	171 ± 7	171 ± 4	168 ± 6
Brain						
Absolute	1.769 ± 0.040	1.954 ± 0.215	1.765 ± 0.021	1.765 ± 0.012	1.813 ± 0.043	1.801 ± 0.018
Relative	9.996 ± 0.268	11.074 ± 1.206	11.049 ± 0.169	10.379 ± 0.405	10.643 ± 0.301	10.754 ± 0.370
Heart						
Absolute	0.62 ± 0.02	0.63 ± 0.03	0.58 ± 0.02	0.61 ± 0.03	0.63 ± 0.02	0.62 ± 0.02
Relative	3.506 ± 0.078	3.575 ± 0.139	3.630 ± 0.133	3.567 ± 0.050	3.700 ± 0.089	3.731 ± 0.197
R. Kidney						
Absolute	0.77 ± 0.03	0.75 ± 0.03	0.68 ± 0.02	0.71 ± 0.03	0.76 ± 0.02	0.75 ± 0.03
Relative	4.320 ± 0.079	4.265 ± 0.100	4.265 ± 0.055	4.140 ± 0.024	4.461 ± 0.083	4.470 ± 0.088
Liver						
Absolute	8.63 ± 0.16	9.16 ± 0.26	7.80 ± 0.14	8.57 ± 0.55	8.77 ± 0.31[b]	8.64 ± 0.36
Relative	48.722 ± 0.714	51.887 ± 1.248	48.856 ± 1.013	49.967 ± 1.908	52.422 ± 1.172[b]	51.442 ± 1.822
Lung						
Absolute	0.97 ± 0.02	0.94 ± 0.03	0.87 ± 0.01	0.92 ± 0.04	0.91 ± 0.02	1.00 ± 0.05
Relative	5.484 ± 0.170	5.323 ± 0.041	5.432 ± 0.119	5.399 ± 0.147	5.330 ± 0.133	5.939 ± 0.274
R. Testis						
Absolute	1.032 ± 0.017	1.012 ± 0.058	0.965 ± 0.050	1.012 ± 0.040	1.014 ± 0.023[b]	0.954 ± 0.081
Relative	5.832 ± 0.098	5.728 ± 0.280	6.023 ± 0.219	5.920 ± 0.098	5.878 ± 0.178[b]	5.635 ± 0.341
Thymus						
Absolute	0.412 ± 0.008	0.439 ± 0.008	0.413 ± 0.018	0.436 ± 0.028	0.447 ± 0.016	0.428 ± 0.030
Relative	2.326 ± 0.056	2.490 ± 0.051	2.586 ± 0.112	2.550 ± 0.138	2.621 ± 0.085	2.569 ± 0.256
Female						
n	5	5	5	5	5	2
Necropsy body wt	123 ± 3	122 ± 3	124 ± 2	120 ± 3	124 ± 3	125 ± 4
Brain						
Absolute	1.625 ± 0.029	1.624 ± 0.029	1.642 ± 0.012	1.653 ± 0.039	1.625 ± 0.038[b]	1.704 ± 0.056
Relative	13.188 ± 0.252	13.376 ± 0.477	13.300 ± 0.335	13.850 ± 0.403	13.281 ± 0.279[b]	13.662 ± 0.037
Heart						
Absolute	0.47 ± 0.01	0.47 ± 0.02	0.48 ± 0.01	0.46 ± 0.01	0.48 ± 0.01	0.48 ± 0.03
Relative	3.810 ± 0.086	3.872 ± 0.089	3.883 ± 0.097	3.830 ± 0.087	3.857 ± 0.144	3.831 ± 0.073
R. Kidney						
Absolute	0.57 ± 0.01	0.56 ± 0.02	0.56 ± 0.01	0.55 ± 0.01	0.58 ± 0.02	0.60 ± 0.02
Relative	4.641 ± 0.081	4.555 ± 0.075	4.544 ± 0.161	4.617 ± 0.094	4.665 ± 0.086	4.812 ± 0.017
Liver						
Absolute	5.43 ± 0.19	5.44 ± 0.20	5.42 ± 0.09	5.29 ± 0.09	5.51 ± 0.19	5.20 ± 0.08
Relative	43.988 ± 0.632	44.664 ± 0.947	43.950 ± 1.328	44.308 ± 0.897	44.427 ± 0.591	41.720 ± 0.798
Lung						
Absolute	0.77 ± 0.04	0.82 ± 0.02	0.83 ± 0.03	0.81 ± 0.05	0.82 ± 0.03	0.81 ± 0.05
Relative	6.226 ± 0.283	6.739 ± 0.237	6.703 ± 0.349	6.723 ± 0.243	6.675 ± 0.330	6.501 ± 0.176
Thymus						
Absolute	0.353 ± 0.010	0.402 ± 0.021	0.357 ± 0.025	0.358 ± 0.018	0.371 ± 0.014	0.238 ± 0.021**
Relative	2.874 ± 0.118	3.299 ± 0.135	2.901 ± 0.252	2.992 ± 0.139	3.011 ± 0.174	1.901 ± 0.097*

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n= 4

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of α,β-Thujone[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	4
Necropsy body wt	174 ± 6	180 ± 6	172 ± 8	141 ± 5**	168 ± 6	170 ± 4
Brain						
Absolute	1.795 ± 0.062	1.862 ± 0.022	1.837 ± 0.020	1.813 ± 0.037	1.851 ± 0.039	1.837 ± 0.048
Relative	10.327 ± 0.310	10.360 ± 0.311	10.775 ± 0.566	12.970 ± 0.558**	11.091 ± 0.426	10.804 ± 0.386
Heart						
Absolute	0.67 ± 0.03	0.67 ± 0.02	0.66 ± 0.03	0.54 ± 0.02**	0.65 ± 0.01	0.68 ± 0.02
Relative	3.860 ± 0.087	3.738 ± 0.043	3.813 ± 0.084	3.834 ± 0.118	3.881 ± 0.067	3.978 ± 0.069
R. Kidney						
Absolute	0.78 ± 0.02	0.78 ± 0.02	0.79 ± 0.04	0.70 ± 0.02	0.76 ± 0.02	0.79 ± 0.03
Relative	4.466 ± 0.092	4.310 ± 0.065	4.588 ± 0.087	4.991 ± 0.140**	4.557 ± 0.043	4.634 ± 0.067
Liver						
Absolute	8.80 ± 0.40	8.99 ± 0.46	8.91 ± 0.40	6.76 ± 0.39*	8.46 ± 0.36	9.03 ± 0.62
Relative	50.461 ± 0.739	49.761 ± 1.287	51.800 ± 1.090	47.966 ± 1.321	50.451 ± 0.767	52.850 ± 2.351
Lung						
Absolute	1.01 ± 0.06	1.02 ± 0.05	1.04 ± 0.03	0.83 ± 0.02*	0.96 ± 0.04	0.92 ± 0.03
Relative	5.817 ± 0.305	5.663 ± 0.223	6.136 ± 0.446	5.907 ± 0.168	5.720 ± 0.157	5.433 ± 0.321
R. Testes						
Absolute	1.071 ± 0.027	1.045 ± 0.043	1.006 ± 0.063	1.028 ± 0.051	1.016 ± 0.033	0.991 ± 0.117
Relative	6.169 ± 0.194	5.788 ± 0.069	5.836 ± 0.218	7.358 ± 0.501	6.063 ± 0.097	5.777 ± 0.589
Thymus						
Absolute	0.411 ± 0.019	0.426 ± 0.026	0.441 ± 0.013	0.303 ± 0.032**	0.397 ± 0.009	0.416 ± 0.020
Relative	2.364 ± 0.075	2.377 ± 0.168	2.589 ± 0.155	2.143 ± 0.178	2.376 ± 0.068	2.439 ± 0.063
Female						
n	5	5	5	5	5	5
Necropsy body wt	134 ± 3	128 ± 3	131 ± 3	133 ± 2	130 ± 5	130 ± 5
Brain						
Absolute	1.671 ± 0.038	1.761 ± 0.011	1.708 ± 0.045	1.678 ± 0.053	1.719 ± 0.022	1.727 ± 0.034
Relative	12.543 ± 0.464	13.827 ± 0.299	13.031 ± 0.293	12.674 ± 0.503	13.277 ± 0.530	13.373 ± 0.583
Heart						
Absolute	0.53 ± 0.02	0.51 ± 0.01	0.52 ± 0.01	0.53 ± 0.03	0.49 ± 0.02	0.49 ± 0.02
Relative	3.971 ± 0.088	3.998 ± 0.045	3.967 ± 0.095	3.951 ± 0.144	3.772 ± 0.130	3.785 ± 0.067
R. Kidney						
Absolute	0.60 ± 0.01	0.60 ± 0.01	0.61 ± 0.01	0.60 ± 0.01	0.59 ± 0.03	0.61 ± 0.03
Relative	4.521 ± 0.110	4.719 ± 0.096	4.643 ± 0.102	4.507 ± 0.123	4.521 ± 0.085	4.670 ± 0.137
Liver						
Absolute	6.20 ± 0.21	5.69 ± 0.10	5.71 ± 0.10	5.72 ± 0.22	5.72 ± 0.29	5.87 ± 0.24
Relative	46.387 ± 0.585	44.660 ± 0.801	43.562 ± 1.043	43.057 ± 1.200	43.861 ± 0.925	45.211 ± 0.939
Lung						
Absolute	0.92 ± 0.07	0.86 ± 0.06	0.81 ± 0.02	0.83 ± 0.02	0.79 ± 0.02	0.79 ± 0.01
Relative	6.891 ± 0.523	6.761 ± 0.434	6.192 ± 0.135	6.242 ± 0.105	6.116 ± 0.297	6.131 ± 0.233
Thymus						
Absolute	0.346 ± 0.035	0.368 ± 0.009	0.351 ± 0.016	0.345 ± 0.017	0.331 ± 0.021	0.347 ± 0.021
Relative	2.589 ± 0.252	2.892 ± 0.114	2.681 ± 0.122	2.597 ± 0.122	2.559 ± 0.180	2.676 ± 0.150

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Male						
n	10	10	10	10	8	2
Necropsy body wt	339 ± 13	347 ± 2	344 ± 4	339 ± 4	338 ± 7	323 ± 5
Heart						
Absolute	0.90 ± 0.05	0.88 ± 0.01	0.87 ± 0.01	0.85 ± 0.01	0.86 ± 0.01	0.86 ± 0.01
Relative	2.633 ± 0.073	2.542 ± 0.037	2.519 ± 0.031	2.506 ± 0.024	2.558 ± 0.025	2.649 ± 0.024
R. Kidney						
Absolute	1.04 ± 0.04	1.11 ± 0.01	1.09 ± 0.02	1.09 ± 0.02	1.06 ± 0.02	1.12 ± 0.08
Relative	3.059 ± 0.043	3.184 ± 0.033	3.178 ± 0.041	3.210 ± 0.075	3.144 ± 0.037	3.474 ± 0.300**
Liver						
Absolute	11.18 ± 0.60	11.77 ± 0.15	11.79 ± 0.20	11.34 ± 0.23	11.40 ± 0.28	11.90 ± 0.17
Relative	32.790 ± 0.703	33.912 ± 0.374	34.263 ± 0.308	33.448 ± 0.442	33.717 ± 0.273	36.860 ± 1.065**
Lung						
Absolute	1.28 ± 0.06	1.38 ± 0.03	1.36 ± 0.03	1.35 ± 0.04	1.35 ± 0.02	1.41 ± 0.02
Relative	3.776 ± 0.100	3.982 ± 0.092	3.947 ± 0.086	3.970 ± 0.116	3.997 ± 0.104	4.352 ± 0.019*
R. Testes						
Absolute	1.387 ± 0.038	1.415 ± 0.013	1.382 ± 0.013	1.429 ± 0.034	1.396 ± 0.022	1.347 ± 0.062
Relative	4.115 ± 0.082	4.079 ± 0.043	4.024 ± 0.067	4.224 ± 0.121	4.139 ± 0.081	4.174 ± 0.253
Thymus						
Absolute	0.239 ± 0.011	0.261 ± 0.008	0.251 ± 0.009	0.237 ± 0.011	0.201 ± 0.008*	0.178 ± 0.007*
Relative	0.705 ± 0.021	0.751 ± 0.021	0.732 ± 0.030	0.699 ± 0.034	0.593 ± 0.024**	0.550 ± 0.012*
Female						
n	10	10	10	10	2	1[b]
Necropsy body wt	186 ± 3	184 ± 3	183 ± 3	209 ± 4**	218 ± 3**	233
Heart						
Absolute	0.56 ± 0.01	0.57 ± 0.01	0.56 ± 0.01	0.61 ± 0.01**	0.63 ± 0.02**	0.75
Relative	3.013 ± 0.049	3.076 ± 0.038	3.060 ± 0.049	2.918 ± 0.033	2.895 ± 0.057	3.213
R. Kidney						
Absolute	0.63 ± 0.01	0.66 ± 0.01	0.65 ± 0.01	0.70 ± 0.02	0.70 ± 0.03	0.76
Relative	3.378 ± 0.059	3.559 ± 0.045	3.544 ± 0.034	3.376 ± 0.099	3.220 ± 0.177	3.256
Liver						
Absolute	5.39 ± 0.14	5.63 ± 0.14	5.60 ± 0.14	6.69 ± 0.14**	6.62 ± 0.31**	7.69
Relative	28.943 ± 0.533	30.567 ± 0.512*	30.531 ± 0.540*	32.060 ± 0.267**	30.428 ± 1.773*	32.948
Lung						
Absolute	0.91 ± 0.03	0.93 ± 0.03	0.95 ± 0.05	0.99 ± 0.03	1.06 ± 0.02	1.13
Relative	4.908 ± 0.147	5.054 ± 0.158	5.161 ± 0.214	4.729 ± 0.155	4.849 ± 0.010	4.841
Thymus						
Absolute	0.206 ± 0.009	0.192 ± 0.005	0.200 ± 0.010	0.235 ± 0.008	0.230 ± 0.002	0.231
Relative	1.107 ± 0.047	1.040 ± 0.020	1.084 ± 0.040	1.130 ± 0.047	1.057 ± 0.004	0.990

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No standard errors were calculated; less than two measurements were available.

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of α-Thujone[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	1[b]
Necropsy body wt	27.0 ± 0.9	26.2 ± 0.7	26.0 ± 0.8	26.9 ± 0.5	25.8 ± 0.7	26.8
Brain						
Absolute	0.476 ± 0.007	0.487 ± 0.009	0.485 ± 0.011	0.482 ± 0.010	0.485 ± 0.005	0.473
Relative	17.716 ± 0.725	18.616 ± 0.487	18.668 ± 0.167	17.924 ± 0.418	18.798 ± 0.389	17.649
Heart						
Absolute	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13
Relative	4.666 ± 0.140	5.075 ± 0.189	4.815 ± 0.084	4.743 ± 0.129	5.055 ± 0.211	4.925
R. Kidney						
Absolute	0.27 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.27
Relative	9.846 ± 0.198	9.932 ± 0.250	9.756 ± 0.144	9.748 ± 0.166	10.300 ± 0.285	9.888
Liver						
Absolute	1.54 ± 0.09	1.53 ± 0.06	1.44 ± 0.05	1.55 ± 0.03	1.53 ± 0.02	1.67
Relative	56.674 ± 1.809	58.361 ± 1.074	55.269 ± 0.784	57.573 ± 0.997	59.243 ± 1.670	62.127
Lung						
Absolute	0.19 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16
Relative	6.924 ± 0.341	7.264 ± 0.514	6.602 ± 0.128	6.170 ± 0.123	6.619 ± 0.275	5.784
R. Testis						
Absolute	0.109 ± 0.002	0.106 ± 0.001	0.113 ± 0.003	0.107 ± 0.003	0.113 ± 0.001	0.110
Relative	4.074 ± 0.177	4.048 ± 0.105	4.369 ± 0.121	3.968 ± 0.072	4.377 ± 0.133	4.104
Thymus						
Absolute	0.058 ± 0.005	0.055 ± 0.005	0.046 ± 0.006	0.053 ± 0.002	0.051 ± 0.005	0.052
Relative	2.146 ± 0.153	2.072 ± 0.135	1.758 ± 0.180	1.975 ± 0.108	1.964 ± 0.184	1.940
Female						
n	5	5	5	5	5	0
Necropsy body wt	20.7 ± 0.6	20.6 ± 0.6	21.1 ± 0.3	21.0 ± 0.3	20.4 ± 0.4	
Brain						
Absolute	0.481 ± 0.016	0.487 ± 0.014	0.472 ± 0.009	0.477 ± 0.009	0.473 ± 0.006	
Relative	23.344 ± 0.960	23.697 ± 0.283	22.360 ± 0.431	22.781 ± 0.403	23.187 ± 0.659	
Heart						
Absolute	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.01	
Relative	5.554 ± 0.148	5.224 ± 0.181	5.341 ± 0.157	5.258 ± 0.100	5.194 ± 0.208	
R. Kidney						
Absolute	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.00	0.16 ± 0.01	0.17 ± 0.01	
Relative	8.146 ± 0.137	8.213 ± 0.230	8.135 ± 0.080	7.417 ± 0.401	8.241 ± 0.272	
Liver						
Absolute	1.15 ± 0.04	1.18 ± 0.06	1.14 ± 0.05	1.14 ± 0.02	1.11 ± 0.05	
Relative	55.700 ± 0.880	57.313 ± 1.139	54.161 ± 1.642	54.473 ± 0.924	54.222 ± 1.381	
Lung						
Absolute	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.00	0.15 ± 0.01	
Relative	8.231 ± 0.524	7.500 ± 0.215	7.317 ± 0.304	6.993 ± 0.134*	7.311 ± 0.248	
Thymus						
Absolute	0.075 ± 0.005	0.075 ± 0.004	0.073 ± 0.003	0.074 ± 0.005	0.069 ± 0.005	
Relative	3.667 ± 0.311	3.641 ± 0.162	3.473 ± 0.122	3.543 ± 0.237	3.411 ± 0.302	

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

[b] No standard errors were calculated; less than two measurements were available.

TABLE G5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of α,β-Thujone[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	0
Necropsy body wt	25.5 ± 0.4	26.4 ± 0.5	26.8 ± 0.3	26.2 ± 0.5	26.6 ± 0.4	
Brain						
Absolute	0.479 ± 0.003	0.468 ± 0.010	0.473 ± 0.008	0.469 ± 0.005	0.466 ± 0.005	
Relative	18.824 ± 0.375	17.791 ± 0.537	17.661 ± 0.165	17.940 ± 0.303	17.564 ± 0.336	
Heart						
Absolute	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.01	0.15 ± 0.01	
Relative	5.251 ± 0.043	5.295 ± 0.050	5.163 ± 0.096	5.238 ± 0.134	5.484 ± 0.270	
R. Kidney						
Absolute	0.27 ± 0.01	0.29 ± 0.00	0.29 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	
Relative	10.611 ± 0.157	11.063 ± 0.124	10.822 ± 0.164	10.801 ± 0.241	10.752 ± 0.291	
Liver						
Absolute	1.47 ± 0.04	1.55 ± 0.04	1.57 ± 0.02	1.56 ± 0.03	1.54 ± 0.03	
Relative	57.703 ± 0.626	58.803 ± 0.909	58.630 ± 0.452	59.651 ± 1.456	57.925 ± 1.158	
Lung						
Absolute	0.16 ± 0.01	0.17 ± 0.01	0.18 ± 0.01*	0.16 ± 0.00	0.17 ± 0.00	
Relative	6.093 ± 0.351	6.435 ± 0.148	6.746 ± 0.191	6.280 ± 0.084	6.316 ± 0.179	
R. Testes						
Absolute	0.107 ± 0.002	0.107 ± 0.002	0.102 ± 0.006	0.110 ± 0.002	0.112 ± 0.002	
Relative	4.213 ± 0.108	4.077 ± 0.074	3.819 ± 0.254	4.191 ± 0.073	4.235 ± 0.072	
Thymus						
Absolute	0.053 ± 0.002	0.058 ± 0.006	0.050 ± 0.004	0.050 ± 0.005	0.057 ± 0.005	
Relative	2.083 ± 0.083	2.183 ± 0.219	1.866 ± 0.138	1.914 ± 0.183	2.142 ± 0.225	
Female						
n	5	5	5	5	5	3
Necropsy body wt	20.4 ± 0.5	21.5 ± 0.5	21.6 ± 0.4	20.7 ± 0.4	20.7 ± 0.3	21.9 ± 0.6
Brain						
Absolute	0.470 ± 0.007	0.471 ± 0.009	0.468 ± 0.005	0.478 ± 0.009	0.466 ± 0.004	0.490 ± 0.004
Relative	23.064 ± 0.312	21.961 ± 0.386	21.732 ± 0.236	23.108 ± 0.367	22.531 ± 0.432	22.383 ± 0.775
Heart						
Absolute	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.01
Relative	5.723 ± 0.221	5.604 ± 0.093	5.547 ± 0.086	5.714 ± 0.098	5.734 ± 0.167	5.419 ± 0.232
R. Kidney						
Absolute	0.17 ± 0.01	0.18 ± 0.00	0.17 ± 0.01	0.17 ± 0.00	0.17 ± 0.00	0.19 ± 0.01*
Relative	8.405 ± 0.262	8.435 ± 0.209	8.057 ± 0.123	8.420 ± 0.109	8.336 ± 0.185	8.740 ± 0.125
Liver						
Absolute	1.12 ± 0.03	1.24 ± 0.03	1.22 ± 0.05	1.21 ± 0.05	1.17 ± 0.03	1.24 ± 0.05
Relative	54.803 ± 0.116	57.665 ± 0.601	56.559 ± 1.224	58.371 ± 1.302*	56.641 ± 0.817	56.638 ± 1.018
Lung						
Absolute	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Relative	7.455 ± 0.170	7.459 ± 0.212	7.903 ± 0.618	7.361 ± 0.348	7.584 ± 0.301	7.615 ± 0.459
Thymus						
Absolute	0.072 ± 0.007	0.078 ± 0.003	0.072 ± 0.004	0.075 ± 0.002	0.075 ± 0.002	0.069 ± 0.003
Relative	3.507 ± 0.344	3.619 ± 0.103	3.330 ± 0.224	3.656 ± 0.179	3.606 ± 0.119	3.147 ± 0.117

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg
Male					
n	10	10	10	10	1[b]
Necropsy body wt	35.1 ± 0.4	36.9 ± 0.9	36.6 ± 0.8	35.4 ± 0.9	29.8
Heart					
Absolute	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.15 ± 0.01	0.15
Relative	4.453 ± 0.096	4.270 ± 0.079	4.333 ± 0.139	4.293 ± 0.100	5.034
R. Kidney					
Absolute	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.28
Relative	8.387 ± 0.240	8.222 ± 0.253	8.455 ± 0.132	8.439 ± 0.190	9.396
Liver					
Absolute	1.50 ± 0.03	1.60 ± 0.03	1.57 ± 0.05	1.49 ± 0.06	1.37
Relative	42.640 ± 0.462	43.428 ± 0.670	43.005 ± 1.050	41.894 ± 1.124	45.973
Lung					
Absolute	0.22 ± 0.02	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.16
Relative	6.155 ± 0.414	5.564 ± 0.375	5.702 ± 0.441	6.111 ± 0.415	5.369
R. Testes					
Absolute	0.122 ± 0.002	0.118 ± 0.003	0.120 ± 0.004	0.116 ± 0.002	0.108
Relative	3.483 ± 0.050	3.218 ± 0.065*	3.288 ± 0.078	3.298 ± 0.099	3.624
Thymus					
Absolute	0.033 ± 0.002	0.034 ± 0.001	0.034 ± 0.001	0.031 ± 0.001	0.026
Relative	0.936 ± 0.035	0.940 ± 0.052	0.938 ± 0.028	0.872 ± 0.017	0.872
Female					
n	10	10	10	10	3
Necropsy body wt	29.3 ± 1.0	29.1 ± 0.7	28.7 ± 0.9	29.6 ± 0.9	28.2 ± 0.9
Heart					
Absolute	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
Relative	4.359 ± 0.123	4.350 ± 0.120	4.470 ± 0.182	4.353 ± 0.143	4.502 ± 0.124
R. Kidney					
Absolute	0.18 ± 0.00	0.18 ± 0.00	0.19 ± 0.00	0.18 ± 0.00	0.22 ± 0.02**
Relative	6.076 ± 0.205	6.277 ± 0.139	6.582 ± 0.262	6.215 ± 0.158	7.684 ± 0.376**
Liver					
Absolute	1.26 ± 0.05	1.24 ± 0.03	1.24 ± 0.03	1.26 ± 0.02	1.09 ± 0.02
Relative	42.749 ± 0.663	42.852 ± 0.751	43.397 ± 1.610	42.809 ± 0.939	38.752 ± 1.014
Lung					
Absolute	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.00
Relative	7.104 ± 0.424	6.821 ± 0.566	6.933 ± 0.489	6.428 ± 0.244	6.525 ± 0.276
Thymus					
Absolute	0.044 ± 0.002	0.047 ± 0.003	0.044 ± 0.003	0.045 ± 0.002	0.039 ± 0.003
Relative	1.495 ± 0.058	1.627 ± 0.124	1.533 ± 0.076	1.513 ± 0.090	1.381 ± 0.153

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data were available for male or female 75 mg/kg mice due to 100% mortality in those dose groups.
[b] No standard errors were calculated; less than two measurements were available.

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
n	10	10	9	10
Weights (g)				
Necropsy body wt	339 ± 13	347 ± 2	344 ± 4[b]	339 ± 4
L. Cauda epididymis	0.2001 ± 0.0090	0.2081 ± 0.0073	0.2045 ± 0.0046	0.2145 ± 0.0058
L. Epididymis	0.4887 ± 0.0227	0.5080 ± 0.0140	0.5000 ± 0.0111	0.4793 ± 0.0079
L. Testis	1.4920 ± 0.0459	1.5507 ± 0.0106	1.5173 ± 0.0169	1.5859 ± 0.0557
Spermatid measurements				
Spermatid heads (10^6/testis)	186.00 ± 7.86	183.38 ± 4.50	176.39 ± 7.73	182.25 ± 7.09
Spermatid heads (10^6/g testis)	140.0 ± 5.5	133.6 ± 3.0	130.2 ± 6.5	129.0 ± 6.6
Epididymal spermatozoal measurements				
Sperm motility (%)	71.6 ± 1.5	67.2 ± 1.6	69.2 ± 1.6	65.7 ± 2.6
Sperm (10^6/cauda epididymis)	108.9 ± 4.1	99.6 ± 3.6[c]	100.3 ± 5.8	111.0 ± 5.1
Sperm (10^6/g cauda epididymis)	551 ± 24	481 ± 30[c]	495 ± 35	521 ± 30

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=10
[c] n=9

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	186 ± 3	184 ± 3	183 ± 3	209 ± 4**
Proportion of regular cycling females[b]	9/10	10/10	10/10	8/10
Estrous cycle length (days)	5.5 ± 0.31	5.3 ± 0.15	4.9 ± 0.10	5.5 ± 0.42[c]
Estrous stages (% of cycle)				
Diestrus	50.0	54.2	55.8	54.2
Proestrus	14.2	17.5	11.7	10.0
Estrus	25.0	24.2	25.0	23.3
Metestrus	10.8	4.2	7.5	12.5

** Significantly different (P≤0.01) from the vehicle control group by Williams' test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated that female rats in the highest dose group tested (50 mg/kg) were more likely than controls to remain in extended diestrus once they began extended diestrus, but they were slightly less likely to transition into extended diestrus.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	35.2 ± 0.4	36.8 ± 0.9	36.6 ± 0.8	34.4 ± 1.1
L. Cauda epididymis	0.0239 ± 0.0018	0.0301 ± 0.0014*	0.0316 ± 0.0017**	0.0278 ± 0.0011
L. Epididymis	0.0526 ± 0.0020	0.0599 ± 0.0024	0.0611 ± 0.0020*	0.0608 ± 0.0026*
L. Testis	0.1270 ± 0.0011	0.1238 ± 0.0027	0.1275 ± 0.0031	0.1232 ± 0.0020
Spermatid measurements				
Spermatid heads (10^6/testis)	22.12 ± 0.35	21.50 ± 1.00	23.17 ± 0.78	20.98 ± 0.91
Spermatid heads (10^6/g testis)	204.4 ± 6.3	194.9 ± 5.5	209.9 ± 10.1	194.2 ± 8.1
Epididymal spermatozoal measurements				
Sperm motility (%)	68.4 ± 2.4	58.8 ± 3.6	66.2 ± 2.6	62.1 ± 2.3
Sperm (10^6/cauda epididymis)	17.3 ± 1.2	17.4 ± 1.22[b]	17.5 ± 1.1	16.6 ± 0.7
Sperm (10^6/g cauda epididymis)	753 ± 66	567 ± 51	564 ± 37	598 ± 17

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
** P≤0.01
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=9

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of α,β-Thujone[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	29.3 ± 1.0	29.1 ± 0.7	28.7 ± 0.9	29.6 ± 0.9
Proportion of regular cycling females[b]	7/10	7/10	7/10	9/10
Estrous cycle length (days)	4.7 ± 0.40	4.6 ± 0.51	4.3 ± 0.17[c]	4.3 ± 0.12
Estrous stages (% of cycle)				
Diestrus	37.5	32.5	33.6	38.3
Proestrus	0.0	0.0	0.0	0.0
Estrus	45.8	47.5	46.2	42.5
Metestrus	16.7	20.0	20.2	19.2

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated no differences.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

α-Thujone

α-Thujone was obtained from Fluka Chemical Corporation (Milwaukee, WI) in one lot (10825/1) that was used in the 2-week studies. Identity and purity analyses were conducted by the analytical chemistry laboratory (Battelle Columbus Operations, Chemistry Support Services, Columbus, OH); the study laboratory (BioReliance Corporation, Rockville, MD) performed identity analyses. Galbraith Laboratories, Inc. (Knoxville, TN), performed Karl Fischer titration and elemental analyses. Reports on analyses performed in support of the α-thujone studies are on file at the National Institute of Environmental Health Sciences.

Lot 10825/1 of the chemical, a clear, colorless liquid, was identified as α-thujone by the analytical chemistry laboratory using infrared (IR) spectroscopy, proton and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and optical rotation analyses. The study laboratory confirmed the identity of the test article using IR spectroscopy. All spectra were consistent with the structure of α-thujone, and representative IR and proton NMR spectra are presented in Figures I1 and I2, respectively. Measured optical rotation values were consistent with those provided in the manufacturer's Certificate of Analysis.

The purity of lot 10825/1 was determined by elemental analyses and gas chromatography (GC) with flame ionization detection (FID) by system A (Table I1).

For lot 108251/1, Karl Fischer titration indicated an average water content of 0.04%, and results of elemental analyses for carbon and hydrogen were generally consistent with the theoretical values for α-thujone. GC/FID indicated one major peak and three minor peaks with areas greater than 0.1% of the total peak area. The overall purity of lot 10825/1 was determined to be approximately 99%.

To ensure stability, the bulk chemical was stored at approximately 5° C under a headspace of inert gas in amber glass bottles sealed with Teflon®-lined lids. Analyses of the bulk chemical before and after the 2-week studies were conducted by the study laboratory using GC/FID by system B, and no degradation of the bulk chemical was detected.

α,β-Thujone

α,β-Thujone was obtained from Fluka Chemical Corporation (Milwaukee, WI) in five lots (341637/1 196, 350864/1 897, 288156/1 493, 350864/1 14698, and 431314/1 44801). The analytical chemistry laboratory combined and homogenized lots 341637/1 196, 350864/1 897, 288156/1 493, and 350864/1 14698 and assigned a new lot number (121698) to the resulting mixture. Lot 121698 was used in the 2-week studies conducted by BioReliance Corporation. Lot 431314/1 44801 was homogenized by the analytical chemistry laboratory and reassigned lot number E58/L-2 by the study laboratory (Southern Research Institute, Birmingham, AL) and was used in the 3-month and 2-year studies. Identity and purity analyses were performed by the analytical chemistry laboratory and the study laboratories. Galbraith Laboratories, Inc., performed Karl Fischer titration and elemental analyses. Reports on analyses performed in support of the α,β-thujone studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a light-yellow liquid, were identified as α,β-thujone by the analytical chemistry laboratory and the study laboratories using IR spectroscopy; the analytical chemistry laboratory also used proton- and ^{13}C-NMR spectroscopy, GC coupled with mass spectrometry (MS), and optical rotation analyses. All spectra were consistent with the structures of α,β-thujone (*Aldrich*, 1993). Representative IR and proton NMR spectra are presented in Figures I3 and I4, respectively. Values determined for specific rotation for each lot were consistent with those reported in the manufacturer's Certificate of Analysis.

Purity was determined by the analytical chemistry laboratory using GC/FID by system A (Table I1). In attempts to identify the impurities, the analytical chemistry laboratory obtained spectra of the test chemical using GC/MS by system C (lot 121698) or system D (lot E58/L-2) that were compared to literature spectra (NIST, 2010); tentative identifications were made for fenchone and camphor. The retention times of these compounds were compared to the spectra of commercially obtained standards to further confirm their identity using GC/FID by system E. A standard addition study was performed using GC coupled with Fourier transform IR spectroscopy by system F to quantify the amounts of α-thujone, β-thujone, fenchone, and camphor present in the test chemical.

For lot 121698, Karl Fischer titration indicated an average water content of 0.17%, and elemental analyses for carbon, hydrogen, and oxygen were generally consistent with theoretical values, with oxygen content slightly higher than expected. GC/FID using system A indicated two major peaks and six impurities greater than 0.1% of the total peak area. The combined results of the purity analyses were determined to be approximately 71% α-thujone, 12% β-thujone, 13% fenchone, 3% camphor, and approximately 1% unidentified impurities. The overall purity was determined to be approximately 83% α,β-thujone, consistent with the manufacturer's Certificate of Analysis.

For lot E58/L-2, Karl Fischer titration indicated an average water content of 0.10%, and elemental analyses for carbon, hydrogen, and oxygen were generally consistent with theoretical values, with oxygen content slightly higher than expected. GC/FID using system A indicated two major peaks and five impurities greater than 0.1% of the total peak area. The combined results of purity analyses were determined to be approximately 70% α-thujone, 11% β-thujone, 16% fenchone, 2% camphor, and 0.5% unidentified impurities. The overall purity was determined to be approximately 81% α,β-thujone, consistent with the manufacturer's Certificate of Analysis.

To ensure stability, the bulk chemical was stored in amber glass bottles under a headspace of inert gas, sealed with Teflon®-lined lids at approximately 5° C. Periodic reanalyses of the bulk chemical were performed by the study laboratories using GC/FID by system G before and after all of the studies and approximately every 6 months during the 2-year studies. No degradation of the bulk chemical was detected.

Methylcellulose

Methylcellulose was obtained in one lot (984735) from Fisher Scientific (Pittsburgh, PA) for the 2-week studies and in one lot (31K0155) from Sigma-Aldrich (St. Louis, MO) for the 3-month and 2-year studies. Aqueous 0.5% methylcellulose was chosen as an alternative to corn oil at a time when it was considered important to minimize oil in the overall animal diet following the change to the NTP-2000 feed. The identity of each lot was confirmed by IR spectroscopy by Research Triangle Institute, Research Triangle Park, NC (lot 984735) and the study laboratory at Southern Research Institute (lot 31K0155). All spectra were consistent with the structure of methylcellulose. The average methoxyl contents determined by Galbraith Laboratories, Inc., were 29.13% and 30.0% for lots 984735 and 31K0155, respectively, which are in the acceptable range for methylcellulose.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing the appropriate amount of α-thujone or α,β-thujone with 0.5% methylcellulose in deionized water to give the required concentrations (Table I2). For the 2-week studies, dose formulations were stored at room temperature in amber glass vials under a headspace of inert gas sealed with Teflon®-lined septa and crimped caps and used within 29 days. For the 3-month and 2-year studies, dose formulations were stored refrigerated in amber glass bottles (3-month studies) sealed with Teflon®-lined lids or Teflon® bottles (3-month and 2-year studies) sealed with Teflon®-lined lids for up to 42 days.

Studies to determine the resuspendability and gavagability of a 30 mg/mL dose formulation, homogeneity of 0.05 and 30 mg/mL dose formulations, and stability of a 0.05 mg/mL dose formulation were performed by the analytical chemistry laboratory using a GC/FID system similar to system B (Table I1); the study laboratories also performed homogeneity studies of 0.1 and 20 mg/mL dose formulations using GC/FID by system B. Dose formulations were confirmed to be resuspendable by vigorous stirring for 10 minutes prior to analysis, and homogeneity and gavagability were confirmed. Stability of the dose formulations was confirmed for at least 47 (α-thujone) or 42 (α,β-thujone) days for dose formulations stored in sealed amber glass bottles at room temperature or 5° C and for at least 3 hours under simulated animal room conditions. The analytical method for formulation analyses required

extraction of thujones with ethyl acetate using propiophenone as an internal standard and GC/FID analysis of the extract.

Periodic analyses of the dose formulations of α-thujone and α,β-thujone were conducted by the study laboratories using GC/FID by system B. Throughout the 2-week and 3-month studies, difficulties were experienced by the study laboratory with resuspension of the formulations for analyses, a problem not seen at the analytical chemistry laboratory and apparently due to scale-up to large formulations and use over a dosing period. This issue was particularly noticeable in the analysis of samples from the animal rooms. During the 2-week studies, the dose formulations were analyzed two (mice and α-thujone rats) or three (α,β-thujone rats) times. Of the α-thujone dose formulations analyzed, five of seven for rats and five of seven for mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; one of eight for rats and one of eight for mice were within 10% of the target concentrations. Of the α,β-thujone dose formulations analyzed for the 2-week studies, four of five for rats and all five for mice were within 10% of the target concentrations (Table I4); one of five animal room samples for rats and none of five for mice were within 10% of the target concentrations. Additional formulation and animal room analyses were added during the 3-month studies to help resolve the formulation analysis problem. During the 3-month studies, the dose formulations were analyzed at least monthly; animal room samples were also analyzed (Table I5). Of the dose formulations analyzed, all 20 for rats and all 19 for mice were within 10% of the target concentrations; 11 of 20 animal room samples for rats and none of the 19 animal room samples for mice were within 10% of the target concentrations. A set of special experiments was undertaken prior to the beginning of the 2-year study to resolve the issue of low animal room concentrations. These experiments involved changing parameters such as dosing bottles and stir bar sizes, shaking the formulations and not stirring them, and minimization of air exposure during dosing. Parameters that proved most important were those that minimized adsorption to container walls and losses due to volatility. The use of Teflon® containers and keeping dosing bottles covered as much as possible overcame most of the problem. During the 2-year studies, the dose formulations were analyzed approximately every 8 weeks; animal room samples were also analyzed (Table I6). Of the dose formulations analyzed, all 94 for rats and all 122 for mice were within 10% of the target concentrations; 100 of 114 animal room samples for rats and 110 of 169 animal room samples for mice were within 10% of the target concentrations.

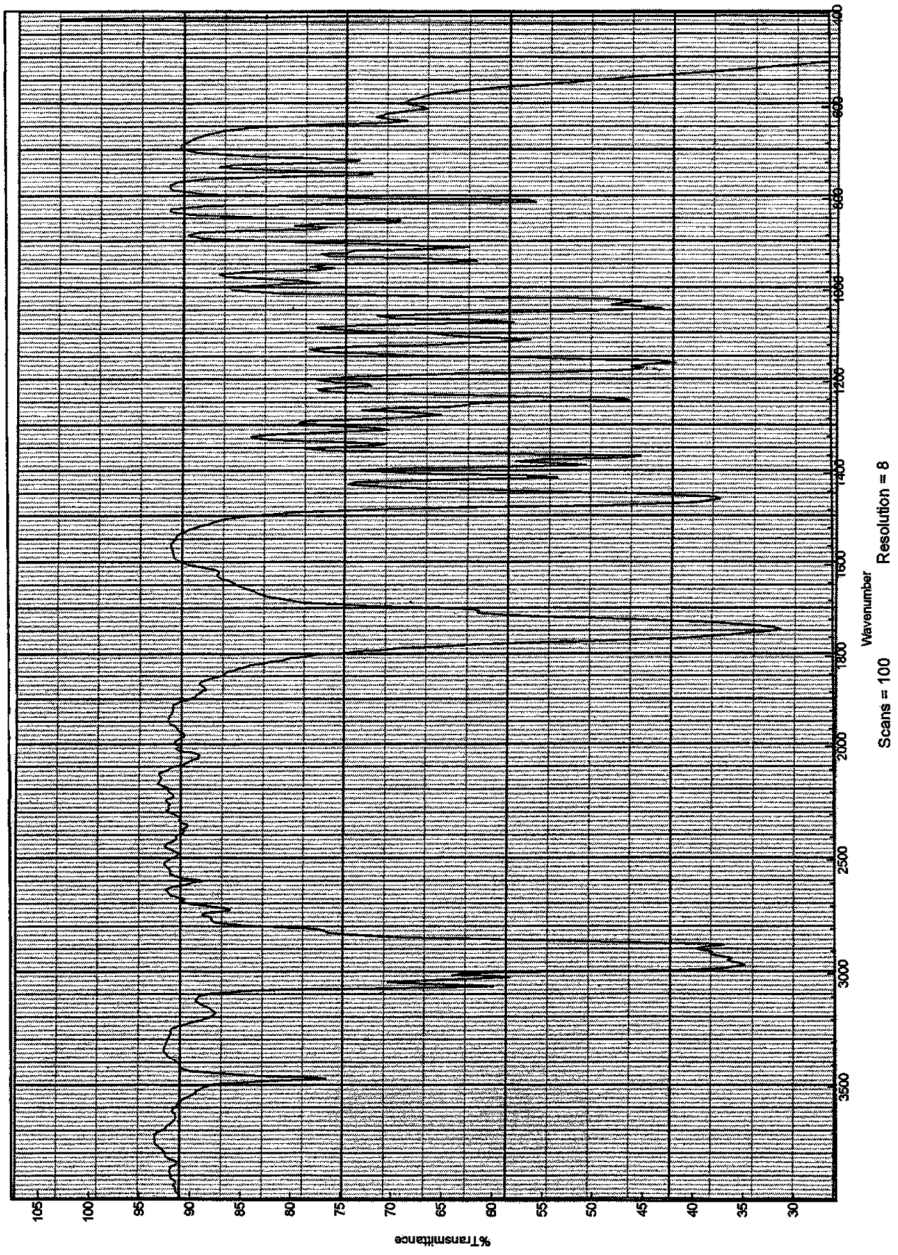


FIGURE I1
Infrared Absorption Spectrum of α-Thujone

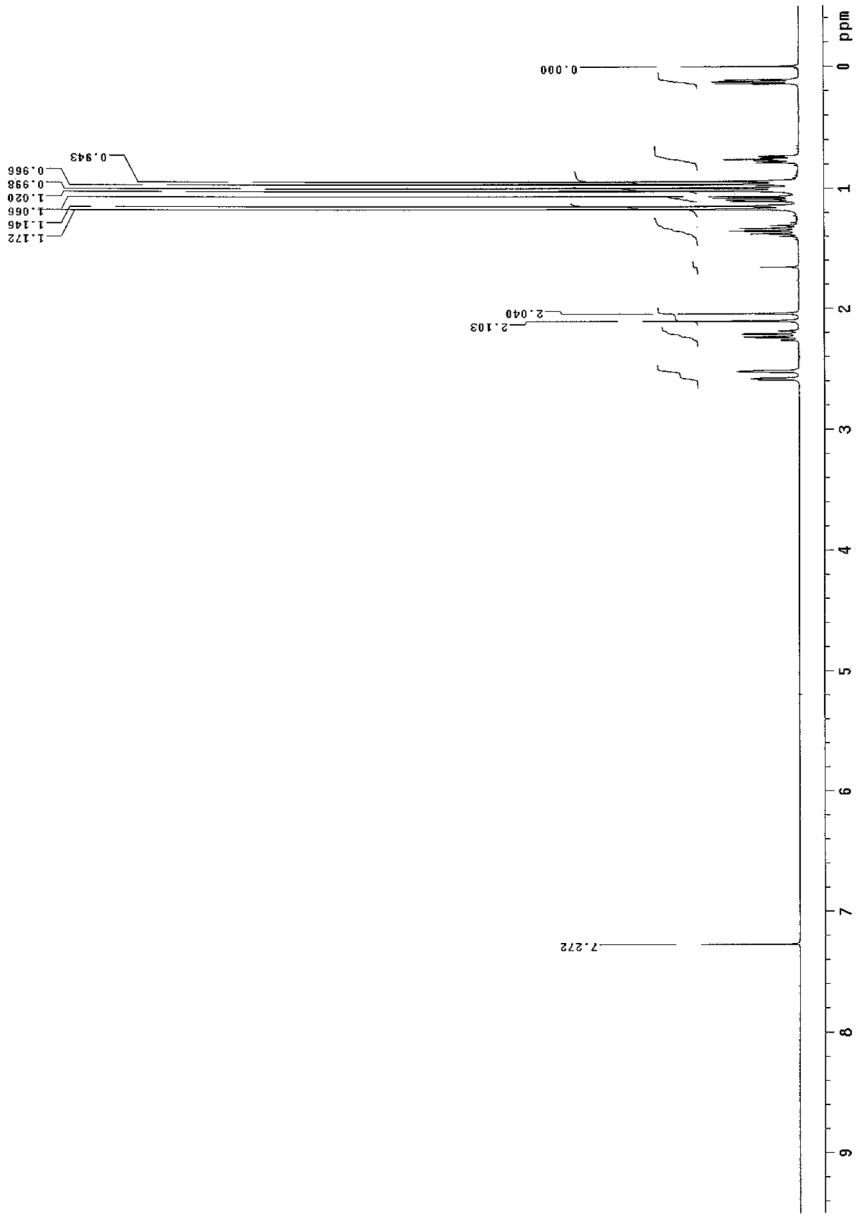


FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of α-Thujone

TABLE I1
Gas Chromatography Systems Used in the Gavage Studies of α-Thujone and α,β-Thujone[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	Stabilwax, 30 m × 0.53 mm, 1.0 µm film (Restek, Bellefonte, PA)	Helium at 8 mL/minute	50° C, then 10° C/minute to 210° C, held for 4 minutes
System B			
Flame ionization	DB™-WAX, 30 m × 0.25 mm, 0.25 µm film (Agilent Technologies, Inc., Palo Alto, CA)	Nitrogen at 11 mL/minute	50° C, then 10° C/minute to 200° C
System C			
Mass spectrometry	Stabilwax DA, 60 m × 0.25 mm, 0.25 µm film (Restek)	Helium at 2 mL/minute	70° C for 2 minutes, then 3° C/minute to 150° C, held for 2 minutes
System D			
Mass spectrometry	Stabilwax, 30 m × 0.32 mm, 0.32 µm film (Restek)	Helium at 1.5 mL/minute	50° C, then 10° C/minute to 210° C, held for 4 minutes
System E			
Fourier transform infrared spectroscopy	Stabilwax, 60 m × 0.25 mm, 1.0 µm film (Restek)	Helium at 2 mL/minute	70° C for 1 minute, then 3° C/minute to 150° C, held for 1 minute
System F			
Fourier transform infrared spectroscopy	Stabilwax DA, 60 m × 0.25 mm, 0.25 µm film (Restek)	Helium at 1.4 mL/minute	60° C for 2 minutes, then 10° C/minute to 250° C
System G			
Flame ionization	Agilent J&W DB™-WAX, 30 m × 0.25 mm, 0.25 µm film (Agilent Technologies)	Helium at ~1 mL/minute	40° C, then 8° C/minute to 160° C

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Palo Alto, CA; Systems A, B, C, D, E, and F), and Hewlett-Packard (Palo Alto, CA; System G).

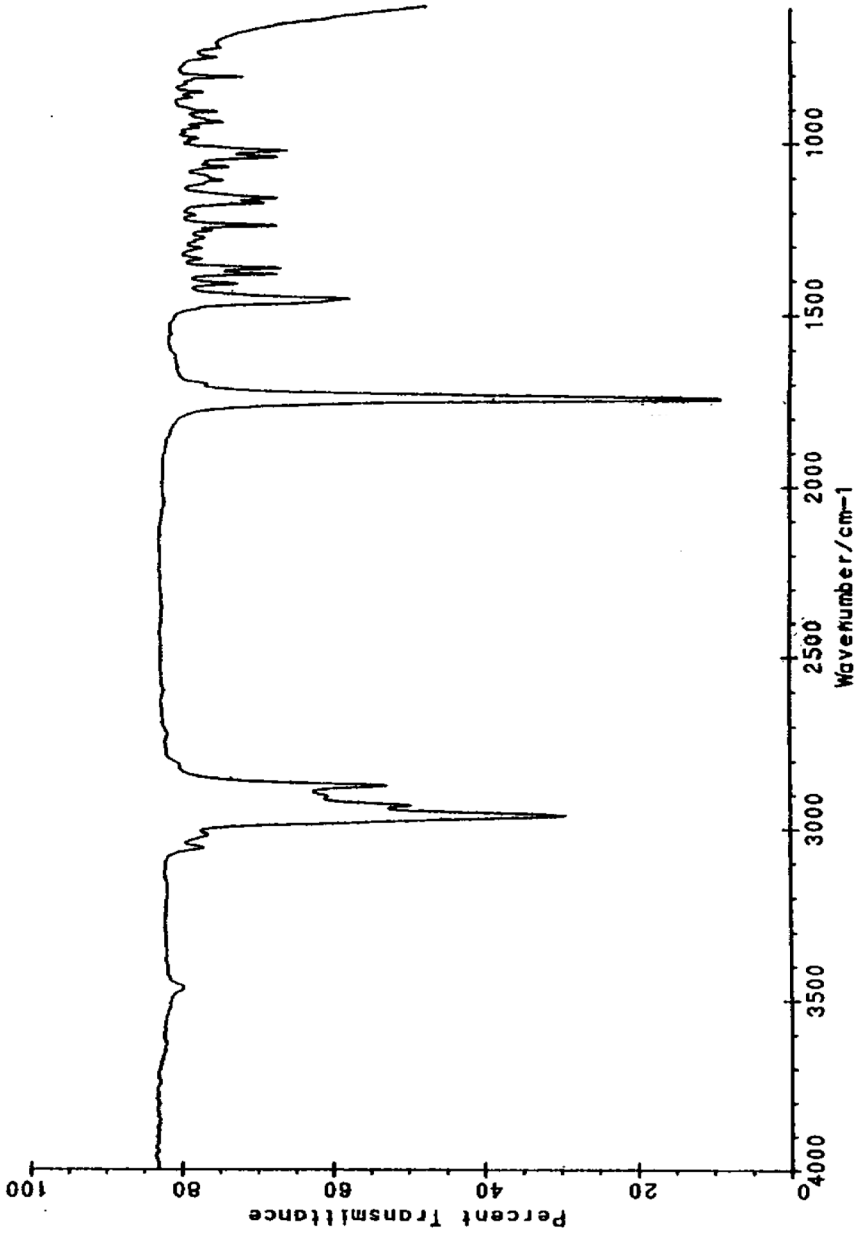


FIGURE I3
Infrared Absorption Spectrum of α,β-Thujone

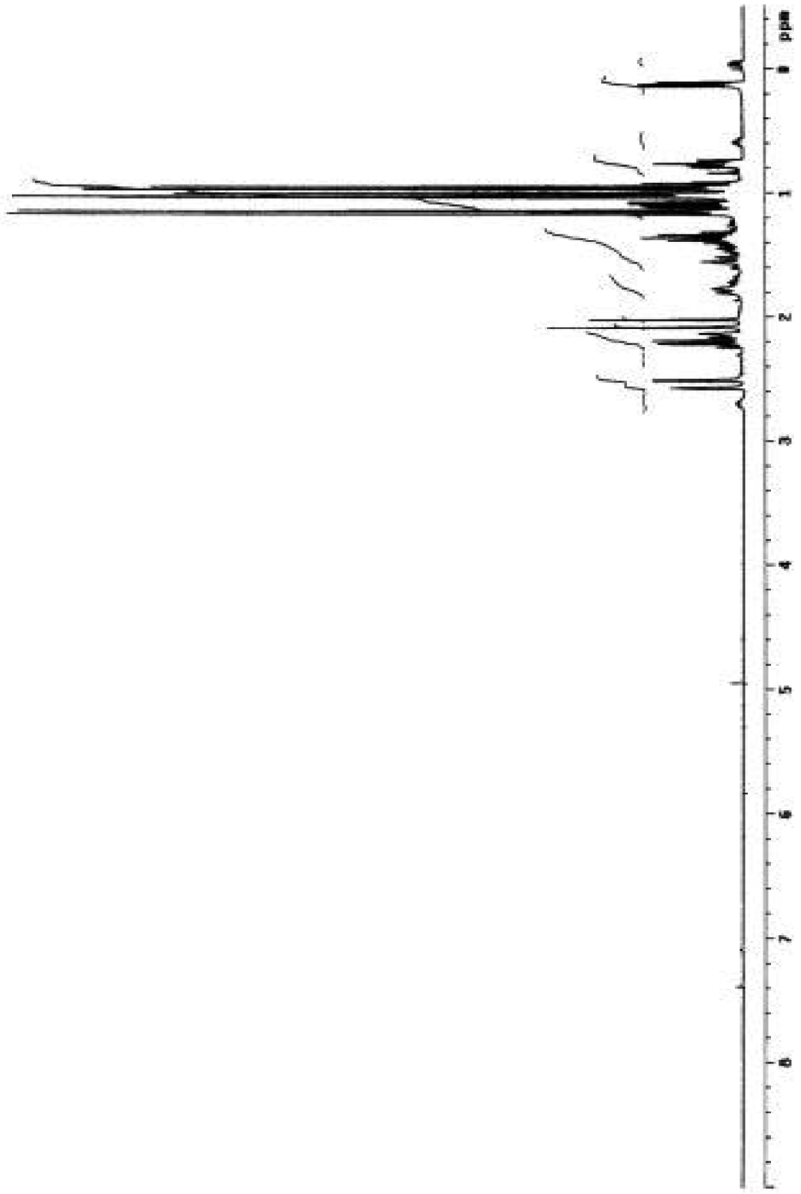


FIGURE I4
Proton Nuclear Magnetic Resonance Spectrum of α,β-Thujone

TABLE I2
Preparation and Storage of Dose Formulations in the Gavage Studies of α-Thujone and α,β-Thujone

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
The dosing vehicle was prepared by mixing methylcellulose with deionized water to form a 0.5% solution. The required amount of α-thujone was added to a specified amount of 0.5% aqueous methylcellulose in appropriately sized calibrated glass bottles. The bottles were shaken and stirred overnight using a magnetic stirrer. The required amount of α,β-thujone was added to a specified amount of 0.5% aqueous methylcellulose in appropriately sized calibrated glass bottles. The bottles were shaken vigorously for 1 minute, then placed in a polytron and mixed for 45 minutes, shaken for 1 minute, stirred for 5 minutes, and shaken for 1 minute. The doses were prepared two (mice and α-thujone rats) or three (α,β-thujone rats) times.	The 0.5% methylcellulose vehicle was prepared by adding the required amount of methylcellulose to hot, deionized water, stirring, and then cooling to room temperature. For formulations requiring less than 1 g of α,β-thujone, the test chemical was drawn into a glass syringe and transferred to a weighed mixing container; the appropriate amount of 0.5% methylcellulose was then added, the container sealed, shaken vigorously for at least 1 minute, mixed with a polytron homogenizer for at least 45 minutes, shaken for at least 1 minute, stirred for at least 5 minutes, and then shaken for at least 1 minute. For formulations requiring more than 1 g, the appropriate amount of α,β-thujone was weighed directly into a glass bottle with a ground glass stopper, the appropriate amount of 0.5% methylcellulose was then added and mixed as described above. The doses were prepared at least monthly.	Same as 3-month studies. The doses were prepared approximately every 8 weeks.
Chemical Lot Number		
10825/1 (α-thujone); 121698 (α,β-thujone)	E58/L-2	E58/L-2
Maximum Storage Time		
47 (α-thujone) or 42 (α,β-thujone) days	42 days	42 days
Storage Conditions		
Stored in amber glass vials under a headspace of inert gas sealed with Teflon®-lined septa and crimped caps at room temperature	Stored refrigerated in sealed amber glass bottles (August 8-September 30, 2002) or Teflon® bottles (October 1-November 12, 2002)	Stored refrigerated in sealed Teflon® bottles
Study Laboratory		
BioReliance Corporation (Rockville, MD)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of α-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
April 11, 2001	April 12, 2001	0.2	0.20	0
		0.6	0.57	–5
		2	1.85	–8
		6	4.55	–24
		20	16	–20
April 17, 2001	April 18, 2001	6	5.9	–2
		20	19.05	–5
April 11 or 17, 2001	May 21, 2001[b]	0.2	0.17	–15
		0.6	0.48	–20
		2	1.70	–15
		6	3.35	–44
		20	16.55	–17
	June 1, 2001[b]	0.2	0.20	0
		0.6	0.50	–17
		6	2.60	–57
Mice				
April 11, 2001	April 12, 2001	0.1	0.10	0
		0.3	0.29	–3
		1	0.89	–11
		3	2.50	–17
		10	9.10	–9
April 17, 2001	April 18, 2001	1	1.1	+10
		3	3.05	+2
April 11 or 17, 2001	May 21, 2001[b]	0.1	0.07	–30
		0.3	0.20	–33
		1	0.70	–30
		3	2.35	–22
		10	7.85	–22
	June 1, 2001[b]	0.1	0.10	0
		3	2.40	–20
		10	7.95	–21

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 0.2 mg/mL=1 mg/kg, 0.6 mg/mL=3 mg/kg, 2 mg/mL=10 mg/kg, 6 mg/mL=30 mg/kg, 20 mg/mL=100 mg/kg. For mice, dosing volume=10 mL/kg; 0.1 mg/mL=1 mg/kg, 0.3 mg/mL=3 mg/kg, 1 mg/mL=10 mg/kg, 3 mg/mL=30 mg/kg, 10 mg/mL=100 mg/kg.
[b] Animal room samples

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
April 25, 2001	April 25, 2001	0.2	0.18	–10
		20	19.5	–3
	June 4, 2001[b]	0.2	0.01	–95
		20	20.3	+2
May 1, 2001	May 1, 2001	2	1.9	–5
	June 4, 2001[b]	2	1.5	–25
May 2, 2001	May 2, 2001	0.6	0.52[c]	–13
		6	5.7	–5
	June 4, 2001[b]	0.6	0.37	–38
		6	0.6	–90
Mice				
May 1, 2001	May 1, 2001	1	0.90	–10
		3	2.8	–7
		10	9.5	–5
	June 4, 2001[b]	1	0.6	–40
		3	1.9	–37
		10	2.9	–71
May 2, 2001	May 2, 2001	0.1	0.09	–10
		0.3	0.28	–7
	June 4, 2001[b]	0.1	0.01	–90
		0.3	0.19	–37

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 0.2 mg/mL=1 mg/kg, 0.6 mg/mL=3 mg/kg, 2 mg/mL=10 mg/kg, 6 mg/mL=30 mg/kg, 20 mg/mL=100 mg/kg. For mice, dosing volume=10 mL/kg; 0.1 mg/mL=1 mg/kg, 0.3 mg/mL=3 mg/kg, 1 mg/mL=10 mg/kg, 3 mg/mL=30 mg/kg, 10 mg/mL=100 mg/kg.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration, but used at NTP's direction.

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
August 1, 2002	August 5-6, 2002	2.5	2.25	–10
		5	4.63	–7
		10	9.67	–3
		15	14.5	–3
		20	19.3	–4
	September 9-10, 2002[b]	2.5	0.881	–65
		5	1.50	–70
		10	6.56	–34
		15	11.7	–22
		20	13.8	–31
August 29, 2002	September 3-4, 2002	2.5	2.33	–7
		5	4.83	–3
		10	9.61	–4
		15	14.8	–1
		20	19.3	–4
	September 23-24, 2002[b]	2.5	1.57	–37
		5	3.31	–34
		10	8.58	–14
		15	12.9	–14
		20	17.7	–12
September 26, 2002	September 30-October 2, 2002	2.5	2.32[c]	–7
		5	4.81[c]	–4
		10	10.0[c]	0
		15	15.3[c]	+2
		20	20.7[c]	+4
	October 14-15, 2002[b]	2.5	1.90	–24
		5	4.62	–8
		10	9.20	–8
		15	14.8	–1
		20	19.9	–1
October 21, 2002	October 22-23, 2002	5	4.72	–6
		10	9.83	–2
		15	14.9	–1
		20	20.6	+3
	November 18-19, 2002[b]	5	4.73	–5
		10	9.01	–10
		15	14.4	–4
		20	19.5	–3
October 24, 2002	October 24, 2002	2.5	2.5	0
	November 18-19, 2002[b]	2.5	2.49	0

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
August 1, 2002	August 5-6, 2002	0.625	0.585	–6
		1.25	1.17	–6
		7.5	7.14	–5
	September 9-10, 2002[b]	0.625	0.224	–64
		1.25	0.506	–60
		7.5	1.99	–74
August 7, 2002	August 7, 2002	2.5	2.33	–7
	September 9-10, 2002[b]	2.5	0.739	–70
August 8, 2002	August 9, 2002	5	4.67	–7
	September 9-10, 2002[b]	5	1.76	–65
August 29, 2002	September 3-4, 2002	0.625	0.594	–5
		2.5	2.33	–7
		5	4.83	–3
	September 23-24, 2002[b]	0.625	0.166	–73
		2.5	1.49	–40
		5	3.52	–30
September 4, 2002	September 5, 2002	1.25	1.20	–4
		7.5	7.48	0
	September 23-24, 2002[b]	1.25	0.336	–73
		7.5	5.35	–29
September 26, 2002	September 30-October 2, 2002	0.625	0.581[c]	–7
		1.25	1.13[c]	–10
		2.5	2.29[c]	–8
		5	4.94[c]	–1
		7.5	7.15[c]	–5
	October 14-15, 2002[b]	0.625	0.366	–41
		1.25	0.777	–38
		2.5	1.17	–53
		5	3.27	–35
		7.5	6.32	–16

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
October 21, 2002[d]	October 22-23, 2002	0.625	0.642	+3
		5	4.72	–6
	November 18-19, 2002[b]	0.625	0.394	–37
		5	3.76	–25
October 24, 2002	October 24, 2002	1.25	1.18	–6
		2.5	2.50	0
	November 18-19, 2002[b]	1.25	0.663	–47
		2.5	1.51	–40

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 2.5 mg/mL=12.5 mg/kg, 5 mg/mL=25 mg/kg, 10 mg/mL=50 mg/kg, 15 mg/mL=75 mg/kg, 20 mg/mL=100 mg/kg. For mice, dosing volume=10 mL/kg; 0.625 mg/mL=6.25 mg/kg, 1.25 mg/mL=12.5 mg/kg, 2.5 mg/mL=25 mg/kg, 5 mg/mL=50 mg/kg, 7.5mg/mL=75 mg/kg.
[b] Animal room samples
[c] Results of quadruplicate analyses
[d] 7.5 mg/mL dose formulation not prepared due to 100% mortality at that dose level

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
May 29, 2003	June 2-3, 2003	2.5	2.59	+4
		5	5.27	+5
		10	10.7	+7
	June 10, 2003[b]	2.5	2.32	–7
		5	4.65	–7
		10	9.78	–2
	June 16, 2003[b]	2.5	2.29	–8
		5	4.54	–9
		10	10.2	+2
June 13, 2003	June 16, 2003	2.5	2.54	+2
	July 3-4, 2003[b]	2.5	2.64	+6
June 17, 2003	June 18, 2003	5	5.03	+1
		10	10.1	+1
	July 3-4, 2003[b]	5	27.6	+452
		10	22.8	+128
June 26, 2003	June 27-28, 2003	2.5	2.59	+4
		5	5.00	0
		10	10.5	+5
	July 11-12, 2003[b]	2.5	2.38	–5
		5	5.12	+2
		10	10.5	+5
	July 18-19, 2003[b]	2.5	2.45	–2
		5	4.94	–1
		10	9.87	–1
July 10, 2003	July 14, 2003	2.5	2.55	+2
		5	5.24	+5
		10	9.93	–1

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
July 24, 2003	July 24-25, 2003	2.5	2.63	+5
		2.5	2.57	+3
		5	4.88	–2
		5	4.88	–2
		10	10.4	+4
		10	10.3	+3
	August 8-9, 2003[b]	2.5	7.05	+182
		2.5	2.84	+14
		5	10.3	+106
		5	5.50	+10
		10	13.6	+36
		10	11.3	+13
	August 14-15, 2003[b]	2.5	3.06	+22
		2.5	2.65	+6
		5	8.09	+62
		5	5.61	+12
		10	14.1	+41
		10	9.67	–3
	August 28-29, 2003[b]	2.5	1.87	–25
		2.5	2.29	–8
		5	4.18	–16
		5	4.51	–10
		10	10.4	+4
		10	9.73	–3
August 21, 2003	August 21-22, 2003	2.5	2.61	+4
		5	5.03	+1
		10	9.95	–1
September 4, 2003	September 5-6, 2003	2.5	2.48	–1
		2.5	2.38	–5
		5	4.71	–6
		5	4.65	–7
		10	9.25	–8
		10	9.30	–7
	September 12-13, 2003[b]	2.5	2.50	0
		2.5	2.51	0
		5	4.72	–6
		5	4.88	–2
		10	9.08	–9
		10	9.02	–10
	September 19-20, 2003[b]	2.5	2.47	–1
		2.5	2.42	–3
		5	4.98	0
		5	4.92	–2
		10	9.48	–5
		10	9.73	–3

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
September 9, 2003	September 9-10, 2003	2.5	2.55	+2
		5	5.03	+1
		10	10.5	+5
	September 25-26, 2003[b]	2.5	2.57	+3
		2.5	2.55	+2
		5	5.21	+4
		5	5.00	0
		10	10.3	+3
		10	10.5	+5
September 18, 2003	September 18-19, 2003	2.5	2.64	+6
		2.5	2.61	+4
		5	4.58	–8
		5	4.64	–7
		10	9.26	–7
		10	9.29	–7
	October 9-10, 2003[b]	2.5	2.54	+2
		2.5	2.50	0
		5	5.02	0
		5	4.98	0
		10	10.1	+1
		10	10.1	+1
October 16, 2003	October 16-17, 2003	2.5	2.68	+7
		2.5	2.62	+5
		5	4.90	–2
		5	4.95	–1
		10	9.92	–1
		10	9.93	–1
	November 6-7, 2003[b]	2.5	2.44	–2
		2.5	2.44	–2
		5	5.28	+6
		5	5.08	+2
		10	10.3	+3
		10	10.2	+2
December 11, 2003	December 11-12, 2003	2.5	2.53	+1
		2.5	2.53	+1
		5	5.28	+6
		5	5.11	+2
		10	10.4	+4
		10	10.0	0
	December 31, 2003-January 1, 2004[b]	2.5	2.38	–5
		2.5	2.40	–4
		5	4.83	–3
		5	4.48	–10
		10	9.13	–9
		10	9.09	–9

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
February 5, 2004	February 5-6, 2004	2.5	2.46	–2
		2.5	2.47	–1
		5	5.09	+2
		5	5.19	+4
		10	9.94	–1
	February 26-27, 2004[b]	2.5	2.54	+2
		2.5	2.50	0
		5	5.12	+2
		5	4.78	–4
		10	9.88	–1
April 1, 2004	April 1-2, 2004	2.5	2.47	–1
		2.5	2.41	–4
		5	4.96	–1
		5	4.98	0
		10	9.88	–1
		10	9.75	–3
	April 22-23, 2004[b]	2.5	2.55	+2
		2.5	2.57	+3
		5	5.15	+3
		5	4.77	–5
		10	10.2	+2
May 26, 2004	May 27-28, 2004	2.5	2.70	+8
		2.5	2.42	–3
		5	4.58	–8
		5	4.70	–6
		10	9.87	–1
	June 17-18, 2004[b]	2.5	2.47	–1
		2.5	2.51	0
		5	4.70	–6
		5	5.09	+2
		10	9.47	–5
July 22, 2004	July 22-23, 2004	2.5	2.60	+4
		2.5	2.60	+4
		5	5.17	+3
		5	5.34	+7
		10	10.1	+1
	August 12-13, 2004[b]	2.5	2.26	–10
		2.5	2.44	–2
		5	4.59	–8
		5	4.56	–9
		10	9.78	–2

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
September 20, 2004	September 20-21, 2004	2.5	2.34	–6
		2.5	2.47	–1
		5	5.14	+3
		5	5.02	0
		10	9.88	–1
	October 7-8, 2004[b]	2.5	2.40	–4
		2.5	2.34	–6
		5	5.01	0
		5	4.39	–12
		10	10.2	+2
November 11, 2004	November 11-12, 2004	2.5	2.59	+4
		2.5	2.54	+2
		5	4.89	–2
		5	5.04	+1
		10	9.77	–2
	December 2-3, 2004[b]	2.5	2.47	–1
		2.5	2.52	+1
		5	5.18	+4
		5	5.02	0
		10	10.0	0
January 6, 2005	January 6-7, 2005	2.5	2.50	0
		2.5	2.50	0
		5	4.84	–3
		5	4.88	–2
		10	9.99	0
	January 27-28, 2005[b]	2.5	2.49	0
		2.5	2.39	–4
		5	4.94	–1
		5	4.95	–1
		10	9.75	–3
March 3, 2005	March 4-5, 2005	2.5	2.64	+6
		2.5	2.66	+6
		5	5.12	+2
		5	5.15	+3
		10	10.6	+6
	March 24-25, 2005[b]	2.5	2.60	+4
		2.5	2.55	+2
		5	5.03	+1
		5	4.95	–1
		10	10.2	+2

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
April 28, 2005	April 28-29, 2005	2.5	2.40	–4
		2.5	2.28	–9
		5	4.93	–1
		5	4.80	–4
		10	9.77	–2
	May 19-20, 2005[b]	2.5	2.51	0
		2.5	2.42	–3
		5	4.97	–1
		5	4.75	–5
		10	9.94	–1
Mice				
May 29, 2003	June 2-3, 2003	0.3	0.293	–2
		0.6	0.616	+3
		1.2	1.20	0
		2.5	2.52	+1
	July 1-2, 2003[b]	0.3	0.264	–12
		0.6	0.516	–14
		1.2	1.00	–17
		2.5	2.21	–12
	July 3-4, 2003[b]	0.3	0.261	–13
		0.6	0.488	–19
		1.2	0.938	–22
		2.5	1.81	–28
June 26, 2003	June 27-28, 2003	0.6	0.611	+2
		1.2	1.18	–2
		2.5	2.55	+2
	July 11-12, 2003[b]	0.6	0.377	–37
		1.2	0.716	–40
		2.5	1.94	–22
	July 18-19, 2003[b]	0.6	0.389	–35
		1.2	0.981	–18
		2.5	2.13	–15
	July 25-26, 2003[b]	0.6	0.482	–20
		0.6	0.431	–28
		1.2	1.02	–15
		1.2	1.03	–14
		2.5	2.21	–12
		2.5	1.94	–22

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
July 1, 2003	July 1-2, 2003	0.3	0.297	–1
	July 11-12, 2003[b]	0.3	0.250	–17
	July 18-19, 2003[b]	0.3	0.259	–14
	July 25-26, 2003[b]	0.3	0.270	–10
		0.3	0.274	–9
July 24, 2003	July 24-25, 2003	0.3	0.274	–9
		0.3	0.299	0
		0.6	0.568	–5
		1.2	1.15	–4
		1.2	1.21	+1
		2.5	2.69	+8
		2.5	2.59	+4
	July 31-August 1, 2003[b]	0.3	0.267	–11
		0.3	0.265	–12
		0.6	0.548	–9
		0.6	0.533	–11
		1.2	1.11	–8
		1.2	1.08	–10
		2.5	2.29	–8
		2.5	2.32	–7
	August 8-9, 2003[b]	0.3	0.274	–9
		0.3	0.265	–12
		0.6	0.545	–9
		0.6	0.548	–9
		1.2	1.19	–1
		1.2	1.15	–4
		2.5	2.36	–6
		2.5	2.51	0
	August 14-15, 2003[b]	0.3	0.270	–10
		0.3	0.267	–11
		0.6	0.514	–14
		0.6	0.497	–17
		1.2	1.18	–2
		1.2	1.15	–4
		2.5	2.33	–7
		2.5	2.32	–7
	August 28-29, 2003[b]	0.3	0.287	–4
		0.3	0.275	–8
		1.2	0.968	–19
		1.2	0.979	–18
		2.5	2.11	–16
		2.5	2.10	–16

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
July 31, 2003	July 31-August 1, 2003	0.6	0.639	+7
	August 28-29, 2003[b]	0.6	0.604	+1
		0.6	0.592	–1
August 21, 2003	August 21-22, 2003	0.3	0.291	–3
		0.6	0.551	–8
		1.2	1.14	–5
		2.5	2.54	+2
September 4, 2003	September 5-6, 2003	0.3	0.300	0
		0.3	0.280	–7
		0.6	0.557	–7
		0.6	0.565	–6
		1.2	1.08	–10
		1.2	1.09	–9
		2.5	2.46	–2
		2.5	2.49	0
	September 12-13, 2003[b]	0.3	0.280	–7
		0.3	0.277	–8
		0.6	0.546	–9
		0.6	0.549	–9
		1.2	1.08	–10
		1.2	1.05	–13
		2.5	2.48	–1
		2.5	2.47	–1
	September 19-20, 2003[b]	0.3	0.267	–11
		0.3	0.271	–10
		0.6	0.514	–14
		0.6	0.518	–14
		1.2	1.06	–12
		1.2	1.06	–12
		2.5	2.36	–6
		2.5	2.29	–8
September 9, 2003	September 9-10, 2003	0.3	0.290	–3
		1.2	1.12	–7
		2.5	2.59	+4
	September 25-26, 2003[b]	0.3	0.281	–6
		0.3	0.273	–9
		1.2	1.10	–8
		1.2	1.06	–12
		2.5	2.53	+1
		2.5	2.59	+4
September 12, 2003	September 12, 2003	0.6	0.575	–4
	September 25-26, 2003[b]	0.6	0.541	–10
		0.6	0.547	–9

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
September 18, 2003	September 18-19, 2003	0.3	0.297	–1
		0.3	0.294	–2
		0.6	0.578	–4
		0.6	0.598	0
		1.2	1.23	+3
		1.2	1.24	+3
		2.5	2.66	+6
		2.5	2.70	+8
	October 9-10, 2003[b]	0.3	0.264	–12
		0.3	0.271	–10
		0.6	0.539	–10
		0.6	0.539	–10
		1.2	1.13	–6
		1.2	1.15	–4
		2.5	2.43	–3
		2.5	2.34	–6
October 16, 2003	October 16-17, 2003	0.3	0.280	–7
		0.3	0.279	–7
		0.6	0.570	–5
		0.6	0.562	–6
		1.2	1.15	–4
		1.2	1.14	–5
		2.5	2.45	–2
		2.5	2.62	+5
	November 6-7, 2003[b]	0.3	0.283	–6
		0.3	0.275	–8
		0.6	0.567	–6
		0.6	0.561	–7
		1.2	1.10	–8
		1.2	1.09	–9
		2.5	2.37	–5
		2.5	2.37	–5
December 11, 2003	December 11-12, 2003	0.3	0.291	–3
		0.3	0.292	–3
		0.6	0.586	–2
		0.6	0.605	+1
		1.2	1.22	+2
		1.2	1.23	+3
		2.5	2.59	+4
		2.5	2.56	+2
	December 31, 2003-January 1, 2004[b]	0.3	0.268	–11
		0.3	0.273	–9
		0.6	0.537	–11
		0.6	0.548	–9
		1.2	1.16	–3
		1.2	1.13	–6
		2.5	2.34	–6
		2.5	2.40	–4

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
February 5, 2004	February 5-6, 2004	0.3	0.290	–3
		0.3	0.295	–2
		0.6	0.625	+4
		0.6	0.606	+1
		1.2	1.19	–1
		1.2	1.21	1
		2.5	2.48	–1
		2.5	2.48	–1
	February 26-27, 2004[b]	0.3	0.289	–4
		0.3	0.283	–6
		0.6	0.586	–2
		0.6	0.598	0
		1.2	1.19	–1
		1.2	1.17	–3
		2.5	2.51	0
		2.5	2.55	+2
April 1, 2004	April 1-2, 2004	0.3	0.283	–6
		0.3	0.288	–4
		0.6	0.574	–4
		0.6	0.573	–5
		1.2	1.18	–2
		1.2	1.21	+1
		2.5	2.51	0
		2.5	2.53	+1
	April 22-23, 2004[b]	0.3	0.277	–8
		0.3	0.285	–5
		0.6	0.560	–7
		0.6	0.564	–6
		1.2	1.11	–8
		1.2	1.15	–4
		2.5	2.32	–7
		2.5	2.35	–6
May 26, 2004	May 27-28, 2004	0.3	0.320	+7
		0.3	0.310	+3
		0.6	0.595	–1
		0.6	0.622	+4
		1.2	1.26	+5
		1.2	1.24	+3
		2.5	2.67	+7
		2.5	2.69	+8
	June 17-18, 2004[b]	0.3	0.307	+2
		0.3	0.299	0
		0.6	0.568	–5
		0.6	0.596	–1
		1.2	1.13	–6
		1.2	1.16	–3
		2.5	2.46	–2

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
July 22, 2004	July 22-23, 2004	0.3	0.291	–3
		0.3	0.299	0
		0.6	0.603	+1
		0.6	0.594	–1
		1.2	1.23	+3
		1.2	1.19	–1
		2.5	2.60	+4
	August 12-13, 2004[b]	0.3	0.289	–4
		0.3	0.274	–9
		0.6	0.541	–10
		0.6	0.537	–11
		1.2	1.10	–8
		1.2	1.13	–6
		2.5	2.39	–4
September 20, 2004	September 20-21, 2004	0.3	0.270	–10
		0.3	0.283	–6
		0.6	0.543	–10
		0.6	0.555	–8
		1.2	1.15	–4
		1.2	1.10	–8
		2.5	2.38	–5
	October 7-8, 2004[b]	0.3	0.290	–3
		0.3	0.281	–6
		0.6	0.563	–6
		0.6	0.556	–7
		1.2	1.12	–7
		1.2	1.13	–6
		2.5	2.22	–11
November 11, 2004	November 11-12, 2004	0.3	0.281	–6
		0.6	0.572	–5
		0.6	0.578	–4
		1.2	1.31	+9
		2.5	2.53	+1
	December 2-3, 2004[b]	0.3	0.245	–18
		0.3	0.306	2
		0.6	0.534	–11
		0.6	0.564	–6
		1.2	1.22	2
		1.2	1.15	–4
		2.5	1.96	–22
November 12, 2004	November 12-15, 2004	0.3	0.298	–1
		1.2	1.19	–1

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of α,β-Thujone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
January 6, 2005	January 6-7, 2005	0.3	0.285	–5
		0.3	0.285	–5
		0.6	0.563	–6
		0.6	0.572	–5
		1.2	1.19	–1
		2.5	2.63	+5
	January 27-28, 2005[b]	0.3	0.272	–9
		0.3	0.264	–12
		0.6	0.470	–22
		0.6	0.487	–19
		1.2	1.16	–3
		1.2	1.15	–4
		2.5	2.19	–12
January 10, 2005	January 10, 2005	1.2	1.19	–1
March 3, 2005	March 3-4, 2005	0.6	0.645	+8
		0.6	0.619	+3
		1.2	1.19	–1
		1.2	1.27	+6
		2.5	2.61	+4
	March 24-25, 2005[b]	0.6	0.535	–11
		0.6	0.528	–12
		1.2	1.06	–12
		1.2	1.08	–10
		2.5	2.40	–4
March 7, 2005	March 8, 2005	0.3	0.291	–3
		0.3	0.321	+7
	March 24-25, 2005[b]	0.3	0.276	–8
		0.3	0.275	–8
April 28, 2005	April 28-29, 2005	0.3	0.271	–10
		0.3	0.292	–3
		0.6	0.579	–4
		0.6	0.577	–4
		1.2	1.18	–2
		1.2	1.13	–6
		2.5	2.46	–2
	May 19-20, 2005[b]	0.3	0.263	–12
		0.3	0.276	–8
		0.6	0.537	–11
		0.6	0.547	–9
		1.2	1.07	–11
		1.2	1.02	–15
		2.5	2.36	–6

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 2.5 mg/mL=12.5 mg/kg, 5 mg/mL=25 mg/kg, 10 mg/mL=50 mg/kg. For mice, dosing volume=10 mL/kg; 0.3 mg/mL=3 mg/kg, 0.6 mg/mL=6 mg/kg, 1.2 mg/mL=12 mg/kg, 2.5 mg/mL=25 mg/kg.
[b] Animal room samples

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.8 ± 0.64	13.7 – 16.1	25
Crude fat (% by weight)	8.1 ± 0.36	7.4 – 9.0	25
Crude fiber (% by weight)	9.2 ± 0.44	8.2 – 9.9	25
Ash (% by weight)	5.0 ± 0.24	4.4 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.775 ± 0.068	0.670 – 0.970	20
Cystine	0.222 ± 0.025	0.150 – 0.250	20
Glycine	0.701 ± 0.043	0.620 – 0.800	20
Histidine	0.356 ± 0.081	0.270 – 0.680	20
Isoleucine	0.543 ± 0.045	0.430 – 0.660	20
Leucine	1.094 ± 0.069	0.960 – 1.240	20
Lysine	0.706 ± 0.115	0.310 – 0.840	20
Methionine	0.408 ± 0.048	0.260 – 0.490	20
Phenylalanine	0.626 ± 0.041	0.540 – 0.720	20
Threonine	0.502 ± 0.044	0.430 – 0.610	20
Tryptophan	0.147 ± 0.027	0.110 – 0.200	20
Tyrosine	0.394 ± 0.058	0.280 – 0.540	20
Valine	0.666 ± 0.045	0.550 – 0.730	20
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.231	3.49 – 4.54	20
Linolenic	0.30 ± 0.031	0.21 – 0.35	20
Vitamins			
Vitamin A (IU/kg)	4,807 ± 1,220	3,230 – 8,900	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.8 ± 19.39	52.0 – 124.0	20
Thiamine (ppm)[b]	8.8 ± 3.6	6.40 – 25.2	25
Riboflavin (ppm)	7.1 ± 1.96	4.20 – 11.20	20
Niacin (ppm)	78.5 ± 9.39	66.4 – 98.2	20
Pantothenic acid (ppm)	26.8 ± 13.16	17.4 – 81.0	20
Pyridoxine (ppm)[b]	9.46 ± 2.06	6.4 – 13.7	20
Folic acid (ppm)	1.65 ± 0.50	1.15 – 3.27	20
Biotin (ppm)	0.319 ± 0.11	0.200 – 0.704	20
Vitamin B_{12} (ppb)	53.9 ± 41.6	18.3 – 174.0	20
Choline (ppm)[b]	2,939 ± 399	2,000 – 3,790	20
Minerals			
Calcium (%)	0.962 ± 0.043	0.884 – 1.030	25
Phosphorus (%)	0.580 ± 0.026	0.538 – 0.623	25
Potassium (%)	0.664 ± 0.028	0.626 – 0.732	20
Chloride (%)	0.386 ± 0.040	0.300 – 0.474	20
Sodium (%)	0.190 ± 0.016	0.160 – 0.222	20
Magnesium (%)	0.217 ± 0.065	0.185 – 0.490	20
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	184 ± 40.7	135 – 311	20
Manganese (ppm)	51.8 ± 7.31	21.0 – 73.1	20
Zinc (ppm)	53.5 ± 8.85	43.3 – 78.5	20
Copper (ppm)	7.05 ± 2.677	3.21 – 16.30	20
Iodine (ppm)	0.496 ± 0.215	0.158 – 0.972	20
Chromium (ppm)	0.674 ± 0.283	0.330 – 1.380	19
Cobalt (ppm)	0.27 ± 0.164	0.133 – 0.864	18

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.27 ± 0.136	0.14 – 0.50	25
Cadmium (ppm)	0.07 ± 0.021	0.036 – 0.101	25
Lead (ppm)	0.09 ± 0.024	0.05 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.21 ± 0.057	0.16 – 0.45	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	13.2 ± 4.04	7.89 – 24.4	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 0	10	25
Coliform (MPN/g)	3.0 ± 0.0	3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.2 ± 1.72	2.3 – 8.5	25
N-Nitrosodimethylamine (ppb)[e]	2.3 ± 1.20	1.1 – 5.6	25
N-Nitrosopyrrolidine (ppb)[e]	2.0 ± 0.74	1.0 – 4.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.121 ± 0.136	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.158 ± 0.176	0.020 – 0.589	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

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 and the study animals are 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.

Serum samples were collected from five male and female vehicle control rats and mice at the end of the 3-month studies, from five male and female sentinel rats and mice at 6, 12, and 18 months, and from five randomly selected 25 mg/kg male and female rats and male mice and five randomly selected 12 mg/kg female mice at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated; fecal samples were collected from five male and five female mice at 18 months in the 2-year study for *Helicobacter* species by polymerase chain reaction testing. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
3-Month Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
RCV/SDA	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination
RCV/SDA	6 months

Method and Test	Time of Collection
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus-FL	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	
Immunofluorescence Assay	
Parvovirus	Study termination
Reovirus 3	Study termination
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
MMV, VP2 (minute virus of mice, viral protein 2)	12 and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months
Mouse adenoma virus-1	Study termination
MHV	6, 12, and 18 months, study termination
MPV, VP2 (mouse parvovirus, viral protein 2)	12 and 18 months, study termination
M. arthritidis	6 months, study termination
M. pulmonis	6 months, study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Mouse adenoma virus-FL	18 months
MCMV (mouse cytomegalovirus)	6 months, study termination
MPV	12 months
M. arthritidis	Study termination
Parvovirus	6 months
PVM	Study termination
Reovirus 3	Study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

APPENDIX L
SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

INTRODUCTION

Single administration toxicokinetic studies of α- and α,β-thujone were designed in F344/N rats and B6C3F1 mice to estimate toxicokinetic parameters for α-thujone and to estimate the extent of bioavailability after oral administration. Male and female rats received a single intravenous dose of α-thujone at 1.6 mg/kg or α,β-thujone at 3.0 mg/kg or a single gavage dose of α-thujone or α,β-thujone at 25 and 50 mg/kg. Male and female mice received a single intravenous dose of α-thujone at 3.2 mg/kg and α,β-thujone at 6.0 mg/kg or a single gavage dose of α- or α,β-thujone at 40 and 80 mg/kg. Plasma and brain levels of α-thujone were used to calculate toxicokinetic parameters of α-thujone following administration of α- or α,β-thujone.

MATERIALS AND METHODS

α-Thujone and α,β-thujone were received as single lots (10825/1 and 431314/1 44801, respectively) from Fluka Chemical Corporation (Milwaukee, WI) that were used in the toxicokinetic studies. Procurement and characterization details for these test articles are provided in Appendix I.

Intravenous dose formulations were prepared in Cremophor®:ethanol:water [1:1:8 (v:v:v)]. Gavage dose formulations were prepared in 0.5% aqueous methylcellulose according to procedures described in Appendix I. All formulations were stored at 5° C, were analyzed before use, and met the acceptance criteria.

F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). Rats and mice used for the intravenous phase of the study had cannulae surgically implanted into the jugular vein by the supplier. Animals were quarantined 4 to 13 days prior to dosing. At dosing, rats were 13 to 14 weeks old and mice were 12 to 15 weeks old. Non-fasted animals were weighed on the morning of dosing for calculation of the dosing volume. Thirty animals of each sex were given a single intravenous dose of 1.6 (rats) or 3.2 (mice) mg/kg α-thujone or 3.0 (rats) or 6.0 (mice) mg/kg α,β-thujone. Twenty-four animals of each sex were given a single oral gavage dose of α-thujone or α,β-thujone at doses of 25 or 50 (rats) or 40 or 80 (mice) mg/kg. In intravenous studies, each dose was administered as a bolus push via the jugular vein cannulus in a volume of 2 (rats) or 4 (mice) mL/kg. In oral gavage studies, each dose was administered in a volume of 5 (rats) or 10 (mice) mL/kg. Table L1 summarizes the experimental design of these studies.

After dosing, blood was collected under CO_2:O_2 (70:30) anesthesia via closed-chest cardiac puncture into 2-mL tubes containing EDTA, mixed gently, and placed on wet ice. Plasma was separated from the blood (target time interval was within 60 minutes of collection) by centrifugation and kept on dry ice until transferred to an approximately –70º C freezer. After blood collection, euthanasia was performed via CO_2 asphyxiation, and brains were collected and stored at –20° C. Three animals in each group were bled at each time point. Target times for blood and brain collection for the intravenous phases of the studies were as follows: male rats at 5, 10, 20, and 45 minutes and 1, 2, 4, 6, 8, and 12 hours; female rats at 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, and 3 hours; and male and female mice at 2, 5, 7, 10, 15, 20, 30, and 45 minutes and 1 and 1.5 hours. Target times for blood and brain collection for the gavage phases of the studies were as follows: male and female rats at 2, 5, 10, and 30 minutes and 1.5, 3, 6, and 12 hours; and male and female mice at 2, 5, 10, 20, and 40 minutes and 1.5, 2 (40 mg/kg females only), 3, 4 (80 mg/kg females only), 5 (40 mg/kg males only), and 6 (80 mg/kg males only) hours.

The quantitation of α-thujone in plasma and brain was done using a validated method. Briefly, 50 to 200 µL of plasma samples were combined in a headspace vial with 0.3 mL of 200 mg/mL aqueous sodium sulfate containing 57 ng/mL fenchone (to assure proper injection of the sample) and 1 mL of water, and the vial was crimp sealed. For samples less than 200 µL, additional blank plasma was added to bring the total volume to 200 µL. Approximately 200 mg (exact weight was recorded) of brain samples were combined in headspace vials with 0.2 mL of saline, 0.3 mL of 200 mg/mL aqueous sodium sulfate containing 140 ng/mL pulegone (the internal standard), and 1 mL of

water. Brain samples were quickly mashed with the flat side of a scalpel, and the vials were crimp sealed. Prior to sampling 1 mL from the headspace for analysis, sample vials were incubated for 15 minutes at 80° C. Single injections were made from each vial headspace using a headspace sampler (CTC Analytics Model HS500; Carrboro, NC) onto a gas chromatograph/mass spectrometer system (Agilent 6890 Plus GC with a 5973N mass spectrometer; Agilent Technologies, Palo Alto, CA) operated in electron impact ionization mode. The chromatographic system used a Stabilwax column (30 m × 0.32 mm ID, 1.0 µm film thickness) (Restek, Bellefonte, PA) with an oven temperature program of 50° C (held for 1 minute) to 210° C at 10° C/minute and then held for 4 minutes. Ions monitored in selected ion monitoring mode were 81 amu for α-thujone, pulegone, and fenchone.

Calibrations for both plasma and brain analyses were performed using six-point standard curves prepared in rat plasma or brain. For plasma analysis, a 1/x weighted linear regression equation was calculated for the analyte relating the response of the analyte to its concentration in plasma standards. For brain analysis, a $1/x^2$ weighted linear regression equation was calculated relating the response ratio of the analyte/internal standard to its concentration in brain standards. The use of weighting was found to be necessary to achieve acceptable accuracy at the low concentration of the curves. The method described was found suitable for samples in the range of 2.5 to 600 ng/mL for α-thujone in plasma, and 10 to 600 (low range) and 500 to 6,000 (high range) ng/g in brain. The method met acceptability criteria for linearity, precision, and accuracy. The experimental limit of quantitation (LOQ) was 2.5 ng/mL in plasma and 10 ng/g in brain.

TOXICOKINETICS

The plasma and tissue concentration time data sets were evaluated prior to toxicokinetic analysis. In general, relative standard deviations (RSDs) less than approximately 30% were considered to be within an acceptable measure of variability for biological samples. At later time points and especially for mice, the RSDs often approached approximately 60% to 75% and were considered acceptable. However, RSDs of this magnitude were specifically evaluated for evidence of misdosing or aberrant sample collection.

Semi-log plots of the mean plasma and brain concentration time data sets were prepared by sex, dosage, route of administration, and species. Group mean brain concentration time data were analyzed using noncompartmental analysis. The model selection was based on the route of administration. Individual animal data were used to evaluate the shape of the curve for selection of an appropriate compartmental model for plasma data sets. Mean plasma concentration time data sets were used to obtain initial estimates of the primary toxicokinetic parameters for a given noncompartmental model. These initial estimates were obtained because, when used with the compartmental modeling algorithm, they facilitated generation of more reliable final parameter estimates. Final plasma data sets were modeled using compartmental models. The following one- or two-compartment models (see below) were tested based on the appearance of the plasma concentration time curve. For each model, each data set was analyzed without weighting and with weighting factors, e.g., 1/Y, $1/Y^2$, 1/Y predicted (hat), and/or 1/Y predicted^2 (hat^2). The model and weighting factor that resulted in the best goodness-of-fit was used to calculate primary and secondary toxicokinetic parameters. All data were evaluated using a nonlinear least-squares fitting program (WinNonlin, Version 5.0.1; Pharsight Corporation, Mountain View, CA).

$$C_{(t)} = A(e^{-k_{10}t} - e^{-k_{01}t})$$

$$C_{(t)} = Ae^{-\alpha t} + B^{-\beta t} + Ce^{-k_{01}t}$$

Where: $\alpha = 0.5\{[k_{12} + k_{21} + k_{10}] + [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2}\}$
$\beta = 0.5\{[k_{12} + k_{21} + k_{10}] - [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2}\}$

$C_{(t)}$ is the plasma concentration at time t; A, B, and C are the intercepts of the distribution, elimination, and absorption phases with the concentration axis; k is a rate constant (subscripts describe the compartments and directions); and α and β are the first-order hybrid rate constants. Half-lives for the absorption and elimination phases were calculated as $0.693/k_{01}$ and $0.693/k_{10}$, respectively.

The area under the curve (AUC_T) was estimated to the last sampling time point (T) using the trapezoidal rule:

$$AUC_T = \sum \frac{C_{n-1}+C_n}{2} \times (t_n - t_{n-1})$$

where C_{n-1} and C_n are the plasma or brain α-thujone concentrations measured at two consecutive time points, t_{n-1} and t_n, respectively.

The area under the curve extrapolated to infinity (AUC_∞) was estimated as:

$$AUC_\infty = AUC_T + \frac{C_T}{\beta}$$

where C_T is the plasma or brain α-thujone concentration measured at the last time point and β is the first-order hybrid rate constant for the terminal phase.

Absolute bioavailability was expressed as the fraction (F) of the oral dose that reached systemic circulation and was calculated as:

$$F = \frac{Dose_{(iv)} \times AUC_{\infty(oral)}}{Dose_{(oral)} \times AUC_{\infty(iv)}}$$

Clearance (Cl) was calculated from the following:

$$\mathrm{Cl} = \mathrm{Dose} / \mathrm{AUC}_\infty$$

and the overall volume of distribution (V_d) was calculated as
$V_d = Cl/\beta$ (two compartment model) or $V_d = Cl/k_{10}$ (one compartment model).

For the oral data, clearance and volume of distribution were adjusted for bioavailability (F) and were expressed as C_F and V_F, respectively.

RESULTS

Rats

Intravenous Administration of α-Thujone

α-Thujone was administered to groups of jugular vein-cannulated male and female F344/N rats at 1.6 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time point (5 minutes) for both sexes. In males, plasma concentrations remained above the LOQ at all planned collection time points. In females, α-thujone plasma concentrations generally fell below the LOQ after 120 minutes following dosing. There were no aberrant plasma concentration time point values; the RSD values were less than 40%. Plasma concentration time profiles were biphasic for both sexes and were best described by a two-compartment model with first-order elimination and $1/Yhat^2$ weighting (Figure L1). Toxicokinetic parameters estimated using this model are presented in Table L2. The peak plasma concentration, C_0, was similar in males and females. The relatively large volumes of distribution indicate that α-thujone undergoes distribution into a peripheral compartment for both sexes but at a greater extent in males, which is made further evident by the distribution rate constants that indicate there is a faster distribution into and slower distribution out of the peripheral compartment(s) for males. Elimination kinetics indicated that the elimination of α-thujone was fast and sex dependent. The overall half-life of elimination of α-thujone was 24.9 ± 2.6 minutes for males and 8.59 ± 1.15 minutes for females. Clearance of α-thujone from the central compartment was 65.1 ± 2.3 mL/minute per kg for males and 248 ± 20 mL/minute per kg for females. Thus, elimination of α-thujone occurred approximately fourfold slower for males than females. AUC_∞ values further demonstrated that males underwent greater exposure than females at the same dose level.

α-Thujone in the brain was measurable at the earliest target sample collection time point (5 minutes) for both sexes. In males, concentrations were above the LOQ through target collection time point 240 minutes, except in one animal at 240 minutes. In females, concentrations were above the LOQ through target collection time point 180 minutes, except for one rat at the target collection time point 150 minutes. The RSD values indicated there was good agreement among samples for all time points; i.e., RSDs were less than 40%. Brain concentration time profiles were biphasic for both sexes (Figure L2). There was a rapid initial decline phase that was followed by a slower terminal decline phase. Toxicokinetic parameters estimated by noncompartmental analysis are presented in Table L3. Observed α-thujone brain C_{max} values were similar in males and females and were approximately threefold (males) and fourfold (females) greater than that observed in the plasma, i.e., 563 (males) and 408 (females) ng/mL. T_{max} occurred at the first collection time point, and the values were similar for males and females. Half-lives were 60.0 and 43.7 minutes for males and females, respectively. In comparison, terminal half-lives ($t_{1/2\beta}$) for α-thujone in the plasma were much longer for males (201 minutes) but similar for the females (56.7 minutes). The poor agreement between tissue types for males can be attributed to the short terminal linear phase for the brain profile relative to the plasma profile. There was no difference between the sexes for AUC_{∞} in the brain.

The α-thujone brain:plasma ratios were greater in females than in males at each time point (Figure L3). Brain concentrations were greater than plasma concentrations (ratio>1.00) at 5 and 10 minutes after dosing for males and at all time points for females. Furthermore, the magnitude of the female values indicated as much as eight times more α-thujone was present in the brain than in the plasma.

Oral Administration of α-Thujone

α-Thujone was administered to groups of male and female F344/N rats at 25 or 50 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time point (2 minutes) for both dose groups and sexes, except in one female rat in each dose group that was below the LOQ. Although there were no aberrant plasma concentration time point values, replicate concentrations varied widely for some time points resulting in an RSD of 104%. The greater variability following gavage dosing when compared to intravenous dosing suggests that factors affecting oral absorption of α-thujone may have played an important role in the systemic concentrations achieved following oral exposure.

α-Thujone plasma concentration time profiles were best described by a one-compartment model with $1/Yhat^2$ weighting (Figures L4 and L5). The shapes of the profiles were similar for both sexes and dose groups. Estimated toxicokinetic parameters are presented in Table L4. Absorption was very rapid with half-lives of 4.62 minutes or less; there was no evidence of a sex- or dose-related effect. Observed high variability in C_{max} values suggests that α-thujone absorption may be influenced by interfering factors such as food. C_{max} tended to be lower for males than females; and for both sexes, it increased slightly greater than proportionally as the dose was increased. Clearance values suggest that females, but not males, may have exhibited a very slight decrease in elimination with increasing dose. AUC_{∞} values further demonstrated that at a given dose males underwent less exposure than females, but as the dose was increased, the females exhibited a greater than proportional increase in AUC_{∞}.

α-Thujone in the brain was measurable at the earliest target sample collection time point (2 minutes) for both sexes and dose groups. Measurable brain concentrations were observed out to the target collection time point of 360 minutes for the 25 mg/kg groups of males and females and the male 50 mg/kg group, whereas the female 50 mg/kg group (and one of three animals in the female 25 mg/kg group) had measurable concentrations out to the target collection time point of 720 minutes. About half of the time point groups had RSDs less than 40%, indicating that there was good agreement among samples. Within a given time point group, there did not appear to be any correlation of later or earlier time points with lower or higher concentrations. For this reason, the samples were grouped together according to their plasma collection groupings.

For both sexes and dose levels, an aberrant early concentration time point was introduced by plotting the average collection time point for the 2-minute samples. For this reason, this concentration time point was not used in toxicokinetic analysis. Brain concentration time profiles are similar for both doses and sexes (Figures L6 and L7). Toxicokinetic parameters estimated by noncompartmental analysis are presented in Table L5. The C_{max} and AUC_{∞} values were greater in females than in males for both doses and were approximately twofold (males) and threefold or fourfold (females) greater than the values observed in plasma. In comparison, terminal half-life values for α-thujone in the plasma were similar (within twofold) to the brain half-life values. The α-thujone brain:plasma ratios were greater in females than males at both doses (Figure L8). For the males, brain concentrations were similar

to or approximately twofold greater than plasma concentrations between 5 and 180 minutes after dosing, whereas for the females, the brain concentrations were generally two to fourfold greater than in the plasma at all time points.

Intravenous Administration of α,β-Thujone

α,β-Thujone was administered to groups of male and female F344/N rats at 3.0 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time (5 minutes). All α-thujone plasma concentrations were above the LOQ except for two female rats at the 180-minute time point. There were no aberrant plasma concentration time point values that required further explanation. The RSD values were less than 40% for all time points in the male group and for all time points in the female group except 90, 120, and 150 minutes where the RSDs were marginally high (41.2% to 46.8%). Plasma concentration time profiles were biphasic for both male and female rats and were best fit by a two-compartment model with $1/Yhat^2$ weighting (Figure L9). Estimated toxicokinetic parameters are presented in Table L6. There was no apparent sex difference in C_0. Although the volume of distribution to the central compartment was similar for both sexes, the volume of distribution to the peripheral compartment was almost threefold larger for males than females. The rate constant for distribution of α-thujone from the central to the peripheral compartment (k_{12}) occurred at an approximately threefold faster rate for males than females. The relatively large volumes of distribution indicate that α-thujone undergoes distribution into a peripheral compartment in both sexes but to a greater extent in males, which is made further evident by the distribution rate constants, k_{12} and k_{21}, that indicate there is a faster distribution into and slower distribution out of the peripheral compartment(s) for males. Elimination kinetics indicate that the elimination of α-thujone was fast and sex dependent. The overall half-life of elimination of α-thujone was greater in males compared to females. Clearance of α-thujone from the central compartment was less in males compared to females. Thus, elimination of α-thujone occurred approximately threefold slower for males than females. This sex-dependent elimination was present after intravenous dosing of α-thujone. AUC_∞ values further demonstrated that males underwent greater exposure than females at the same dose level. As with the other toxicokinetic parameters, AUC_∞ values were within twofold of the AUC_∞ values after dosing with α-thujone.

α-Thujone in the brain was measurable at the earliest planned sample collection time (5 minutes). All α-thujone brain concentrations were above the LOQ except for males, one at 240 minutes and all at the final three time points of 360, 480, and 720 minutes. There were no aberrant brain concentration time point values that required further explanation. The RSD values were less than 40%, indicating there was good agreement among samples for all time points in both sexes. α-Thujone brain concentration time profiles were biphasic for male and female rats. There was a rapid initial decline phase followed by a slower terminal decline phase; and the profiles for males and females were, in general, similar (Figure L10). Toxicokinetic parameters estimated from the noncompartmental analysis are presented in Table L7. There were no apparent differences in the estimated toxicokinetic parameters between the sexes. Observed maximum α-thujone brain concentrations were approximately threefold (males) and fivefold (females) greater than the values observed in the plasma. Half-life values for α-thujone in the brain were similar for males and females. Terminal half-life values ($t_{1/2\beta}$) for α-thujone in the plasma of females were similar to brain half-life values; but in males, the plasma half-life was much longer (165 minutes) compared to brain half-life. The poor agreement between tissue types for males can be attributed to the short terminal linear phase for the brain profile relative to the plasma profile and was similar to what occurred following an intravenous dose of α-thujone.

The α-thujone brain:plasma ratios were greater in females than males (Figure L11). In females, as much as seven times more α-thujone was present in the brain than in the plasma, compared to four times in males.

Oral Administration of α,β-Thujone

α,β-Thujone was administered to groups of male and female F344/N rats at 25 or 50 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time (2 minutes). For males, plasma concentrations were above the LOQ for all except one rat at 720 minutes in the 25 mg/kg group. For females, all plasma concentrations were above the LOQ except for one rat at the 2-minute time point and all three rats at the final 720-minute time point in the 25 mg/kg group and one rat at the 2-minute and 720-minute time points in the 50 mg/kg group. There were no aberrant plasma concentration time point values that required further explanation. The RSD values indicated there was good agreement among samples for all time points in male rats except for 2 and 10 minutes in the 25 mg/kg group and 2 and 5 minutes in the 50 mg/kg group. Female rats did not show as good agreement as males, as females had RSD values above 40% at 5, 180, and 360 minutes in the 25 mg/kg group and at 5, 30, 90, and 360 minutes in the 50 mg/kg group.

Plasma concentration time profiles in both sexes were best described by a one-compartment model with $1/Yhat^2$ weighting (Figures L12 and L13). Estimated toxicokinetic parameters are presented in (Table L8). Absorption of α-thujone was very rapid following a single gavage administration of α,β-thujone in both males and females from both dose groups. There was no evidence of a sex- or dose-related effect, and absorption rates and half-lives were similar to those of rats that received a gavage administration of α-thujone. Observed C_{max} and AUC_{∞} values increased proportionately with the dose, and no difference was noted between males and females. Although there was an apparent dose-proportional decrease in elimination in female rats, the overall clearance of α-thujone was not affected by dose or sex. Unlike with rats dosed with α-thujone, rats dosed with α,β-thujone did not show females receiving greater exposure than males.

α-Thujone in the brain was measurable at the earliest planned collection time (2 minutes). The only concentrations that were below the LOQ were the 720-minute time point in the 25 and 50 mg/kg male groups, and one sample at the 2-minute time point in the 25 mg/kg male group. There were no aberrant brain concentration time point values that required further explanation. The RSD values indicated there was good agreement among samples for all time points except at 5, 90, and 360 minutes (25 mg/kg males); 2, 5, 90, and 180 minutes (50 mg/kg males); 2, 5, and 180 minutes (25 mg/kg females); and 2, 30, 90, 180, and 360 minutes (50 mg/kg females). Most of these RSDs were marginally high with the first measurable time points for each group often having the highest RSD values due to individual animal variability in absorption. α-Thujone brain concentration time profiles had an initial rising phase followed by a terminal declining phase (Figures L14 and 15). Toxicokinetic parameters estimated from noncompartmental analysis are presented in Table L9. Females had a twofold to fourfold greater C_{max} than males, which was also observed following gavage dosing of α-thujone. C_{max} values were approximately twofold (males) and threefold or fourfold (females) greater than the values in the plasma. The brain half-life values were similar to terminal half-life ($t_{1/2\beta}$) values for α-thujone in the plasma. AUC_{∞} values increased proportionally with dose, with the female rats receiving greater exposure to α-thujone in the brain.

The α-thujone brain:plasma ratios were greater in females than males (Figure L16). For the males, brain concentrations were similar to or approximately twofold greater than most plasma concentrations and no more than fourfold greater between 2 and 360 minutes after dosing, whereas for females, brain concentrations were generally twofold to sevenfold greater than in the plasma at all time points.

Mice

Intravenous Administration of α-Thujone

α-Thujone was administered to groups of jugular vein-cannulated male and female B6C3F1 mice at 3.2 mg/kg. α-Thujone in plasma was measurable at the earliest target sample collection time point (2 minutes). For males, α-thujone plasma concentrations were above the LOQ, except at the 60- and 90-minute time points. For females, α-thujone plasma concentrations were above the LOQ except for one mouse at the 20-minute time point and all of the mice from 30 to 90 minutes (except for one at 30 minutes). There were no aberrant plasma concentration time point values. The RSD values were below 40% for all but 2, 20, and 30 minutes in males and 2 minutes in females. Unlike for rats, α-thujone plasma concentration time profiles were monophasic for both sexes of mice and were best fit by a one-compartment model with first-order elimination and $1/Yhat^2$ weighting (Figure L17). Toxicokinetic parameters are give in Table L10. The peak plasma concentration, C_0, tended to be greater for males than for females. This was also seen in rats, but the difference in mice was less than twofold. The volume of distribution was much greater than the volume of total body water for mice (725 mg/mL) (Davies and Morris, 1993), suggesting that there is distribution to tissue. The elimination of α-thujone was fast and sex dependent. Overall elimination of α-thujone occurred approximately twofold faster in females than in males. The faster elimination of α-thujone also occurred in female rats, but with a greater difference between the sexes. AUC_{∞} values further demonstrated that males underwent greater exposure than females at the same dosage level.

α-Thujone in the brain was measurable at the earliest target sample collection time (2 minutes). For males, concentrations were above the LOQ except for one male mouse at the 45-minute target time and all of the mice at the final two planned collection times of 60 and 90 minutes. For females, concentrations were above the LOQ except for one mouse at 30 minutes, two mice at 45 minutes, and all mice at the final two planned collection times (60 and 90 minutes). There were no aberrant brain concentration time point values. The RSD values were below 40% except at 2, 5, and 20 minutes for male mice and at 2, 10, and 15 minutes for female mice.

α-Thujone brain concentration time profiles were monophasic for mouse plasma for both sexes (Figure L18). These declines were different from the biphasic declines that were seen in the brain of rats. Toxicokinetic parameters estimated from a noncompartmental model are given in Table L11. Observed maximum α-thujone brain concentrations were approximately fourfold to sixfold greater than the α-thujone concentrations in plasma for males and females. T_{max} occurred at the first collection time point (target time was 2 minutes), and the values were similar for males and females. Half-life values for α-thujone in the brain were short and are similar to those observed in mouse plasma. There was no difference in AUC_{∞} values between males and females.

The α-thujone brain:plasma ratios were greater in females than in males (Figure L19). Female brain:plasma ratios were about twofold greater than those in males at any given time point. Furthermore, the magnitude of the female values indicated as much as six times more α-thujone present in the brain than in the plasma.

Oral Administration of α-Thujone

α-Thujone was administered to groups of male and female B6C3F1 mice at 40 or 80 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time (2 minutes). For males, α-thujone plasma concentrations were above the LOQ for the 40 mg/kg group except for one mouse at the 40-minute and all of the mice at the final two time points. For the 80 mg/kg male group, two mice at the final 360-minute time point were below the LOQ. For females, all plasma concentrations were below the LOQ from 10 to 180 minutes except for one at 10 minutes and one at 20 minutes in the 40 mg/kg group. Plasma concentrations in all mice were below the LOQ from 40 to 240 minutes for the 80 mg/kg group except for one at 180 minutes and one at 240 minutes. There were no aberrant plasma concentration time point values. The RSD values indicated there was not as good agreement among samples for all time points as was seen after intravenous administration. Except for 40 mg/kg males at 90 minutes and females at 2 minutes, and 80 mg/kg males at 5 minutes, all other time points for each group had RSD values above 40%. This could be due to variability in absorption across the animals. The shapes of the profiles were similar for both sexes, but not for both dose groups. The two dosage groups yielded different best-fit models due to the limited number of measurable time points in the 40 mg/kg groups. The 40 mg/kg groups were best fit by a one-compartment model with first-order absorption and elimination with $1/Yhat^2$ weighting (Figure L20). The 80 mg/kg groups were best fit by a two-compartment model with first-order absorption and elimination with 1/Y weighting (Figure L21). The absorption phase was not well characterized for all groups in that there were not at least three concentration time points that preceded the peak. Otherwise, the profiles had sufficiently characterized terminal linear phases that were used for determining the toxicokinetic parameters as given in Table L12. Absorption of α-thujone was very rapid with half-life values of 4.15 minutes or less in both sexes and at both doses. C_{max} was almost fourfold greater after a twofold increase in dose showing a greater than proportional change in both males and females, while t_{max} was not influenced by dose or sex. Elimination was fast in both sexes, and there appeared to be no sex or dose effect on the elimination of α-thujone. The lack of measurable terminal time points in both female dosage groups resulted in greater standard errors for the elimination half-lives. Similar to what was seen with C_{max}, the males appeared to show a slightly greater than proportional increase in AUC_{∞} following a twofold increase in dose. Estimated AUC_{∞} values in females had high standard errors probably due to an insufficient number of data points.

α-Thujone in the brain was measurable at the earliest planned sample collection time (2 minutes). For males, α-thujone brain concentrations were above the LOQ except for all of the mice at the final two time points in the 40 mg/kg group and one mouse at the final time point in the 80 mg/kg group. For females, α-thujone brain concentrations were above the LOQ except for two mice at 120 minutes and all three mice at 180 minutes in the 40 mg/kg group and one mouse at the 180 minute time point in the 80 mg/kg group. There were no aberrant brain concentration time point values that required further explanation. The RSD values were less than 40% except at 5, 10, and 20 minutes (40 mg/kg males); 2, 40, and 90 minutes (80 mg/kg males); 5 and 20 minutes (40 mg/kg females); and 2, 5, and 10 minutes (80 mg/kg females). This was likely due to individual variability in absorption of α-thujone following gavage administration. α-Thujone brain concentration time profiles had an initial rising phase to a peak followed by a terminal declining phase (Figures L22 and L23). Toxicokinetic parameters estimated from noncompartmental analysis are presented in Table L13. The C_{max} brain values were approximately twofold to fivefold greater than those observed in the plasma. There was no apparent sex difference. Half-life values for α-thujone in the brain were slightly greater than those observed in plasma. Both sexes showed a slightly greater than proportional increase in AUC_{∞} with dose, which was also observed in the plasma samples.

In males, brain concentrations were similar to or approximately twofold to fourfold greater than plasma concentrations between 5 and 90 minutes after dosing, whereas in females, brain concentrations were generally 1.5-fold to fivefold greater than plasma concentrations at all time points (Figure L24). For the most part, male and female brain:plasma ratios were within twofold of each other, suggesting minimal sex difference; however, there were fewer measurable time points for the females at both dosage levels.

Intravenous Administration of α,β-Thujone

α,β-Thujone was administered to groups of male and female B6C3F1 mice at 6.0 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time (2 minutes). All α-thujone plasma concentrations were above the LOQ except for one male mouse at the 60-minute time point and two and three female mice, respectively, at the 60- and 90-minute time points. There were no aberrant plasma concentration time point values except in one animal that was slightly greater than expected at the 90-minute time point. The RSD values were less than 40% for all time points except at 30 and 90 minutes for the males and 15 and 30 minutes for the females. α-Thujone plasma concentration time profiles were biphasic for both sexes and were best described by a two-compartment model with bolus input, first-order output, and 1/Yhat^2 weighting (Figure L25). Estimated toxicokinetic parameters are presented in Table L14. There was no apparent sex difference in C_0. The relatively large volumes of distribution to the peripheral compartment for both sexes indicate that α-thujone undergoes distribution into a peripheral compartment. The $t_{1/2\alpha}$ values (distribution phase) were similar for males and females, taking into consideration the variability of the estimates. Elimination kinetics indicated that the elimination of α-thujone was fast and sex dependent. The overall half-life elimination of α-thujone was short and similar for males and females. However, the clearance of α-thujone from the central compartment was greater in females. Thus, elimination of α-thujone occurred almost twofold slower for males than females. AUC_∞ values further demonstrated that males underwent greater exposure than females at the same dosage level.

α-Thujone in the brain was measurable at the earliest planned sample collection time (2 minutes). All α-thujone brain concentrations were above the LOQ except in male mice at 60 minutes (one mouse) and 90 minutes (two mice) and in female mice at 45 minutes (one mouse), 60 minutes (two mice), and 90 minutes (three mice). There were no aberrant brain concentration time point values that required further explanation. The RSD values indicated there was good agreement among samples for all time points except for one time point (45 minutes) in male mice (51.2%) and one time point (30 minutes) in female mice (110.3%). These RSDs were marginally high but not unusual for these later time points. α-Thujone brain concentration time profiles were monophasic for both sexes (Figure L26). These declines were similar to those seen in the brain of mice following intravenous administration of α-thujone. Toxicokinetic parameters, estimated from noncompartmental analysis, are presented in Table L15. Observed α-thujone C_{max} values are approximately fivefold (males) and sixfold (females) greater than the values observed in the plasma. The brain concentrations did not show a sex difference and were around twofold greater than the values observed after an intravenous dose of α-thujone. In females, the elimination half-life of α-thujone in the brain was similar to that in plasma, but male mice had an almost fourfold slower elimination from the brain than from plasma. The poorer correlation in males may be due to the fewer number of time points used for the half-life determination in the terminal phase in males. The AUC_∞ value was greater in females compared to males.

The α-thujone brain:plasma ratios were greater in females than in males at all time points, often by about twofold (Figure 27).

Oral Administration of α,β-Thujone

α,β-Thujone was administered to groups of male and female B6C3F1 mice at 40 or 80 mg/kg. α-Thujone in plasma was measurable at the earliest sample collection time (2 minutes). In males, all plasma concentrations were above the LOQ except for two mice at 180 minutes and three at 300 minutes in the 40 mg/kg group and two mice at 360 minutes in the 80 mg/kg group. In females, all plasma concentrations were above the LOQ except for one mouse at 40 minutes and all mice from 90 to 180 minutes in the 40 mg/kg group and one mouse at 90 minutes, two mice at 180 minutes, and three mice at 240 minutes in the 80 mg/kg group. There were no aberrant plasma concentration time point values that required further explanation. The RSD values indicated there was good agreement among samples for only the 2-minute time point in 40 mg/kg males, the 2- and 180-minute time points for 80 mg/kg males, the 5-minute time-point for 40 mg/kg females, and the 40-minute time point for 80 mg/kg females. All other RSDs were above 40%. α-Thujone plasma concentration time profiles were characteristic of oral absorption, as there were initial incline phases to peaks followed by declining phases (Figures L28 and L29). The absorption phases were poorly characterized for all groups because there were not at least three concentration time

points that preceded each peak. Additionally, the profiles lacked sufficient time points for accurate characterization of a process with first-order input. Toxicokinetic parameters estimated from the one-compartment model with 1/Yhat weighting are presented in Table L16. Absorption of α-thujone was very rapid with half-life values of 2.92 minutes or less. There was no evidence of a sex- or dose-related effect on absorption. There was no sex difference in C_{max}, but both sexes showed a greater than proportional increase in C_{max} with dose. Elimination was fast, and there was a trend that suggested it was sex and dose dependent. Apparent clearance values, when the measure of variability was taken into consideration, suggested that both males and females exhibited a decrease in clearance with an increase in dose. Additionally, females had a greater clearance and lower overall half-life of α-thujone in the plasma. AUC_{∞} values further demonstrated that at a given dose, males underwent greater exposure than females, but as the dose was increased, both males and females exhibited a greater than proportional increase in AUC_{∞}.

α-Thujone in the brain was measurable at the earliest targeted sample collection time (2 minutes). All α-thujone brain concentrations were above the LOQ except for all mice at 300 minutes and one at 180 minutes in the 40 mg/kg male group; all mice at 180 minutes, two at 120 minutes, and one at 90 minutes in the 40 mg/kg female group; and two mice at 240 minutes in the 80 mg/kg female group. There were no aberrant brain concentration time point values that required further explanation. The RSD values indicated there was good agreement only at the 2-minute target time in the 40 mg/kg male group; the 2-, 5-, 40-, 90-, and 180-minute target times in the 80 mg/kg male group; the 5-minute target time in the 40 mg/kg female group; and the 5- and 40- minute target times in the 80 mg/kg female group. All other RSDs were above 40%. α-Thujone brain concentration time profiles had an initial rising phase followed by terminal declining phases (Figures L30 and L31). Toxicokinetic parameters, estimated from noncompartmental analysis, are presented in Table L17. C_{max} increased more than proportional to dose, and there was no apparent sex difference. C_{max} values in the brain were approximately threefold to fourfold (males) and threefold to fivefold (females) greater than values observed in the plasma. Half-life values for α-thujone in the brain were lower in males compared to females. In comparison, terminal half-life values for α-thujone were about twofold greater in the brain compared to the plasma in both males and females at both doses. AUC_{∞} values showed a greater than proportional increase with dose in both males and females.

For males, brain concentrations were similar to or approximately twofold to fourfold greater than plasma concentrations between 2 and 90 minutes after dosing except at 40 minutes where the brain:plasma ratio was 16.3; for females, brain concentrations were generally twofold to sixfold greater than plasma concentrations at all time points (Figure L32). Female brain:plasma ratios were greater than male ratios at any given time point except at 2 and 40 minutes.

DISCUSSION AND CONCLUSIONS

This study showed that after intravenous administration of α-thujone or α,β-thujone, α-thujone was distributed into a peripheral compartment for rats and mice and was eliminated from the central compartment. α-Thujone had rapid elimination in rats and mice without showing any apparent dose dependence following administration of both forms. α-Thujone was rapidly distributed to the brain with brain:plasma ratios greater than 1.00 in both rats and mice. The only apparent species differences were that mice had shorter elimination half-lives, faster clearance, and lower AUC_{∞} values than rats. There were apparent sex differences including a slower elimination in male rats, greater tissue distribution in male rats, and greater brain:plasma ratios in female rats and female mice. Unlike in male rats, male mice did not have a significantly greater tissue distribution than females.

Following a single gavage administration of α-thujone or α,β-thujone, α-thujone absorption was very rapid and dose, species, and sex independent. The larger variability observed in the C_{max} and t_{max} values suggests that factors influencing absorption may enhance or delay uptake, thereby affecting peak and time to peak values. Examples of such factors include the presence/absence of food and gastric emptying time. There was no evidence that α-thujone absorption was saturated following administration of α-thujone or α,β-thujone. α-Thujone was distributed into a peripheral compartment in both rats and mice following absorption. C_{max} and AUC_{∞} values increased in a dose-proportional manner, except in female rats following administration of α-thujone and in male and female mice following administration of α,β-thujone where the increase was more than proportional to dose. α-Thujone was rapidly distributed to the brain with brain:plasma ratios greater than 1.00 in both rats and mice. Female rats and mice had greater brain:plasma ratios than their male counterparts although the effect is not as pronounced in female

mice as in female rats. The only apparent species difference occurred in elimination of α-thujone where mice had shorter elimination half-lives, faster clearance, and lower AUC_∞ values than rats.

The AUC_∞ (predicted) after gavage administration of α-thujone was compared to the AUC_∞ (predicted) after intravenous administration of α-thujone with respect to dose in male and female rats and mice to estimate bioavailability of α-thujone following oral administration of α-thujone. The oral bioavailability was 20.5% and 27.1% in male rats and 96.2% and 177% in female rats following administration of 25 and 50 mg/kg, respectively. Female rats had a greater than dose-proportional increase in AUC_∞ suggesting possible saturation of elimination kinetics following administration of a 50 mg/kg dose. Therefore, oral bioavailability in female rats was considered to be best represented by the 25 mg/kg group (96.2%). The oral bioavailability was 10.9% and 16.2% in male mice and 70.8% and 13.1% in female mice following administration of 40 or 80 mg/kg, respectively. AUC_∞ for female mice after a 40 mg/kg dose was potentially overestimated due to an insufficient number of time points with measurable concentrations. Therefore, the oral bioavailability in female mice was considered to be best represented by the 80 mg/kg group (13.1%). Based on these data, it can be concluded that the oral bioavailability of α-thujone was greater in female rats compared to male rats. The oral bioavailability was similar for male and female mice but slightly lower than male rats following administration of α-thujone.

The AUC_∞ (predicted) after gavage administration of α,β-thujone was compared to the AUC_∞ (predicted) after intravenous administration of α,β-thujone with respect to dose in male and female rats and mice to estimate bioavailability of α-thujone following oral administration of α,β-thujone. The oral bioavailability was 23.6% and 21.5% in male rats and 54.4% and 58.5% in female rats following administration of 25 and 50 mg/kg, respectively. The oral bioavailability was 9.56% and 52.9% in male mice and 9.77% and 26.8% in female mice following administration of 40 and 80 mg/kg, respectively. Male and female mice showed a greater than dose-proportional increase in AUC_∞ suggesting possible saturation of elimination kinetics following administration of 80 mg/kg. Based on these data, it can be concluded that the oral bioavailability of α-thujone in female rats was greater than in male rats following administration of α,β-thujone. This was similar to that observed following α-thujone administration, although as with α-thujone administration, no saturation of elimination kinetics was observed in females following α,β-thujone administration. The oral bioavailability of α-thujone was similar for male and female mice.

In NTP single-dose toxicokinetic studies in rats and mice, α-thujone absorption was rapid and dose, species, and sex independent following gavage administration of either α-thujone or α,β-thujone. The oral bioavailability was greater in female rats compared to male rats following administration of both formulations; there was no apparent sex difference in bioavailability in mice. α-Thujone was distributed to the brain in high concentrations. The main sex difference was greater brain:plasma ratios in female rats and female mice. The only apparent species difference was the faster elimination of α-thujone in mice compared to rats.

REFERENCE

Davies, B., and Morris, T. (1993). Physiological parameters in laboratory animals and humans. *Pharm. Res.* **10**, 1093-1095.

TABLE L1
Experimental Design of the Toxicokinetic Studies of α-Thujone and α,β-Thujone

Compound	Route of Administration	Target Dose (mg/kg)
Rats		
α-Thujone	Intravenous	1.6
	Gavage	25 50
α,β-Thujone	Intravenous	3.0
	Gavage	25 50
Mice		
α-Thujone	Intravenous	3.2
	Gavage	40 80
α,β-Thujone	Intravenous	6.0
	Gavage	40 80

TABLE L1
Experimental Design of the Toxicokinetic Studies of α-Thujone and α,β-Thujone

Compound	Route of Administration	Target Dose (mg/kg)
Rats		
α-Thujone	Intravenous	1.6
	Gavage	25 50
α,β-Thujone	Intravenous	3.0
	Gavage	25 50
Mice		
α,-Thujone	Intravenous	3.2
	Gavage	40 80
α,β-Thujone	Intravenous	6.0
	Gavage	40 80

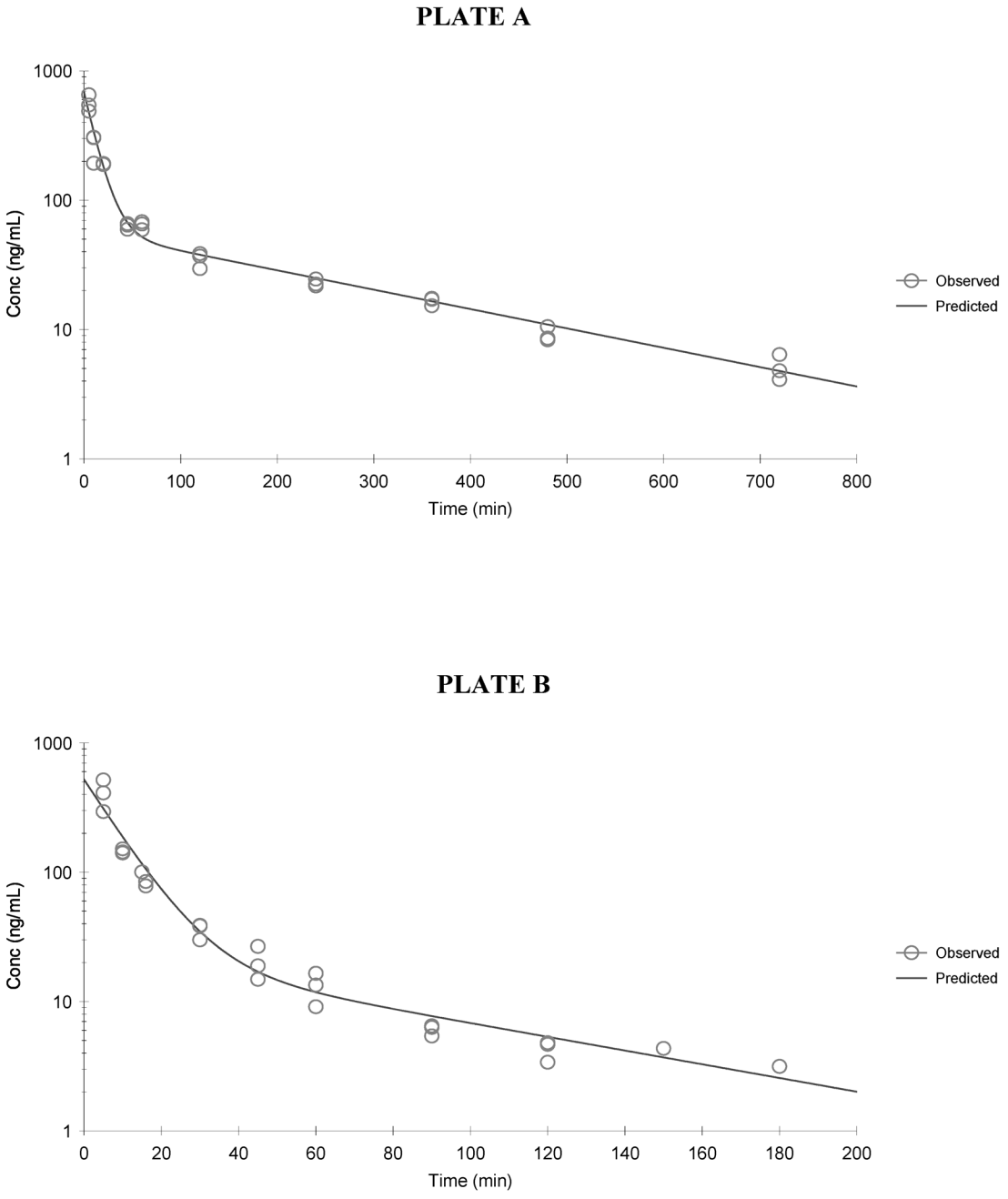


FIGURE L1
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Intravenous Administration of 1.6 mg/kg α-Thujone
Data were fitted using a two-compartment model with $1/Yhat^2$ weighting.

TABLE L2
Toxicokinetic Parameters for α-Thujone in the Plasma of F344/N Rats Following a Single Intravenous Administration of 1.6 mg/kg α-Thujone[a]

	Parameter	Estimate	Standard Error
Male			
	$C_{(5\ min)}$ (obs) (ng/mL)	563	84 (SD)
	$C_{(0\ min)}$ (fitted) (ng/mL)	684	80
	V_d – 1st comp (mL/kg)	2,340	270
	V_d – 2nd comp (mL/kg)	10,700	800
	k_{12} (min^{-1})	0.0445	0.0067
	k_{21} (min^{-1})	0.00973	0.00103
	k_{10} (min^{-1})	0.0278	0.0029
	k_{10} $t_{1/2}$ (min)	24.9	2.6
	Alpha $t_{1/2}$ (min)	8.82	1.07
	Beta $t_{1/2}$ (min)	201	12
	Cl (mL/min/kg)	65.1	2.3
	Cl – 2nd comp (mL/min/kg)	104	11
	MRT (min)	200	11
	AUC_∞ ($ng \bullet mL^{-1} \bullet min$)	24,600	900
Female			
	$C_{(5\ min)}$ (obs) (ng/mL)	408	111 (SD)
	$C_{(0\ min)}$ (fitted) (ng/mL)	522	99
	V_d – 1st comp (mL/kg)	3,070	580
	V_d – 2nd comp (mL/kg)	4,470	1,060
	k_{12} (min^{-1})	0.0241	0.0051
	k_{21} (min^{-1})	0.0165	0.0036
	k_{10} (min^{-1})	0.0807	0.0109
	k_{10} $t_{1/2}$ (min)	8.59	1.15
	Alpha $t_{1/2}$ (min)	6.35	0.91
	Beta $t_{1/2}$ (min)	56.7	11.4
	Cl (mL/min/kg)	248	20
	Cl – 2nd comp (mL/min/kg)	73.8	14.1
	MRT (min)	30.4	4.7
	AUC_∞ ($ng \bullet mL^{-1} \bullet min$)	6,460	520

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation.

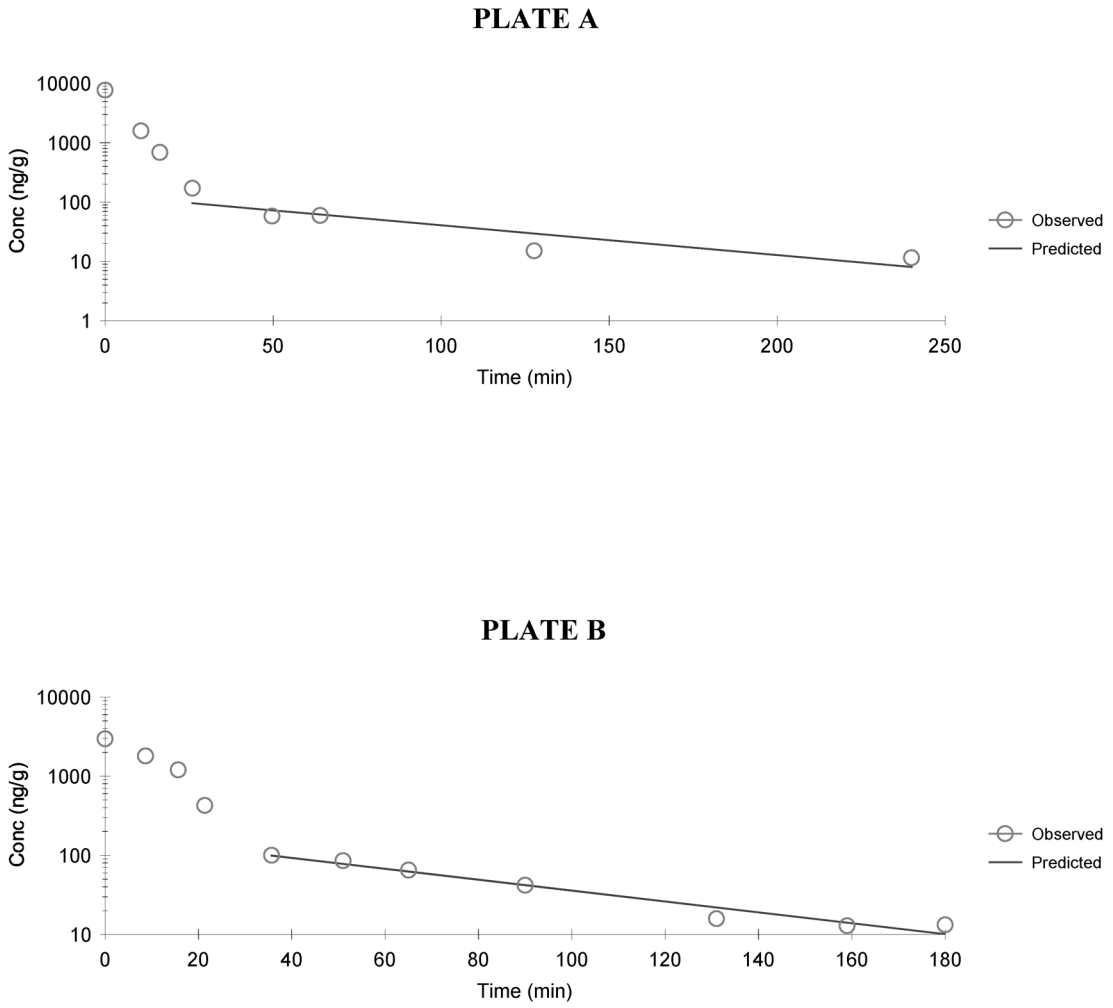


FIGURE L2
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Intravenous Administration of 1.6 mg/kg α-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

TABLE L3
Toxicokinetic Parameters for α-Thujone in the Brain of F344/N Rats Following a Single Intravenous Administration of 1.6 mg/kg α-Thujone[a]

Parameter	Male	Female
C_{max} (obs) (ng/g)	1,590 (SD=520)	1,810 (SD=360)
t_{max} (obs) (min)	10.7	8.67
$t_{1/2}$ (min)	60.0	43.7
AUC_{last} ($ng \bullet g^{-1} \bullet minute$)	67,900	45,400
AUC_{∞_pred} ($ng \bullet g^{-1} \bullet minute$)	68,600	46,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.

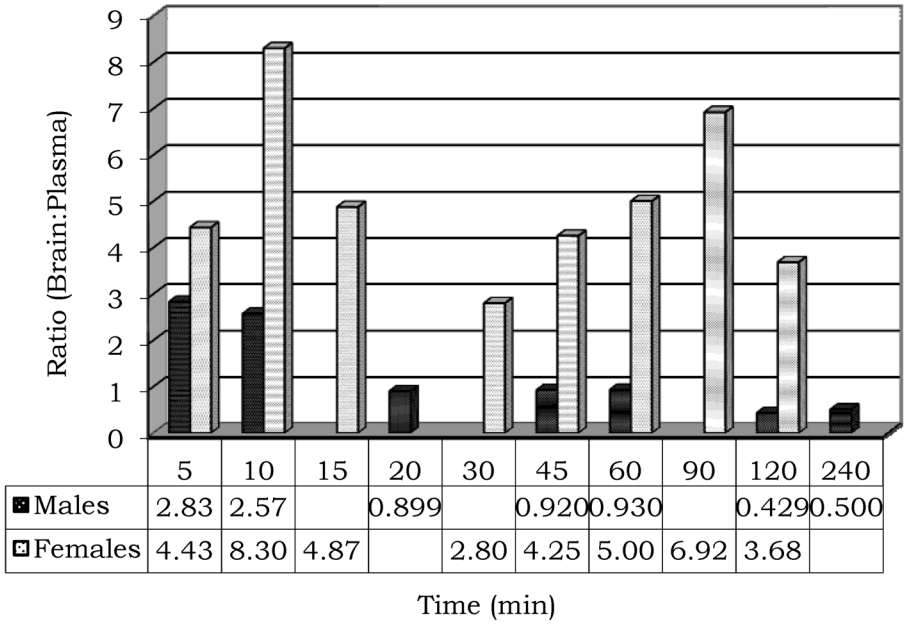


FIGURE L3
Brain:Plasma Concentration Ratios of α-Thujone in F344/N Rats Following a Single Intravenous Administration of 1.6 mg/kg α-Thujone
n>1 Conc/Time Point; Target Times Used

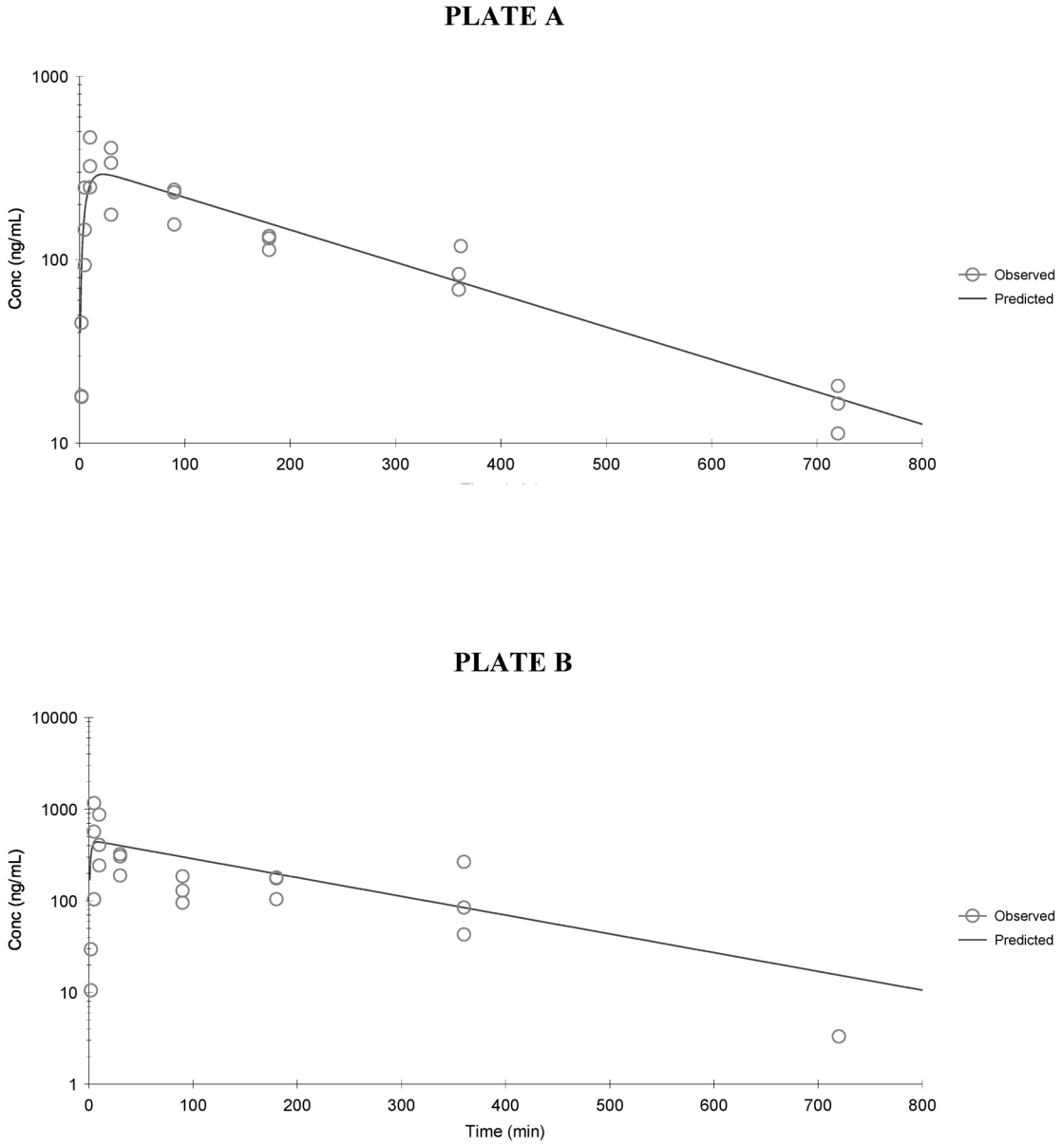


FIGURE L4
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 25 mg/kg α-Thujone
Data were fitted using a one-compartment model with $1/Yhat^2$ weighting.

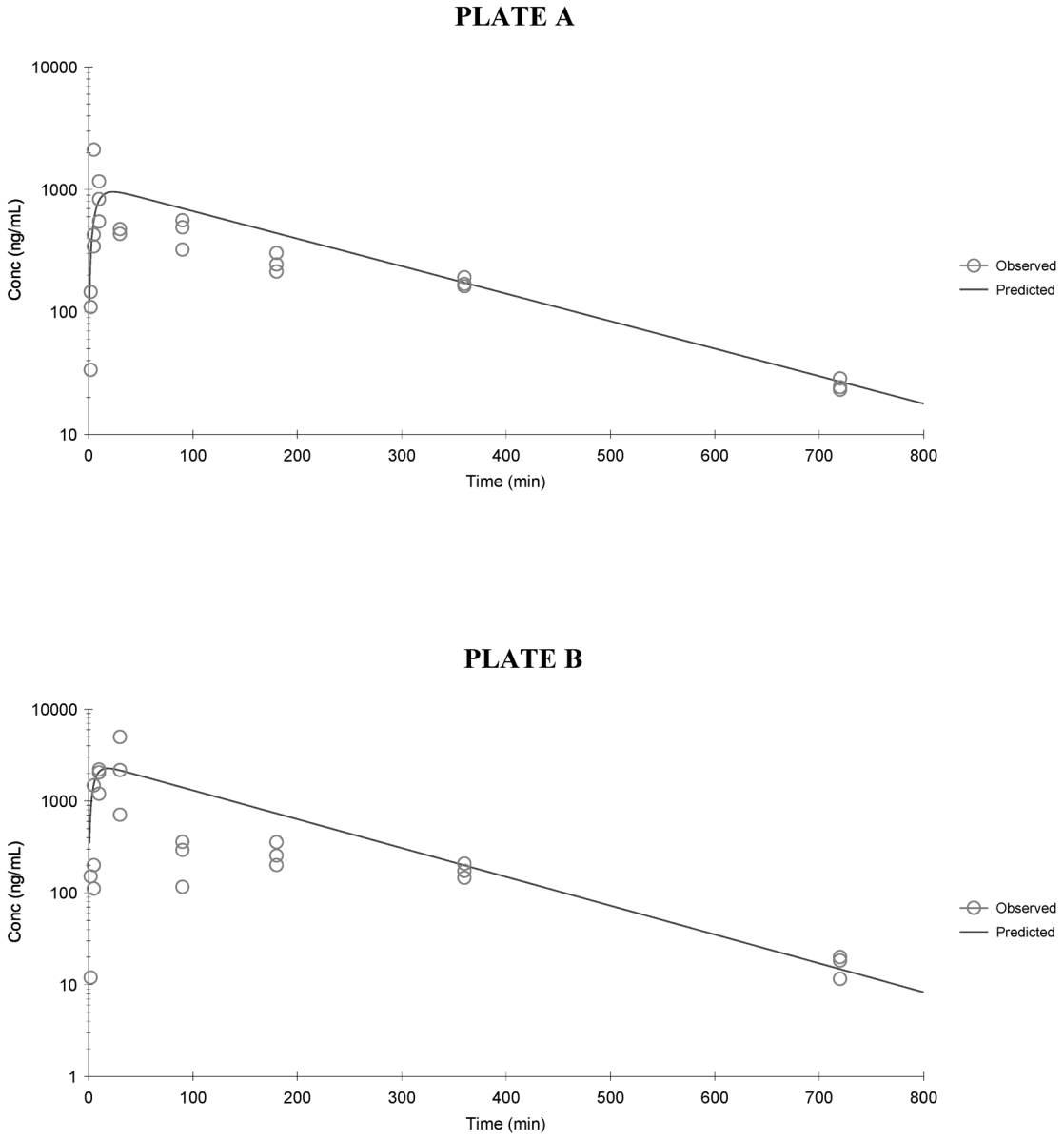


FIGURE L5
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 50 mg/kg α-Thujone
Data were fitted using a one-compartment model with $1/Yhat^2$ weighting.

TABLE L4
Toxicokinetic Parameters for α-Thujone in the Plasma of F344/N Rats Following a Single Gavage Administration of 25 or 50 mg/kg α-Thujone[a]

Dose (mg/kg)	Parameter	Male		Female	
		Estimate	Standard Error	Estimate	Standard Error
25	k_{01} (min^{-1})	0.171	0.053	0.584	0.747
	k_{01} $t_{1/2}$ (min)	4.06	1.27	1.19	1.51
	C_{max} (obs) (ng/mL)	345	110 (SD)	613	532 (SD)
	C_{max} (pred) (ng/mL)	292	39	440	110
	t_{max} (obs) (min)	10.0	0.0	5.00	0.00
	t_{max} (pred) (min)	22.4	5.0	8.31	8.37
	V_d_F (mL/kg)	78,100	11,900	54,600	14,900
	k_{10} (min^{-1})	0.00407	0.00044	0.00472	0.00113
	k_{10} $t_{1/2}$ (min)	170	18	147	35
	Cl_F (mL/min/kg)	318	33	258	56
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	83,400	ND	77,300	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	78,700	8,200	97,100	21,100
50	k_{01} (min^{-1})	0.150	0.078	0.183	0.098
	k_{01} $t_{1/2}$ (min)	4.62	2.40	3.78	2.02
	C_{max} (obs) (ng/mL)	963	1,000 (SD)	2,630	2,180 (SD)
	C_{max} (pred) (ng/mL)	955	215	2,270	450
	t_{max} (obs) (min)	5.00	0.00	30.0	0.0
	t_{max} (pred) (min)	23.3	8.5	18.4	7.0
	V_d_F (mL/kg)	46,400	12,300	19,300	4,500
	k_{10} (min^{-1})	0.00517	0.00076	0.00723	0.00069
	k_{10} $t_{1/2}$ (min)	134	20	95.9	9.1
	Cl_F (mL/min/kg)	240	45	140	25
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	159,000	ND	307,000	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	208,000	39,000	358,000	64,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation; ND=not determined.

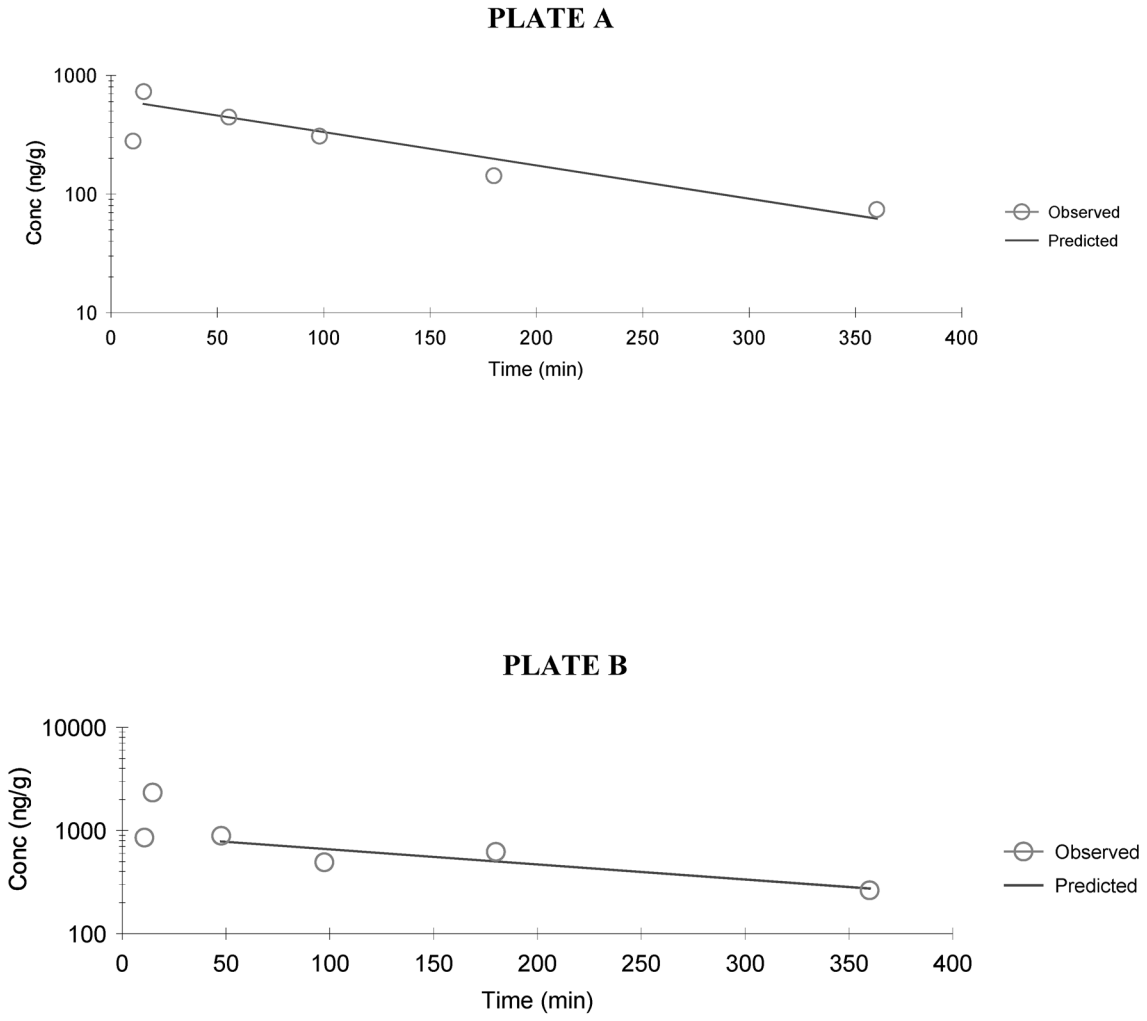


FIGURE L6
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 25 mg/kg α-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

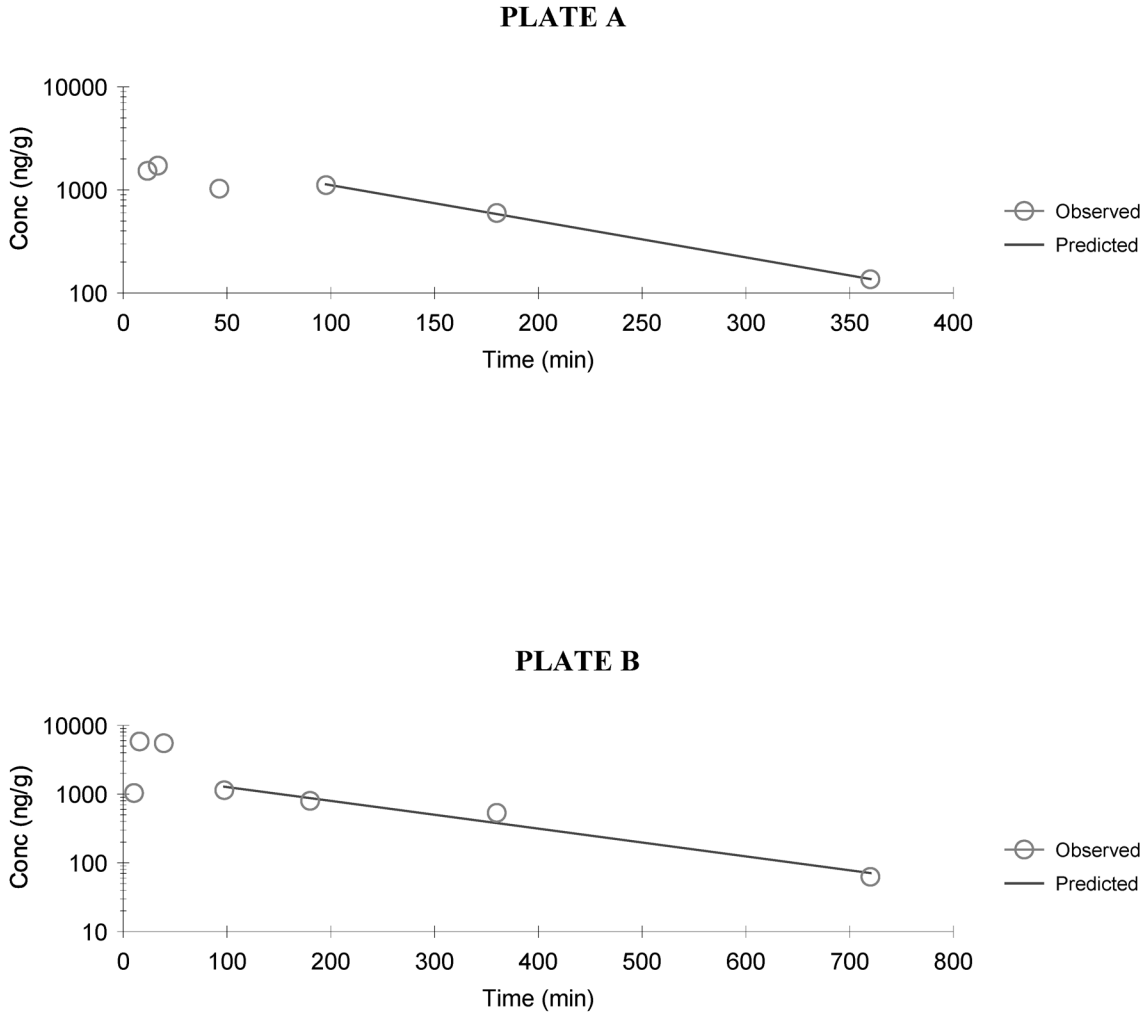
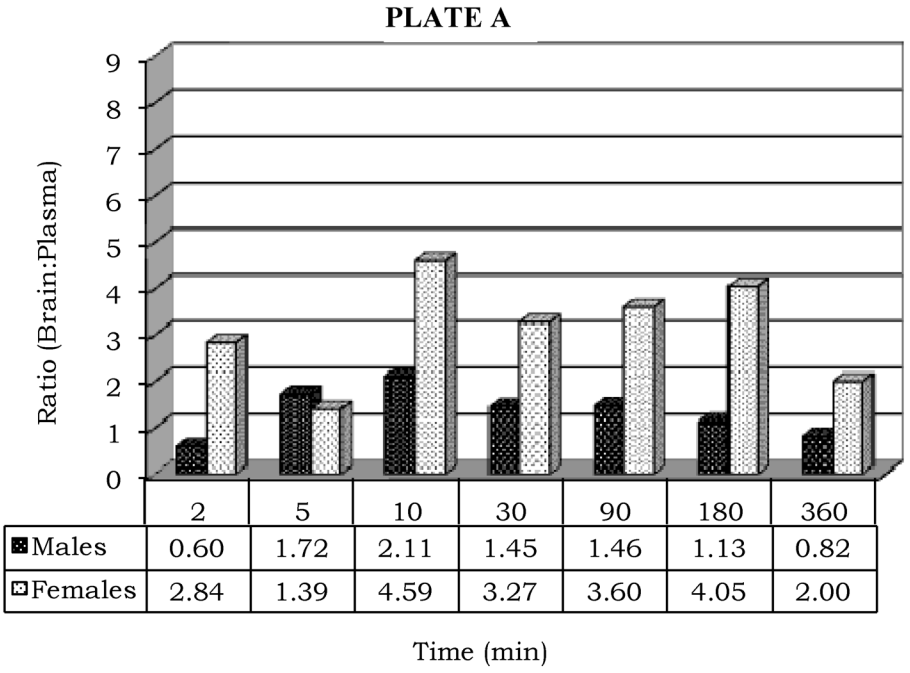


FIGURE L7
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 50 mg/kg α-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

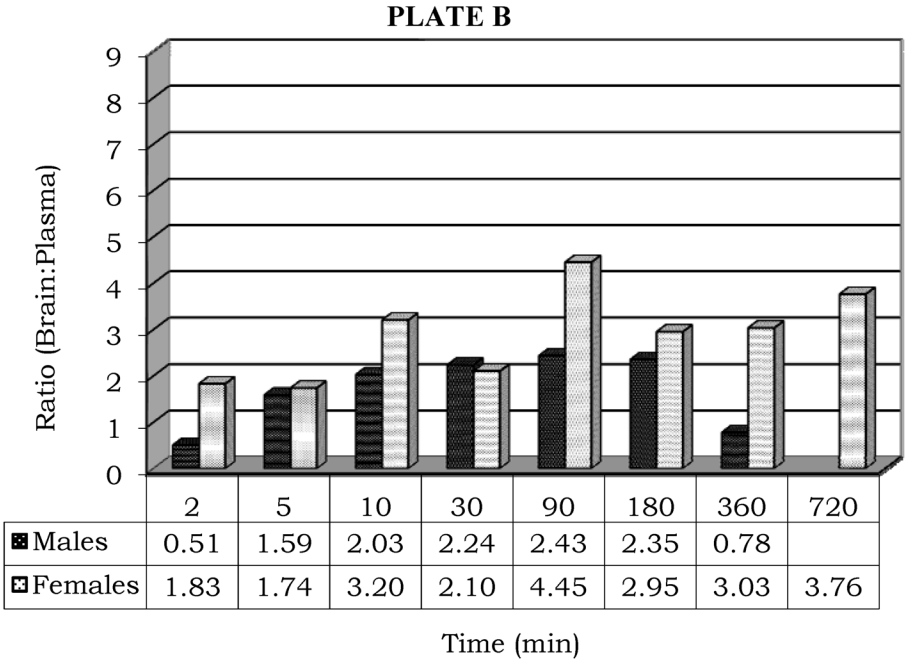
TABLE L5
Toxicokinetic Parameters for α-Thujone in the Brain of F344/N Rats Following a Single Gavage Administration of 25 or 50 mg/kg α-Thujone[a]

Dose (mg/kg)	Parameter	Male	Female
25	C_{max} (obs) (ng/g)	728 (SD=38)	2,330 (SD=1,450)
	t_{max} (obs) (min)	15.3	14.7
	$t_{1/2}$ (min)	107	206
	AUC_{last} (ng•g^{-1}•min)	81,400	224,000
	AUC_{∞_pred} (ng•g^{-1}•min)	91,000	306,000
50	C_{max} (obs) (ng/g)	1,720 (SD=660)	5,820 (SD=1,100)
	t_{max} (obs) (min)	16.7	15.7
	$t_{1/2}$ (min)	86.1	149
	AUC_{last} (ng•g^{-1}•min)	249,000	658,000
	AUC_{∞_pred} (ng•g^{-1}•min)	266,000	673,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.



	2	5	10	30	90	180	360
Males	0.60	1.72	2.11	1.45	1.46	1.13	0.82
Females	2.84	1.39	4.59	3.27	3.60	4.05	2.00



	2	5	10	30	90	180	360	720
Males	0.51	1.59	2.03	2.24	2.43	2.35	0.78	
Females	1.83	1.74	3.20	2.10	4.45	2.95	3.03	3.76

FIGURE L8
Brain:Plasma Concentration Ratios of α-Thujone in F344/N Rats Following a Single Gavage Administration of 25 (Plate A) or 50 (Plate B) mg/kg α-Thujone
n>1 Conc/Time Point; Target Times Used

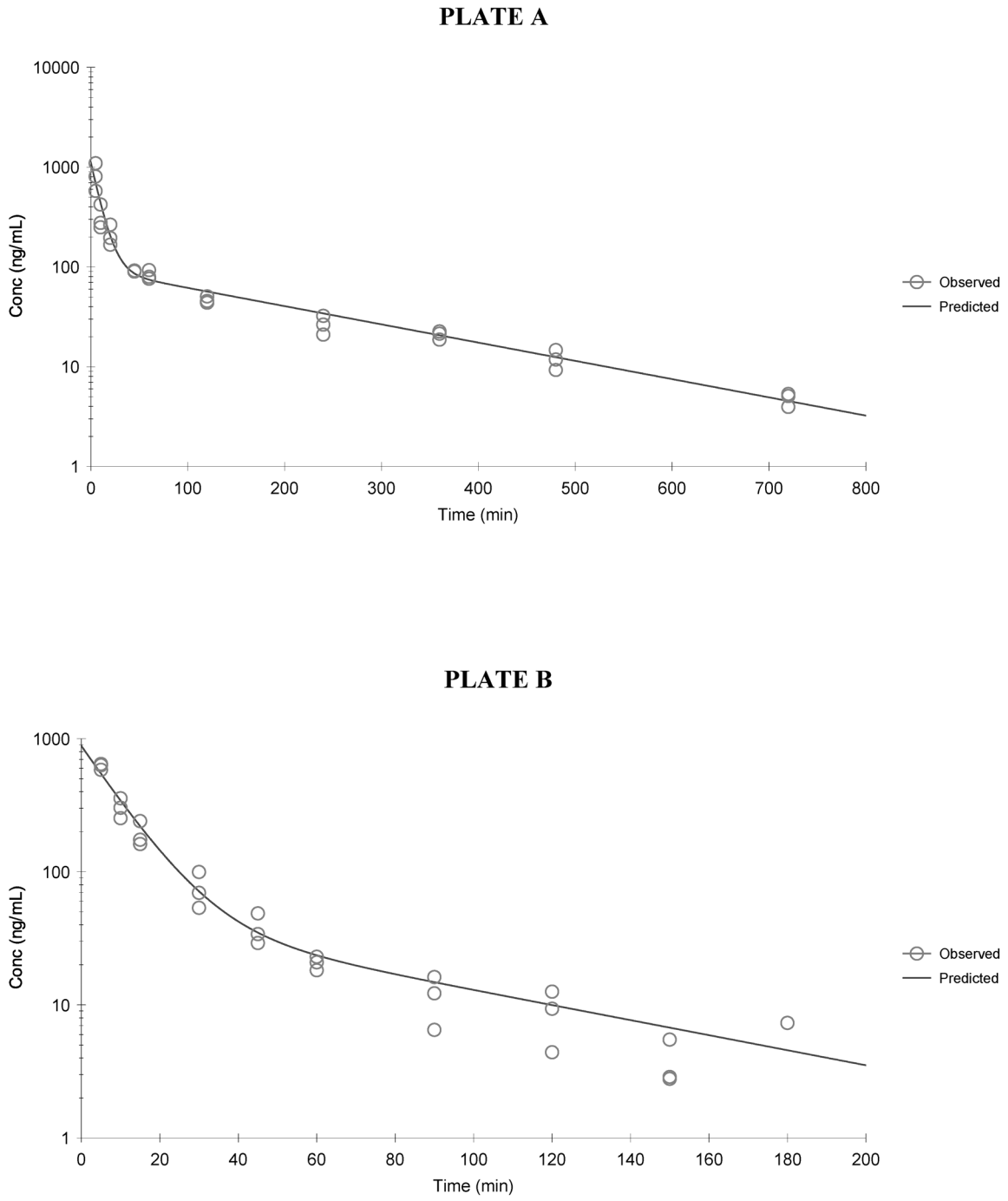


FIGURE L9
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Intravenous Administration of 3.0 mg/kg α,β-Thujone
Data were fitted using a two-compartment model with $1/Yhat^2$ weighting.

TABLE L6
Toxicokinetic Parameters for α-Thujone in the Plasma of F344/N Rats Following a Single Intravenous Administration of 3.0 mg/kg α,β-Thujone[a]

Parameter	Estimate	Standard Error
Male		
$C_{(5\ min)}$ (obs) (ng/mL)	826	259 (SD)
$C_{(0\ min)}$ (fitted) (ng/mL)	1,110	200
V_d – 1st comp (mL/kg)	2,700	490
V_d – 2nd comp (mL/kg)	13,200	1,000
k_{12} (min^{-1})	0.0633	0.0127
k_{21} (min^{-1})	0.0129	0.0014
k_{10} (min^{-1})	0.0349	0.0057
k_{10} $t_{1/2}$ (min)	19.8	3.2
Alpha $t_{1/2}$ (min)	6.48	1.10
Beta $t_{1/2}$ (min)	165	9
Cl (mL/min/kg)	94.2	4.2
Cl – 2nd comp (mL/min/kg)	171	21
MRT (min)	169	9
AUC_∞ (ng•mL^{-1}•min)	31,800	1,400
Female		
$C_{(5\ min)}$ (obs) (ng/mL)	622	33 (SD)
$C_{(0\ min)}$ (fitted) (ng/mL)	885	185
V_d – 1st comp (mL/kg)	3,390	710
V_d – 2nd comp (mL/kg)	4,310	970
k_{12} (min^{-1})	0.0227	0.0061
k_{21} (min^{-1})	0.0178	0.0042
k_{10} (min^{-1})	0.0746	0.0113
k_{10} $t_{1/2}$ (min)	9.29	1.41
Alpha $t_{1/2}$ (min)	6.79	1.17
Beta $t_{1/2}$ (min)	53.2	10.8
Cl (mL/min/kg)	253	22
Cl – 2nd comp (mL/min/kg)	76.8	17.2
MRT (min)	30.5	4.2
AUC_∞ (ng•mL^{-1}•min)	11,900	1,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation.

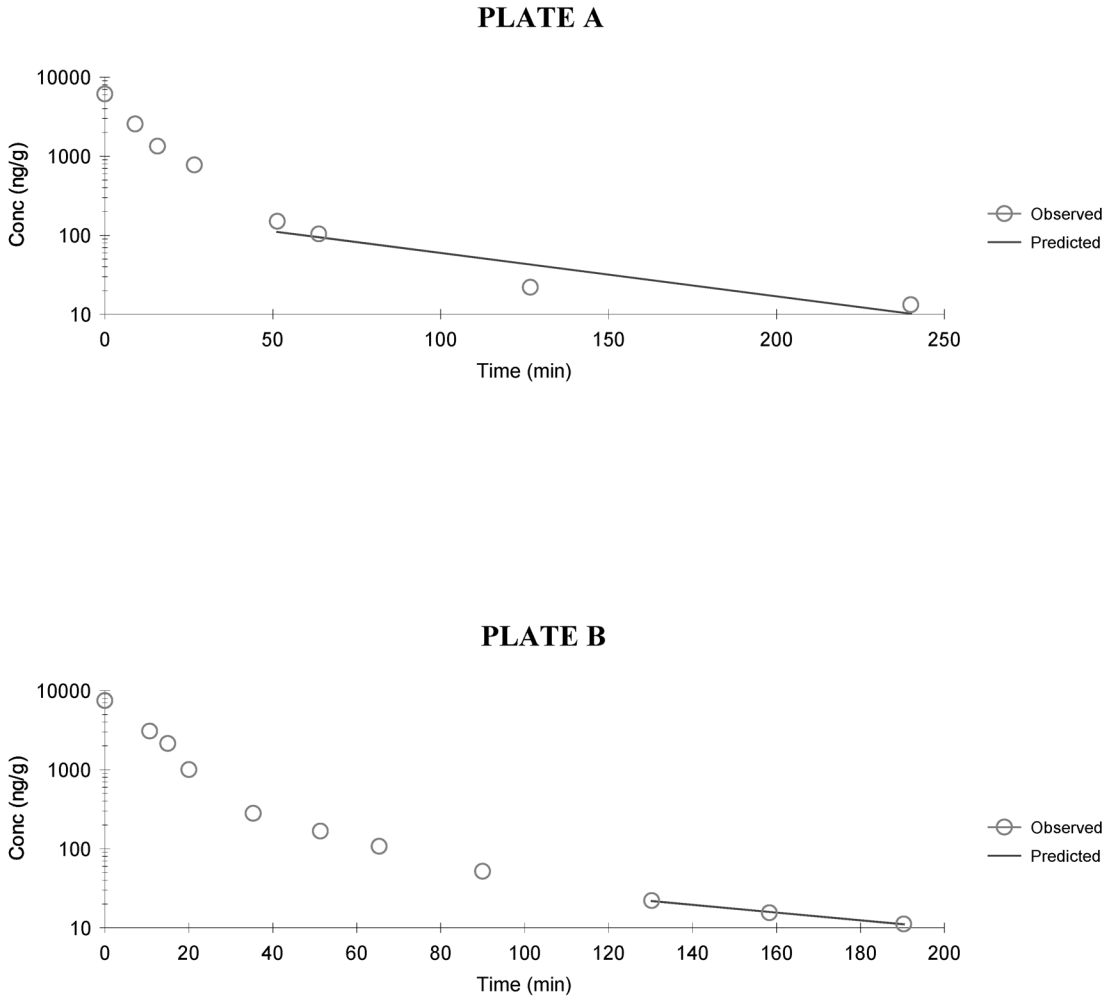


FIGURE L10
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Intravenous Administration of 3.0 mg/kg α,β-Thujone
Data were fitted using a noncompartmental model with uniform weighting

TABLE L7
Toxicokinetic Parameters for α-Thujone in the Brain of F344/N Rats Following a Single Intravenous Administration of 3.0 mg/kg α,β-Thujone[a]

Parameter	Male	Female
C_{max} (obs) (ng/g)	2,560 (SD=590)	3,090 (SD=200)
t_{max} (obs) (min)	9.00	10.7
$t_{1/2}$ (min)	54.9	61.5
AUC_{last} (ng•g^{-1}•min)	82,700	95,600
AUC_{∞_pred} (ng•g^{-1}•min)	83,500	96,600

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.

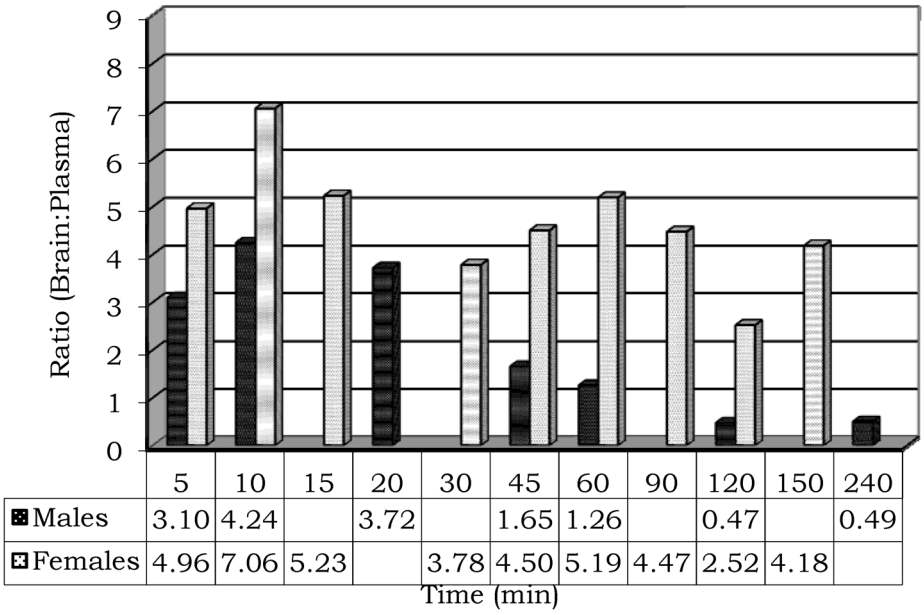


FIGURE L11
Brain:Plasma Concentration Ratios of α-Thujone in F344/N Rats Following a Single Intravenous Administration of 3.0 mg/kg α,β-Thujone
n>1 Conc/Time Point; Target Times Used)

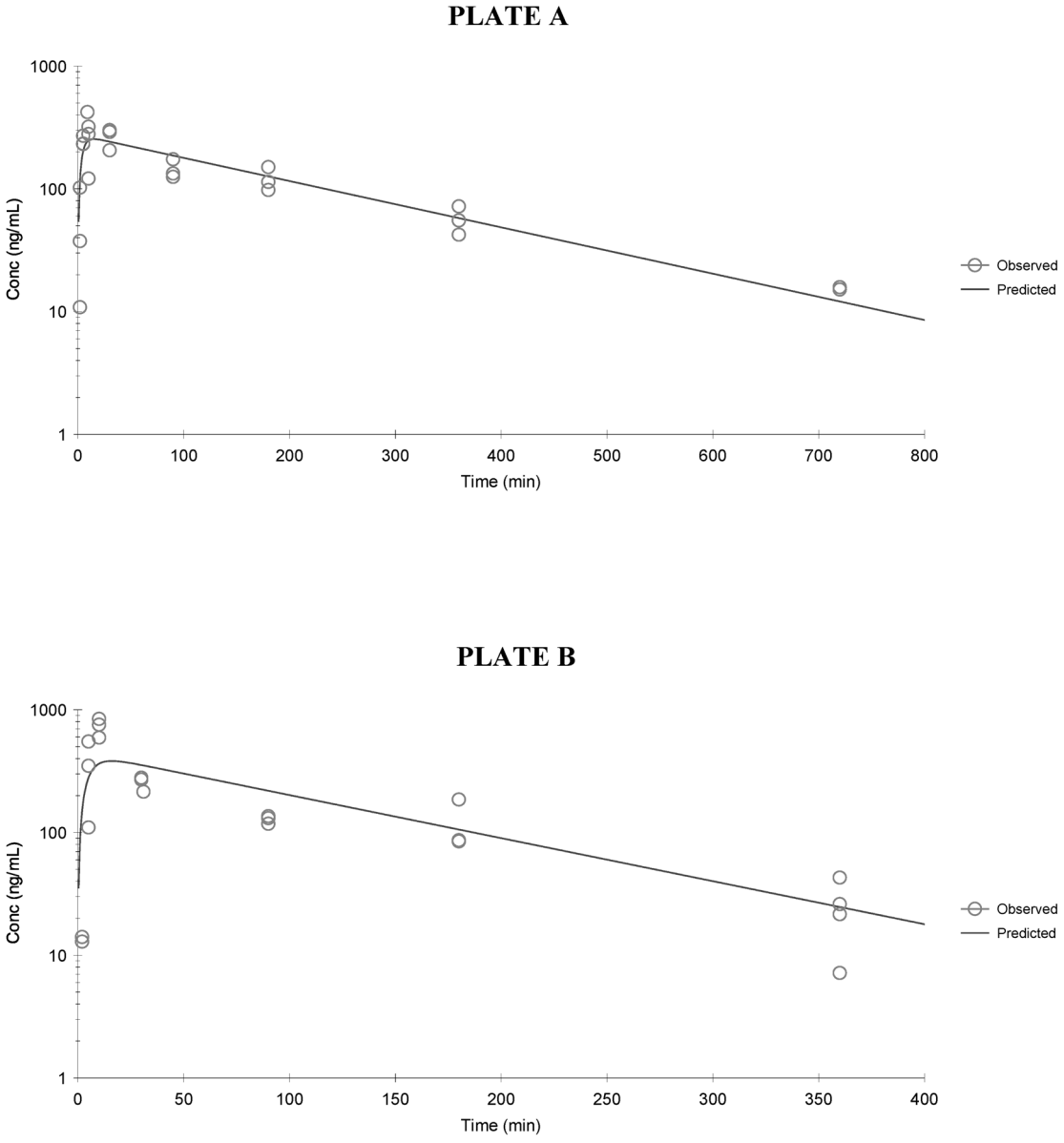


FIGURE L12
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 25 mg/kg α,β-Thujone
Data were fitted using a one-compartment model with $1/Yhat^2$ weighting.

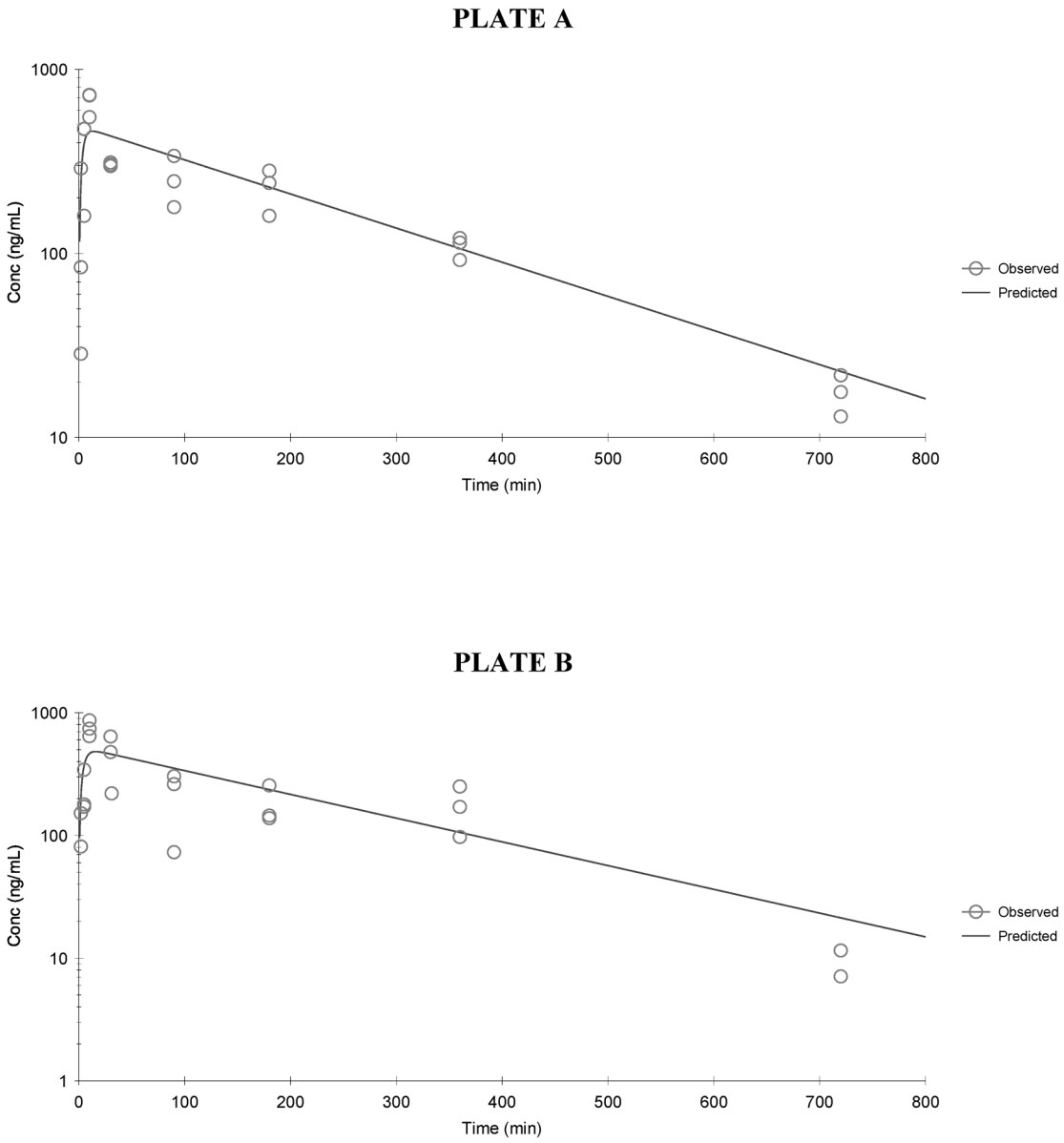


FIGURE L13
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 50 mg/kg α,β-Thujone
Data were fitted using a one-compartment model with $1/Yhat^2$ weighting.

TABLE L8
Toxicokinetic Parameters for α-Thujone in the Plasma of F344/N Rats Following a Single Gavage Administration of 25 or 50 mg/kg α,β-Thujone[a]

Dose (mg/kg)	Parameter	Male		Female	
		Estimate	Standard Error	Estimate	Standard Error
25	k_{01} (min^{-1})	0.281	0.093	0.213	0.135
	k_{01} $t_{1/2}$ (min)	2.47	0.81	3.25	2.07
	C_{max} (obs) (ng/mL)	286	125 (SD)	731	127 (SD)
	C_{max} (pred) (ng/mL)	255	29	383	88
	t_{max} (obs) (min)	9.75	0.50	10.0	0.0
	t_{max} (pred) (min)	15.1	3.7	16.0	7.0
	V_d_F (mL/kg)	92,000	12,000	57,400	16,200
	k_{10} (min^{-1})	0.00435	0.00042	0.00807	0.00142
	k_{10} $t_{1/2}$ (min)	160	16	85.9	15.1
	Cl_F (mL/min/kg)	400	37	464	85
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	57,500	ND	44,300	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	62,600	5,800	53,900	9,800
50	k_{01} (min^{-1})	0.344	0.129	0.259	0.142
	k_{01} $t_{1/2}$ (min)	2.01	0.75	2.68	1.46
	C_{max} (obs) (ng/mL)	666	100 (SD)	752	112 (SD)
	C_{max} (pred) (ng/mL)	462	55	483	87
	t_{max} (obs) (min)	10.0	0.0	10.0	0.0
	t_{max} (pred) (min)	12.9	3.7	16.0	6.5
	V_d_F (mL/kg)	102,000	13,000	96,400	19,600
	k_{10} (min^{-1})	0.00427	0.00039	0.00446	0.00067
	k_{10} $t_{1/2}$ (min)	162	15	156	23
	Cl_F (mL/min/kg)	437	41	430	63
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	96,400	ND	106,000	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	114,000	11,000	116,000	17,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation; ND=not determined.

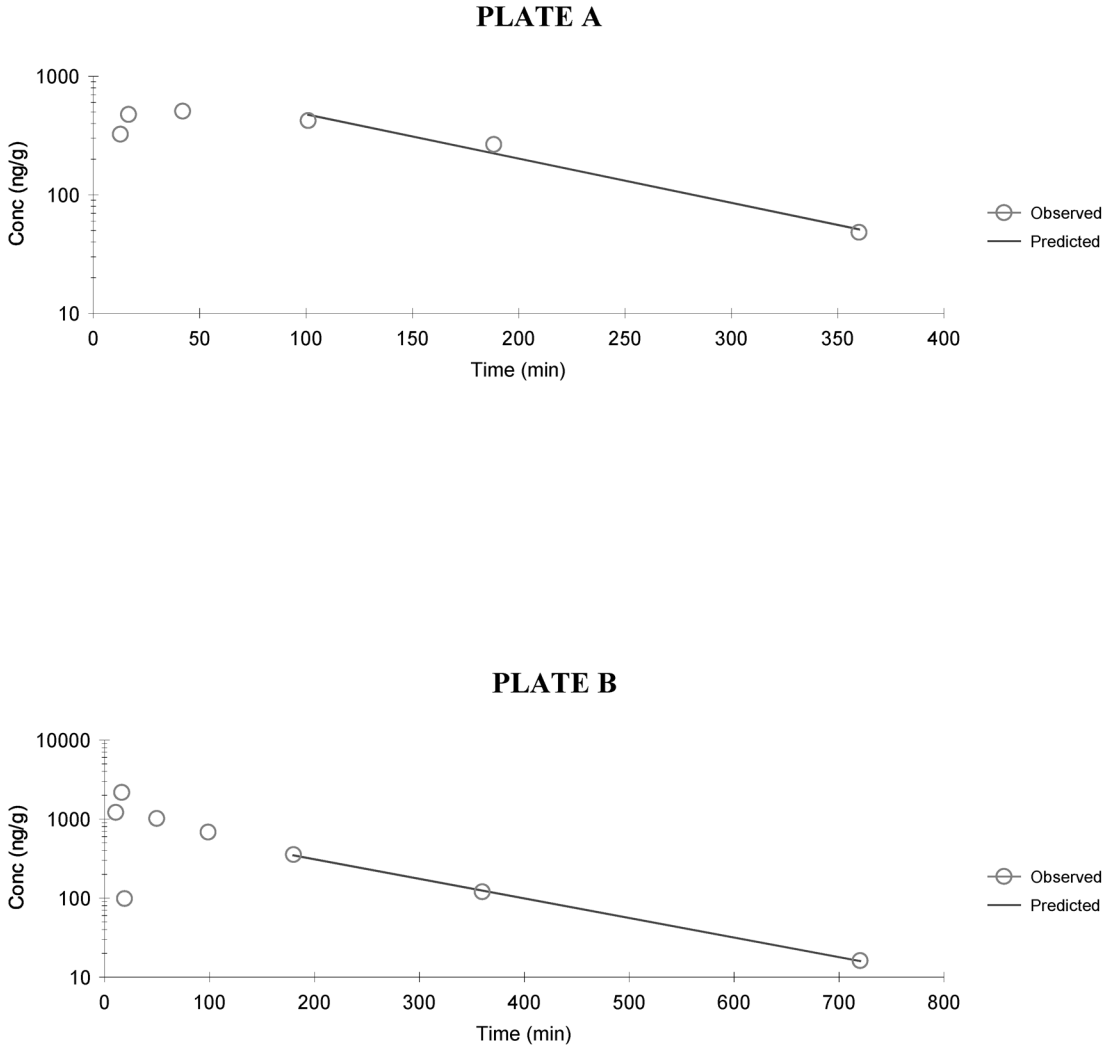


FIGURE L14
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 25 mg/kg α,β-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

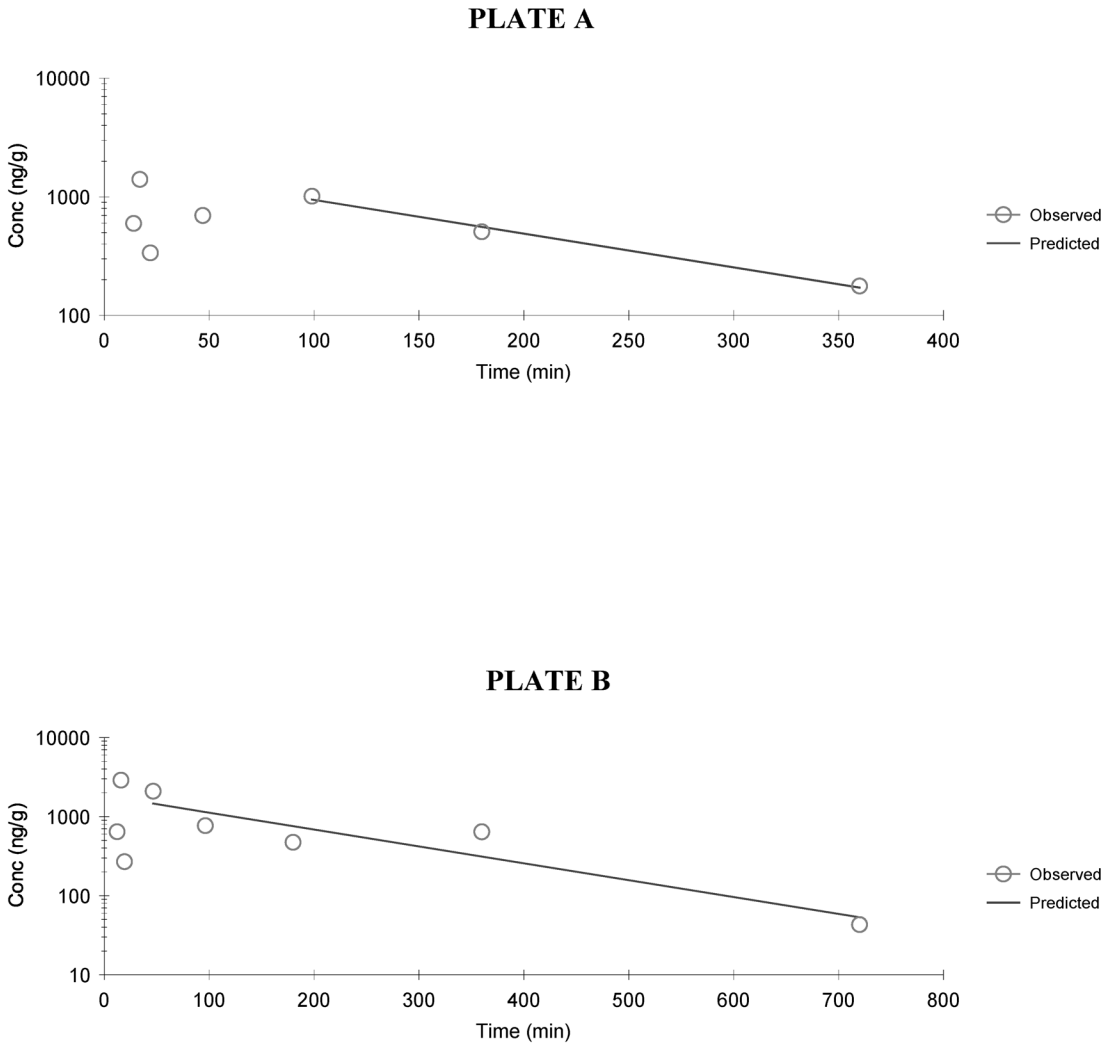
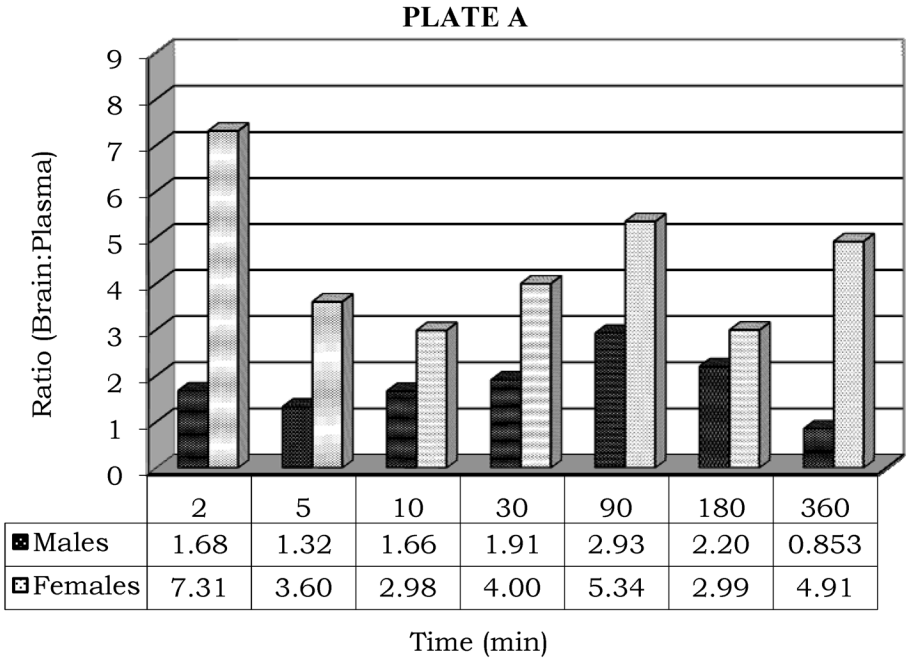


FIGURE L15
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats Following a Single Gavage Administration of 50 mg/kg α,β-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

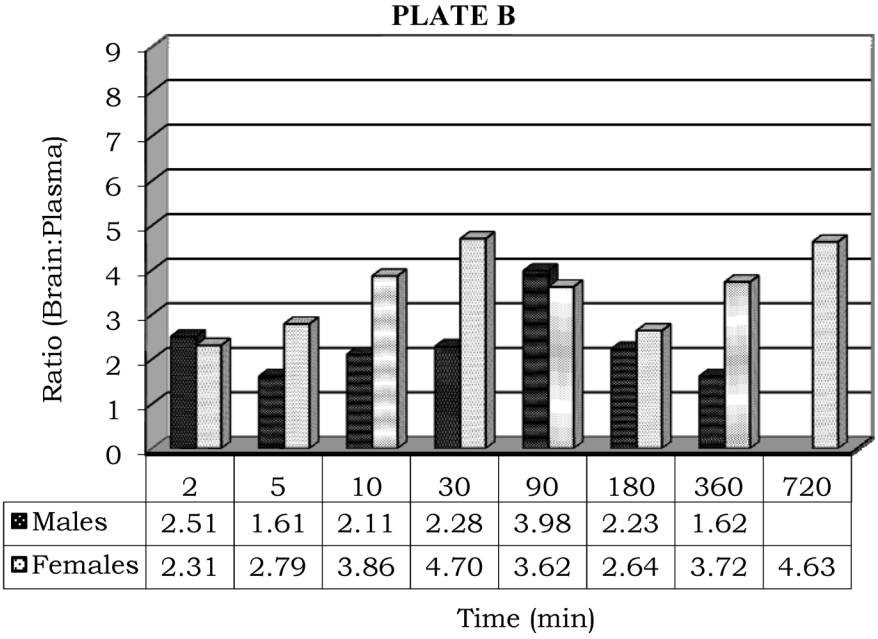
TABLE L9
Toxicokinetic Parameters for α-Thujone in the Brain of F344/N Rats Following a Single Gavage Administration of 25 or 50 mg/kg α,β-Thujone[a]

Dose (mg/kg)	Parameter	Male	Female
25	C_{max} (obs) (ng/g)	508 (SD=75)	2,180 (SD=260)
	t_{max} (obs) (min)	42.0	16.3
	$t_{1/2}$ (min)	80.7	121
	AUC_{last} (ng•g^{-1}•min)	101,000	188,000
	AUC_{∞_pred} (ng•g^{-1}•min)	107,000	191,000
50	C_{max} (obs) (ng/g)	1,400 (SD=210)	2,900 (SD=500)
	t_{max} (obs) (min)	17.0	16.0
	$t_{1/2}$ (min)	106	141
	AUC_{last} (ng•g^{-1}•min)	192,000	396,000
	AUC_{∞_pred} (ng•g^{-1}•min)	218,000	407,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.



	2	5	10	30	90	180	360
Males	1.68	1.32	1.66	1.91	2.93	2.20	0.853
Females	7.31	3.60	2.98	4.00	5.34	2.99	4.91



	2	5	10	30	90	180	360	720
Males	2.51	1.61	2.11	2.28	3.98	2.23	1.62	
Females	2.31	2.79	3.86	4.70	3.62	2.64	3.72	4.63

FIGURE L16
Brain:Plasma Concentration Ratios of α-Thujone in F344/N Rats Following a Single Gavage Administration of 25 (Plate A) or 50 (Plate B) mg/kg α,β-Thujone
n>1 Conc/Time Point; Target Times Used

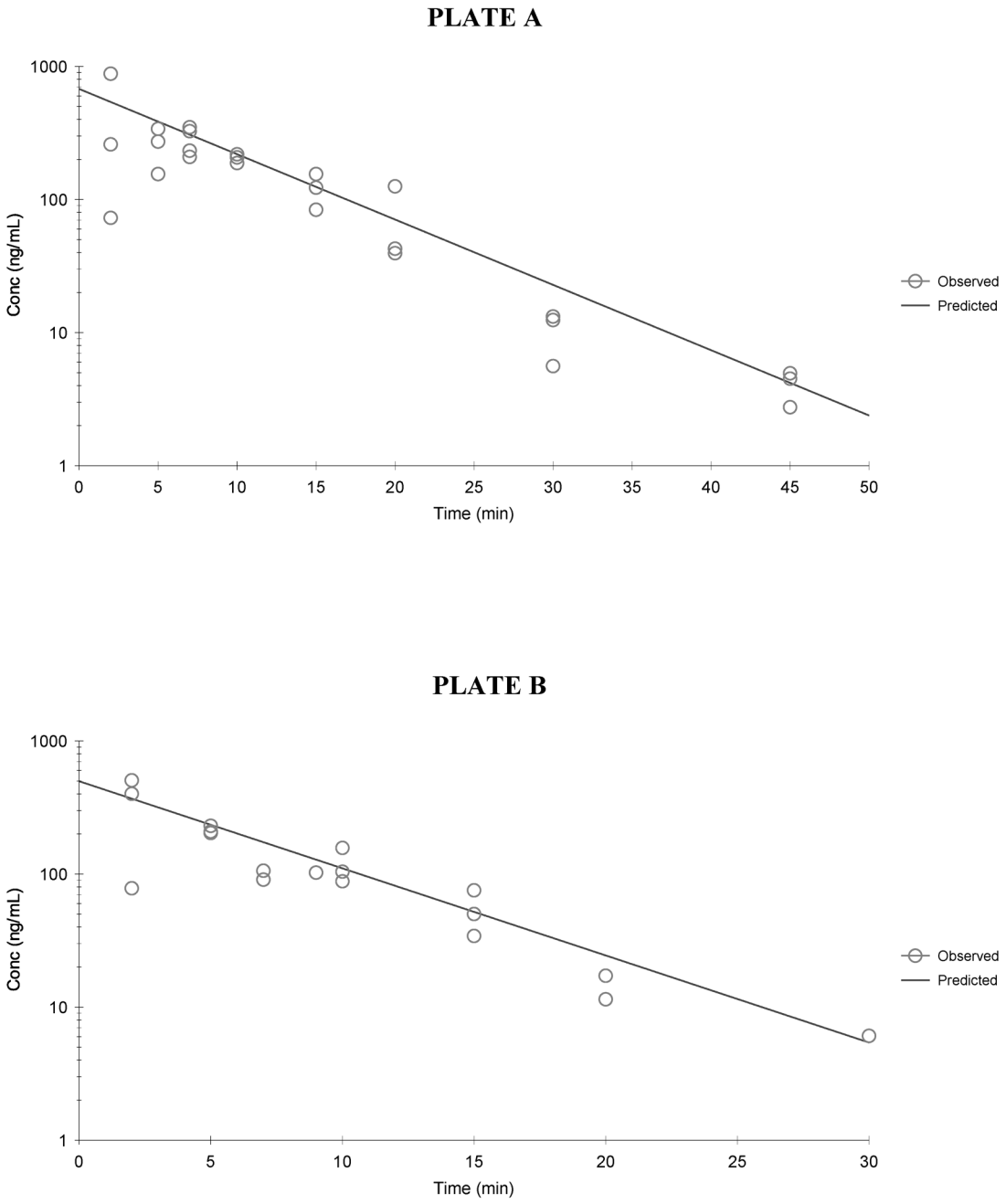


FIGURE L17
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Intravenous Administration of 3.2 mg/kg α-Thujone
Data were fitted using a one-compartment model with $1/Yhat^2$ weighting.

TABLE L10
Toxicokinetic Parameters for α-Thujone in the Plasma of B6C3F1 Mice Following a Single Intravenous Administration of 3.2 mg/kg α-Thujone[a]

Parameter	Estimate	Standard Error
Male		
$C_{(2\ min)}$ (obs) (ng/mL)	404	423 (SD)
$C_{(0\ min)}$ (fitted) (ng/mL)	676	92
V_d (mL/kg)	4,730	640
k_{10} (min^{-1})	0.113	0.006
k_{10} $t_{1/2}$ (min)	6.13	0.35
Cl (mL/min/kg)	535	53
MRT (min)	8.85	0.50
AUC_∞ ($ng \bullet mL^{-1} \bullet min$)	5,990	590
Female		
$C_{(2\ min)}$ (obs) (ng/mL)	328	223 (SD)
$C_{(0\ min)}$ (fitted) (ng/mL)	498	75
V_d (mL/kg)	6,430	970
k_{10} (min^{-1})	0.151	0.012
k_{10} $t_{1/2}$ (min)	4.60	0.36
Cl (mL/min/kg)	969	95
MRT (min)	6.64	0.52
AUC_∞ ($ng \bullet mL^{-1} \bullet min$)	3,300	320

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation.

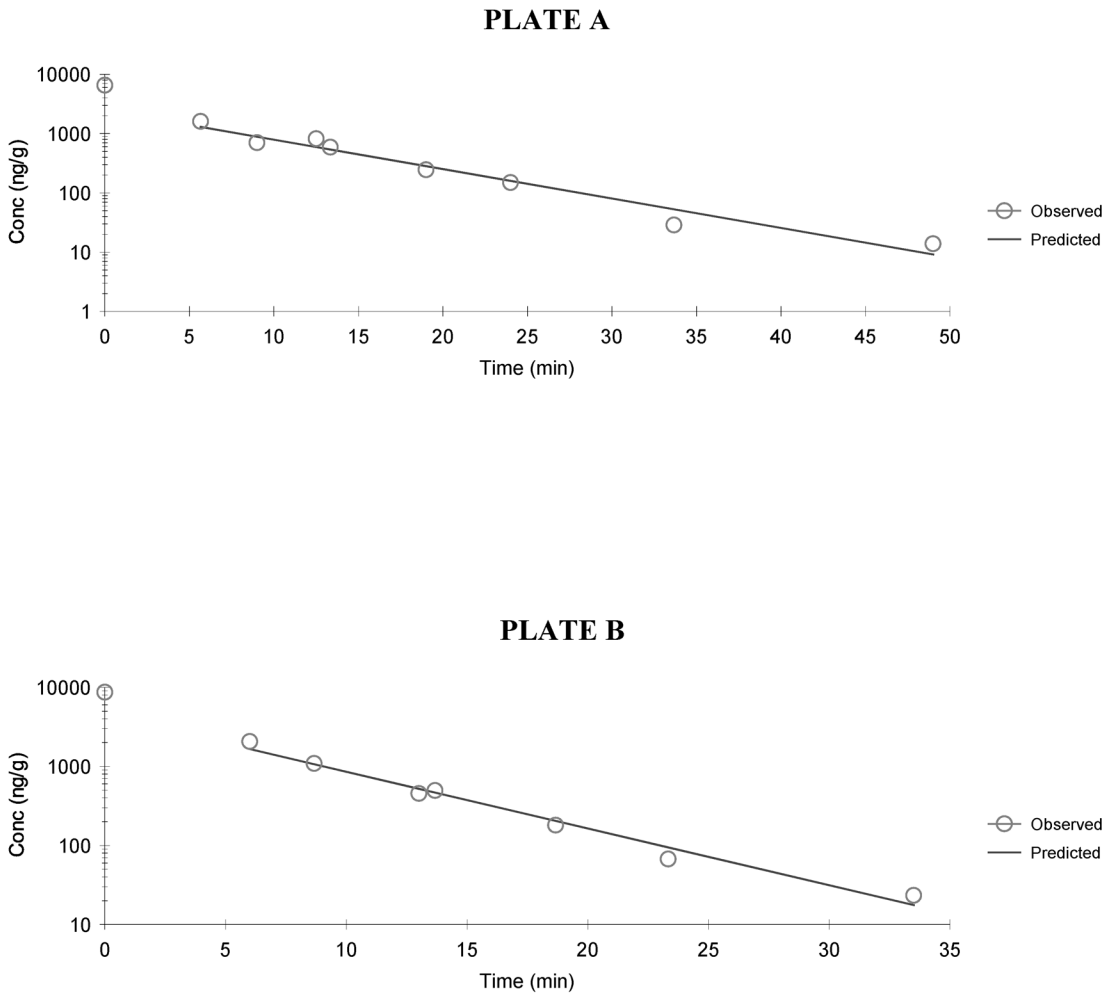


FIGURE L18
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Intravenous Administration of 3.2 mg/kg α-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

TABLE L11
Toxicokinetic Parameters for α-Thujone in the Brain of B6C3F1 Mice Following a Single Intravenous Administration of 3.2 mg/kg α-Thujone

Parameter	Male	Female
C_{max} (obs) (ng/g)	1,610 (SD=2,180)	2,070 (SD=1,720)
t_{max} (obs) (min)	5.67	6.00
$t_{1/2}$ (min)	6.07	4.19
AUC_{last} (ng•g^{-1}•min)	34,800	43,000
AUC_{∞_pred} (ng•g^{-1}•min)	34,900	43,100

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.

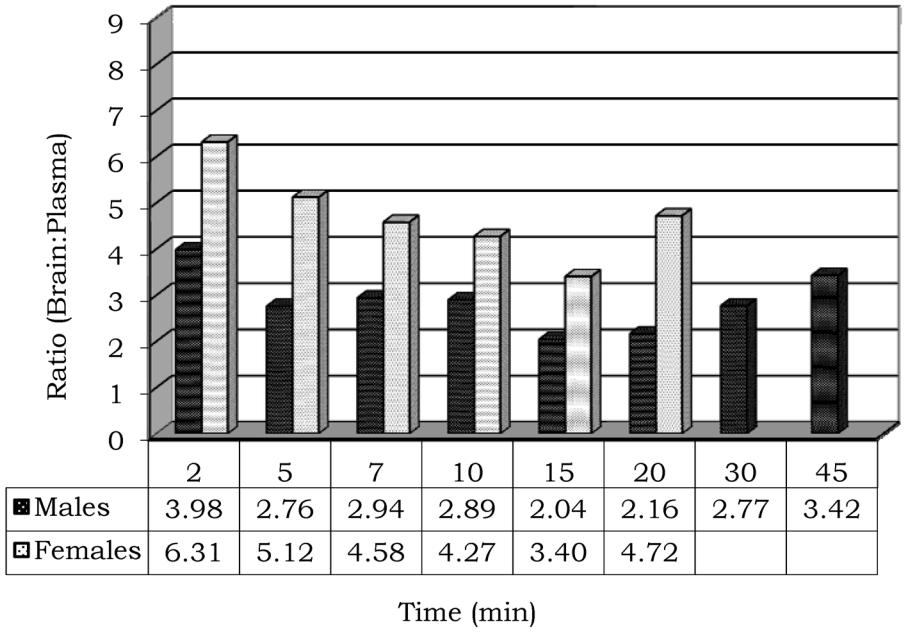


FIGURE L19
Brain:Plasma Concentration Ratios of α-Thujone in B6C3F1 Mice Following a Single Intravenous Administration of 3.2 mg/kg α-Thujone
n>1 Conc/Time Point; Target Times Used

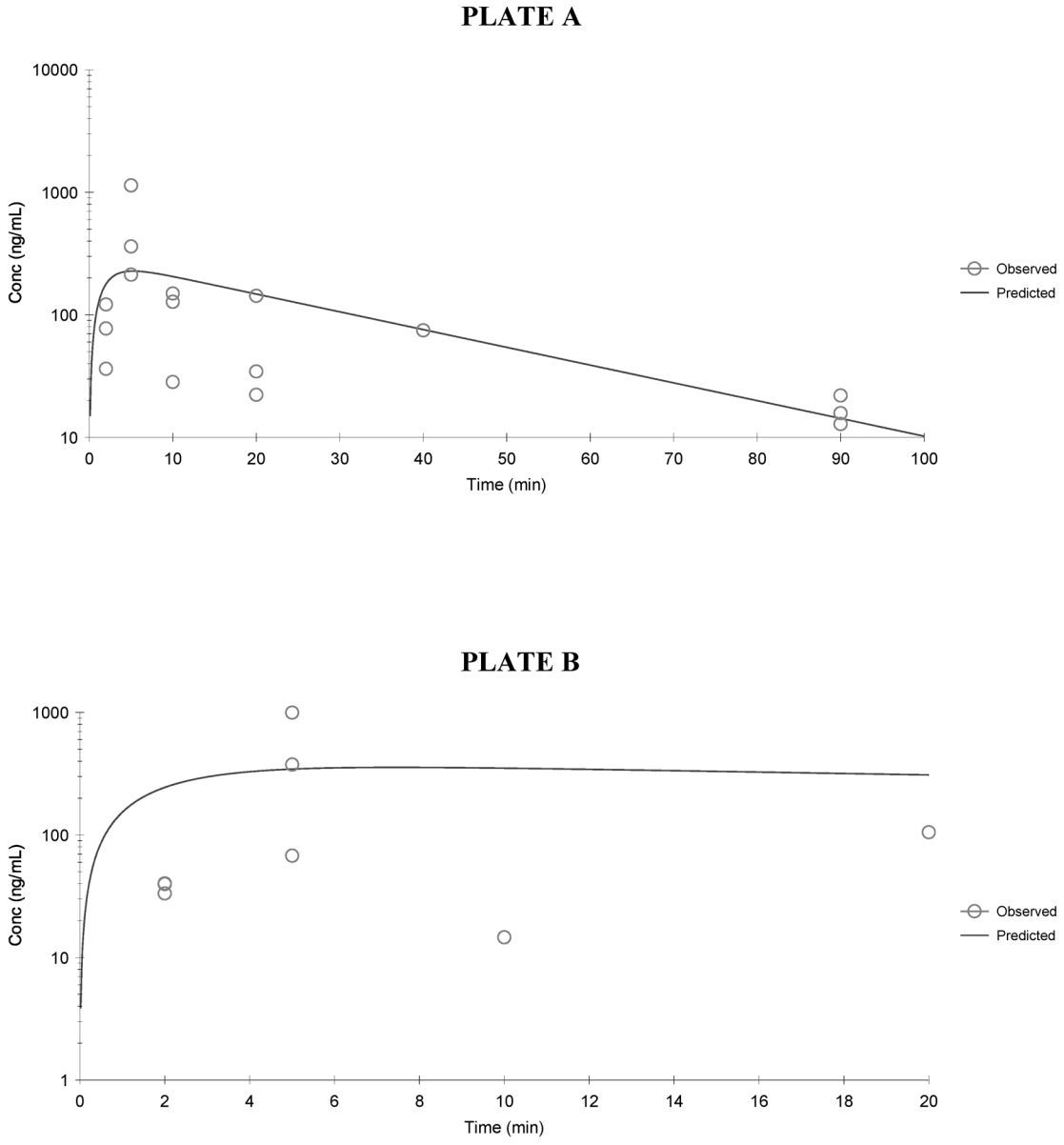


FIGURE L20
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 40 mg/kg α-Thujone
Data were fitted using a one-compartment model with $1/Yhat^2$ weighting.

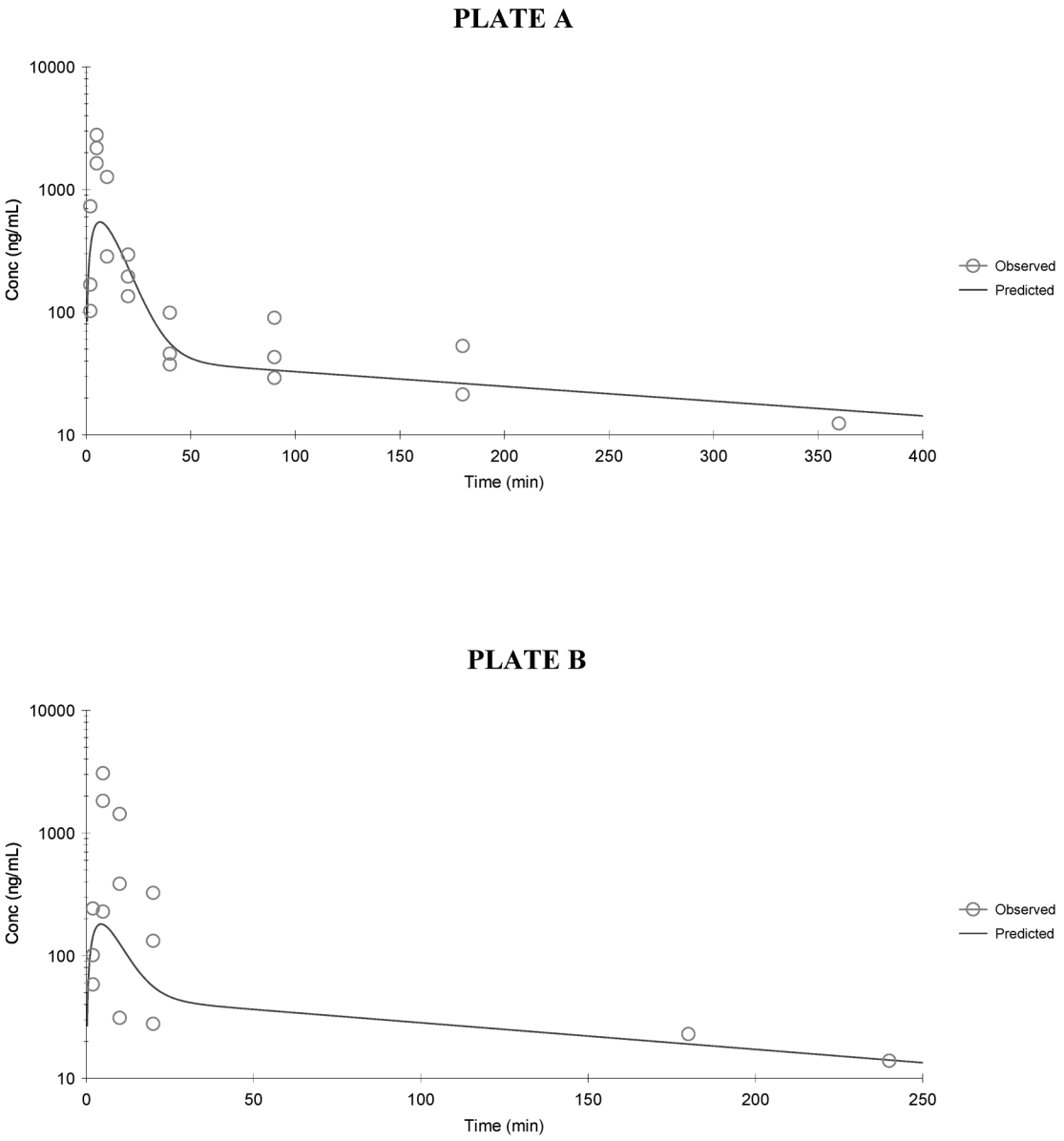


FIGURE L21
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 80 mg/kg α-Thujone
Data were fitted using a two-compartment model with 1/Y weighting.

TABLE L12
Toxicokinetic Parameters for α-Thujone in the Plasma of B6C3F1 Mice Following a Single Gavage Administration of 40 or 80 mg/kg α-Thujone[a]

Dose (mg/kg)	Parameter	Male		Female	
		Estimate	Standard Error	Estimate	Standard Error
40	k_{01} (min^{-1})	0.574	0.923	0.499	1.49
	k_{01} $t_{1/2}$ (min)	1.21	1.94	1.39	4.15
	C_{max} (obs) (ng/mL)	571	497 (SD)	480	473 (SD)
	C_{max} (pred) (ng/mL)	228	86	356	263
	t_{max} (obs) (min)	5.00	0.00	5.00	0.00
	t_{max} (pred) (min)	5.26	5.76	7.44	11.57
	V_d_F (mL/kg)	147,000	71,000	102,000	155,000
	k_{10} (min^{-1})	0.0334	0.0112	0.0135	0.132
	k_{10} $t_{1/2}$ (min)	20.8	7.0	51.5	506
	Cl_F (mL/min/kg)	4,920	1,650	1,370	11,700
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	8,760	ND	1,460	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	8,140	2,730	29,200	248,000
80	k_{01} (min^{-1})	0.167	1.92	0.257	76.6
	k_{01} $t_{1/2}$ (min)	4.15	47.6	2.69	802
	C_{max} (obs) (ng/mL)	2,200	600 (SD)	1,710	1,420 (SD)
	C_{max} (pred) (ng/mL)	544	197	181	221
	t_{max} (obs) (min)	5.00	0.00	5.00	0.00
	t_{max} (pred) (min)	6.59	2.58	4.44	11.2
	V_d_F (mL/kg)	59,100	650,000	180,000	ND
	k_{10} (min^{-1})	0.0556	0.677	0.0410	12.2
	k_{10} $t_{1/2}$ (min)	12.5	151	16.9	4,900
	Cl_F (mL/min/kg)	3,290	8,300	7,380	30,400
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	35,300	ND	21,300	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	24,300	61,000	10,800	42,400

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation; ND=not determined.

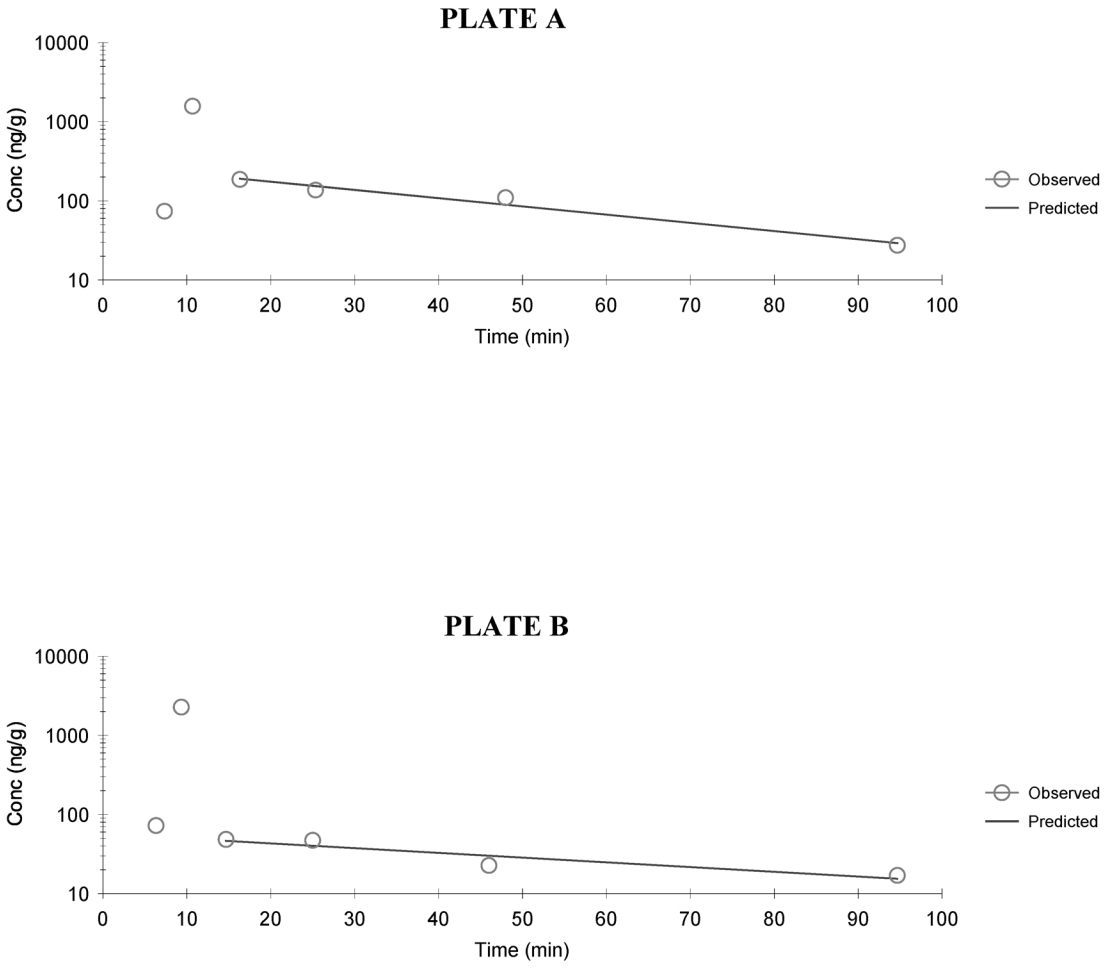


FIGURE L22
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 40 mg/kg α-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

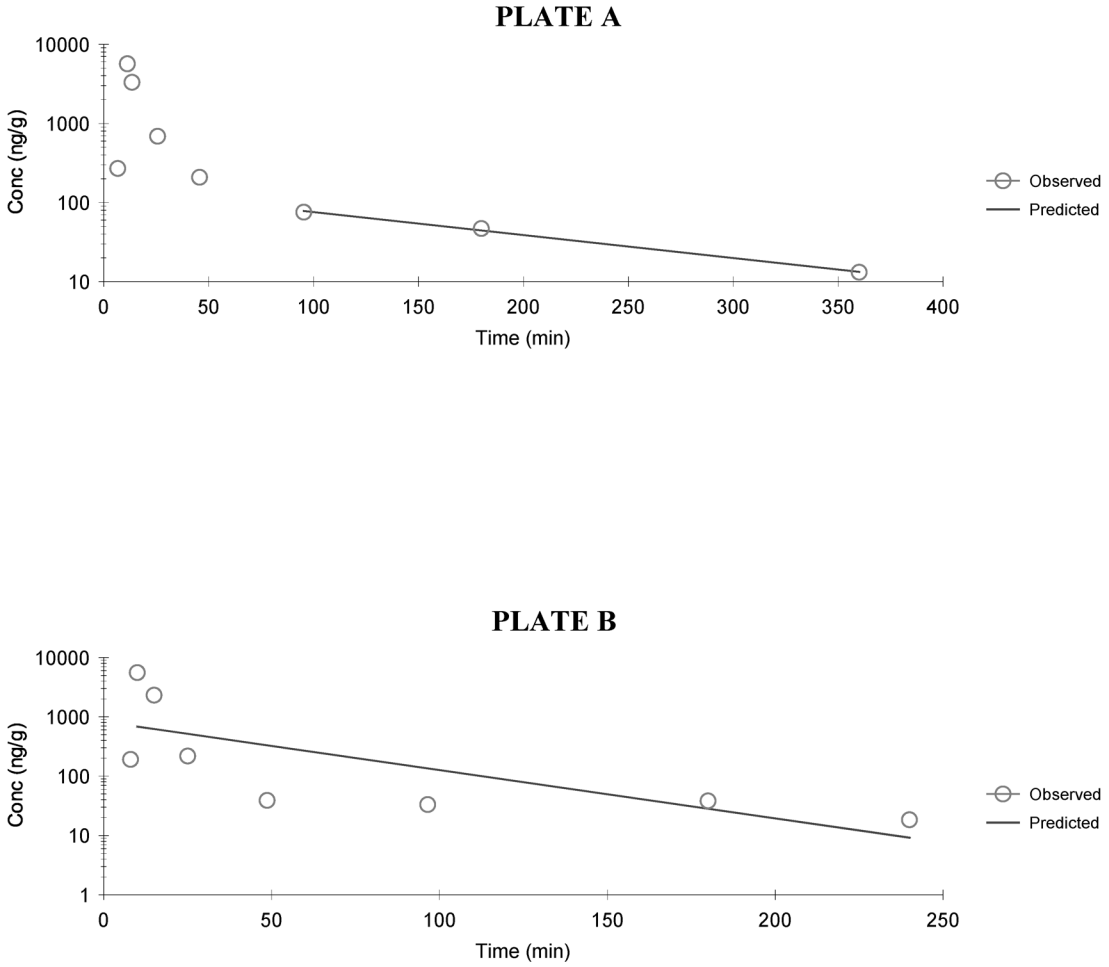
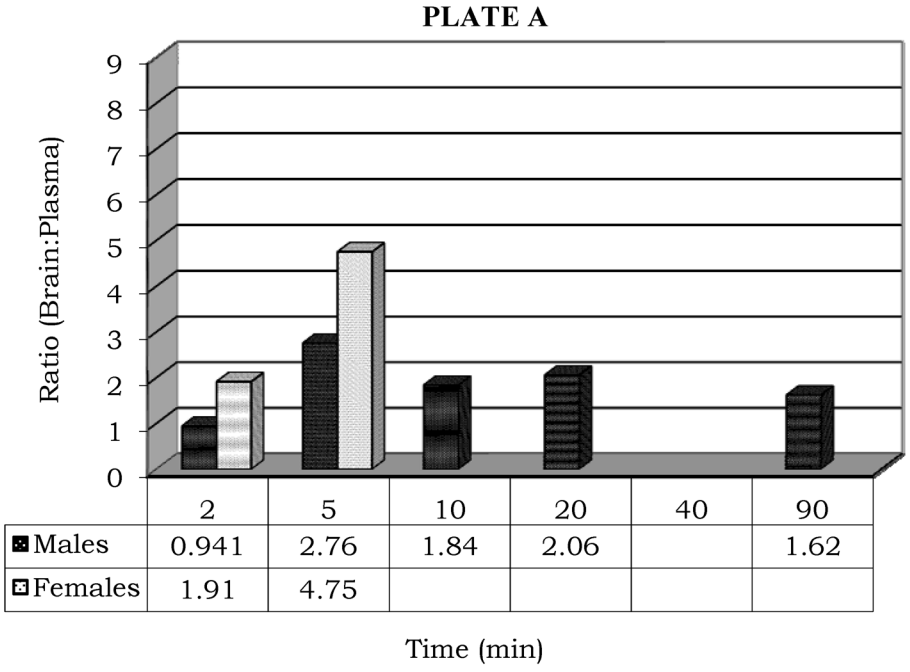


FIGURE L23
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 80 mg/kg α-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

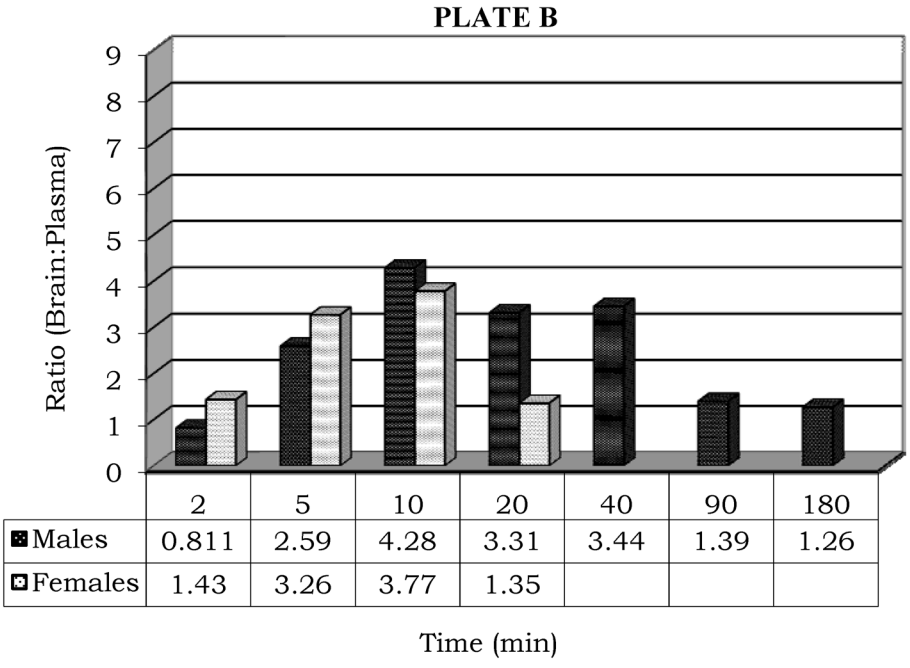
TABLE L13
Toxicokinetic Parameters for α-Thujone in the Brain of B6C3F1 Mice Following a Single Gavage Administration of 40 or 80 mg/kg α-Thujone[a]

Dose (mg/kg)	Parameter	Male	Female
40	C_{max} (obs) (ng/g)	1,580 (SD=1,500)	2280 (SD=1,250)
	t_{max} (obs) (min)	10.7	9.33
	$t_{1/2}$ (min)	29.0	50.4
	$AUC_{\infty\ last}$ ($ng \bullet g^{-1} \bullet min$)	15,400	12,100
	AUC_{∞_pred} ($ng \bullet g^{-1} \bullet min$)	16,700	13,300
80	C_{max} (obs) (ng/g)	5,690 (SD=865)	5,580 (SD=4,160)
	t_{max} (obs) (min)	11.3	10.0
	$t_{1/2}$ (min)	104	37.0
	$AUC_{\infty\ last}$ ($ng \bullet g^{-1} \bullet min$)	75,500	48,500
	AUC_{∞_pred} ($ng \bullet g^{-1} \bullet min$)	77,500	48,900

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.



	2	5	10	20	40	90
Males	0.941	2.76	1.84	2.06		1.62
Females	1.91	4.75				



	2	5	10	20	40	90	180
Males	0.811	2.59	4.28	3.31	3.44	1.39	1.26
Females	1.43	3.26	3.77	1.35			

FIGURE L24
Brain:Plasma Concentration Ratios of α-Thujone in B6C3F1 Mice Following a Single Gavage Administration of 40 (Plate A) or 80 (Plate B) mg/kg α-Thujone
n>1 Conc/Time Point; Target Times Used

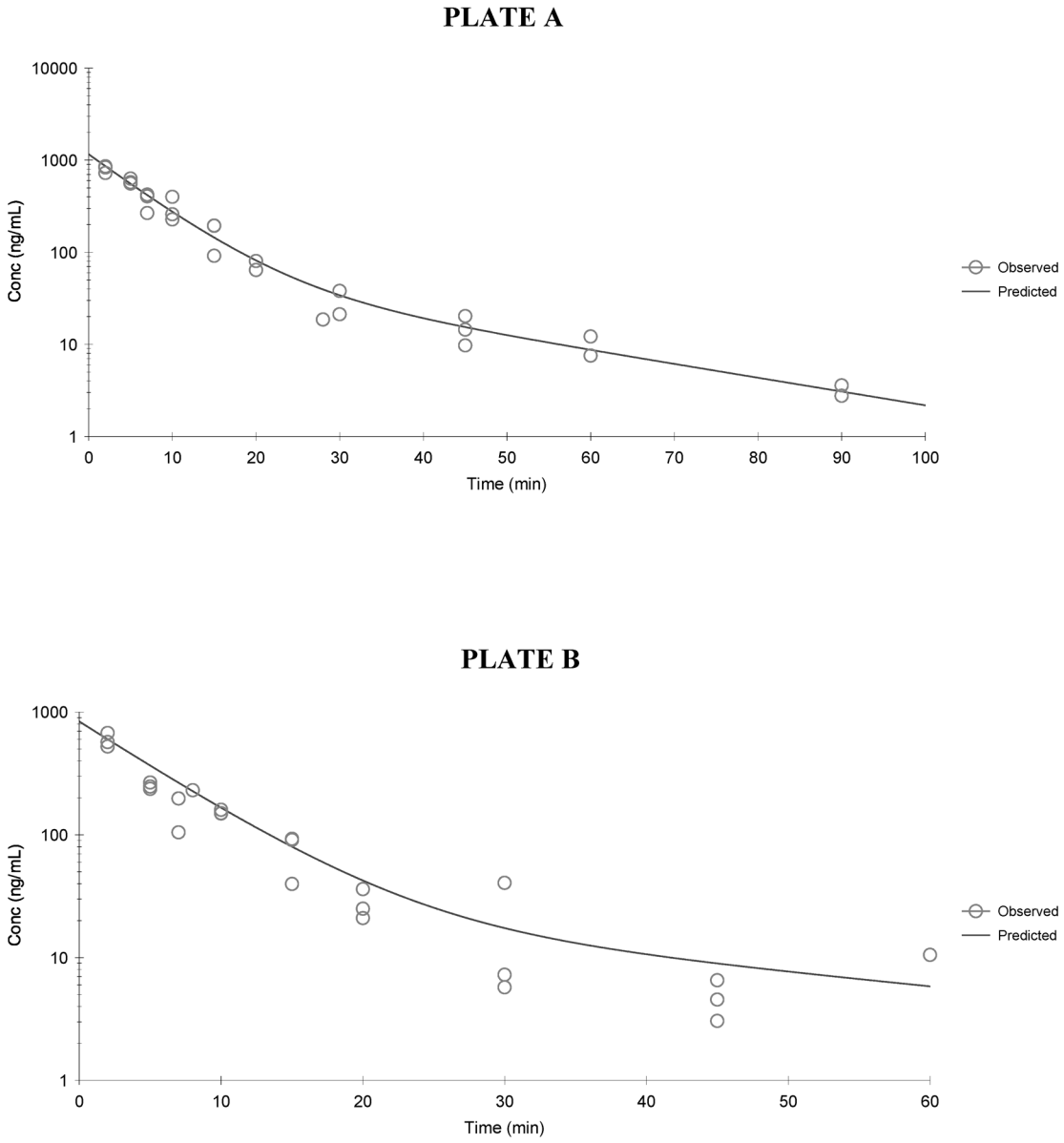


FIGURE L25
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Intravenous Administration of 6.0 mg/kg α,β-Thujone
Data were fitted using a two-compartment model with $1/Yhat^2$ weighting.

TABLE L14
Toxicokinetic Parameters for α-Thujone in the Plasma of B6C3F1 Mice Following a Single Intravenous Administration of 6.0 mg/kg α,β-Thujone in Mice[a]

Parameter	Estimate	Standard Error
Male		
$C_{(2\ min)}$ (obs) (ng/mL)	806	70 (SD)
$C_{(0\ min)}$ (fitted) (ng/mL)	1,160	160
V_d – 1st comp (mL/kg)	5,170	730
V_d – 2nd comp (mL/kg)	2,470	410
k_{12} (min^{-1})	0.0199	0.0059
k_{21} (min^{-1})	0.0416	0.0086
k_{10} (min^{-1})	0.130	0.012
k_{10} $t_{1/2}$ (min)	5.34	0.51
Alpha $t_{1/2}$ (min)	4.42	0.54
Beta $t_{1/2}$ (min)	20.1	3.4
Cl (mL/min/kg)	671	45
Cl – 2nd comp (mL/min/kg)	103	25
MRT (min)	11.4	0.8
AUC_∞ (ng•mL^{-1}•min)	8,940	600
Female		
$C_{(2\ min)}$ (obs) (ng/mL)	589	78 (SD)
$C_{(0\ min)}$ (fitted) (ng/mL)	837	234
V_d – 1st comp (mL/kg)	7,170	2,000
V_d – 2nd comp (mL/kg)	5,100	6,160
k_{12} (min^{-1})	0.0222	0.0086
k_{21} (min^{-1})	0.0312	0.0363
k_{10} (min^{-1})	0.145	0.031
k_{10} $t_{1/2}$ (min)	4.78	1.02
Alpha $t_{1/2}$ (min)	4.03	0.97
Beta $t_{1/2}$ (min)	26.4	29.9
Cl (mL/min/kg)	1,040	140
Cl – 2nd comp (mL/min/kg)	159	57
MRT (min)	11.8	7.1
AUC_∞ (ng•mL^{-1}•min)	5,760	780

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation.

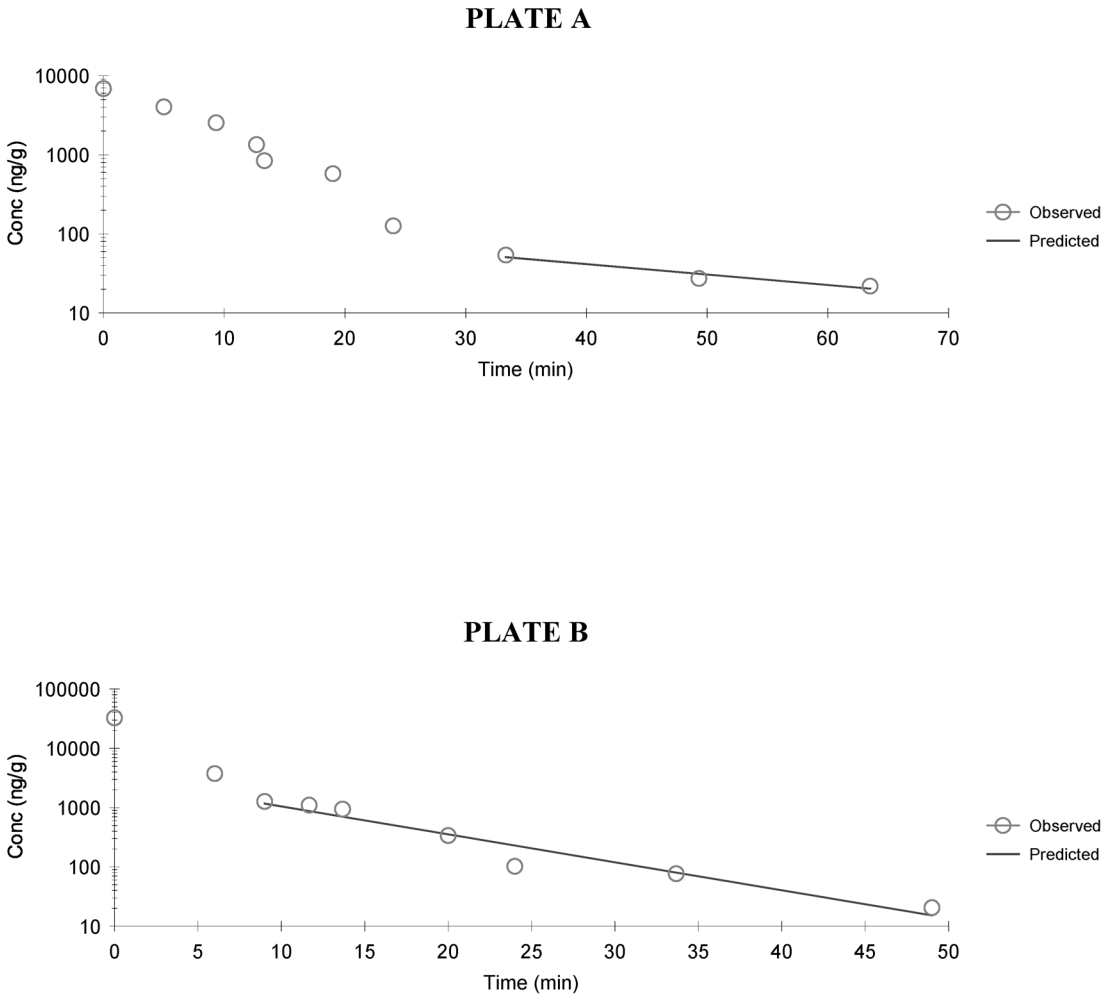


FIGURE L26
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Intravenous Administration of 6.0 mg/kg α,β-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

TABLE L15
Toxicokinetic Parameters for α-Thujone in the Brain of B6C3F1 Mice Following a Single Intravenous Administration of 6.0 mg/kg α,β-Thujone[a]

Parameter	Male	Female
C_{max} (obs) (ng/g)	4,030 (SD=400)	3,760 (SD=920)
t_{max} (obs) (min)	5.00	6.00
$t_{1/2}$ (min)	22.8	6.39
AUC_{last} ($ng \bullet g^{-1} \bullet min$)	56,200	128,000
AUC_{∞_pred} ($ng \bullet g^{-1} \bullet min$)	56,900	129,000

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.

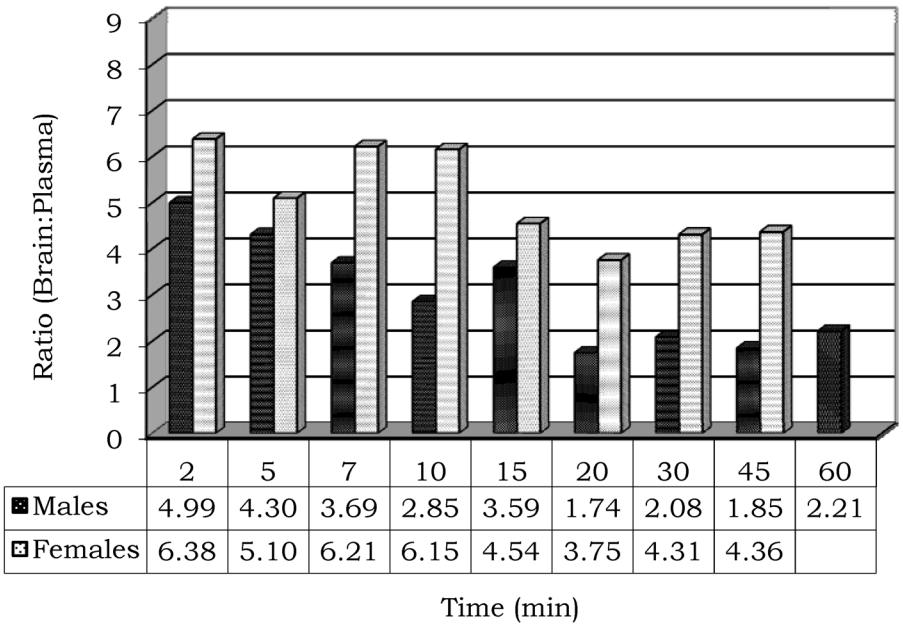


FIGURE L27
Brain:Plasma Concentration Ratios of α-Thujone in B6C3F1 Mice Following a Single Intravenous Administration of 6.0 mg/kg α,β-Thujone
n>1 Conc/Time Points; Target Times Used

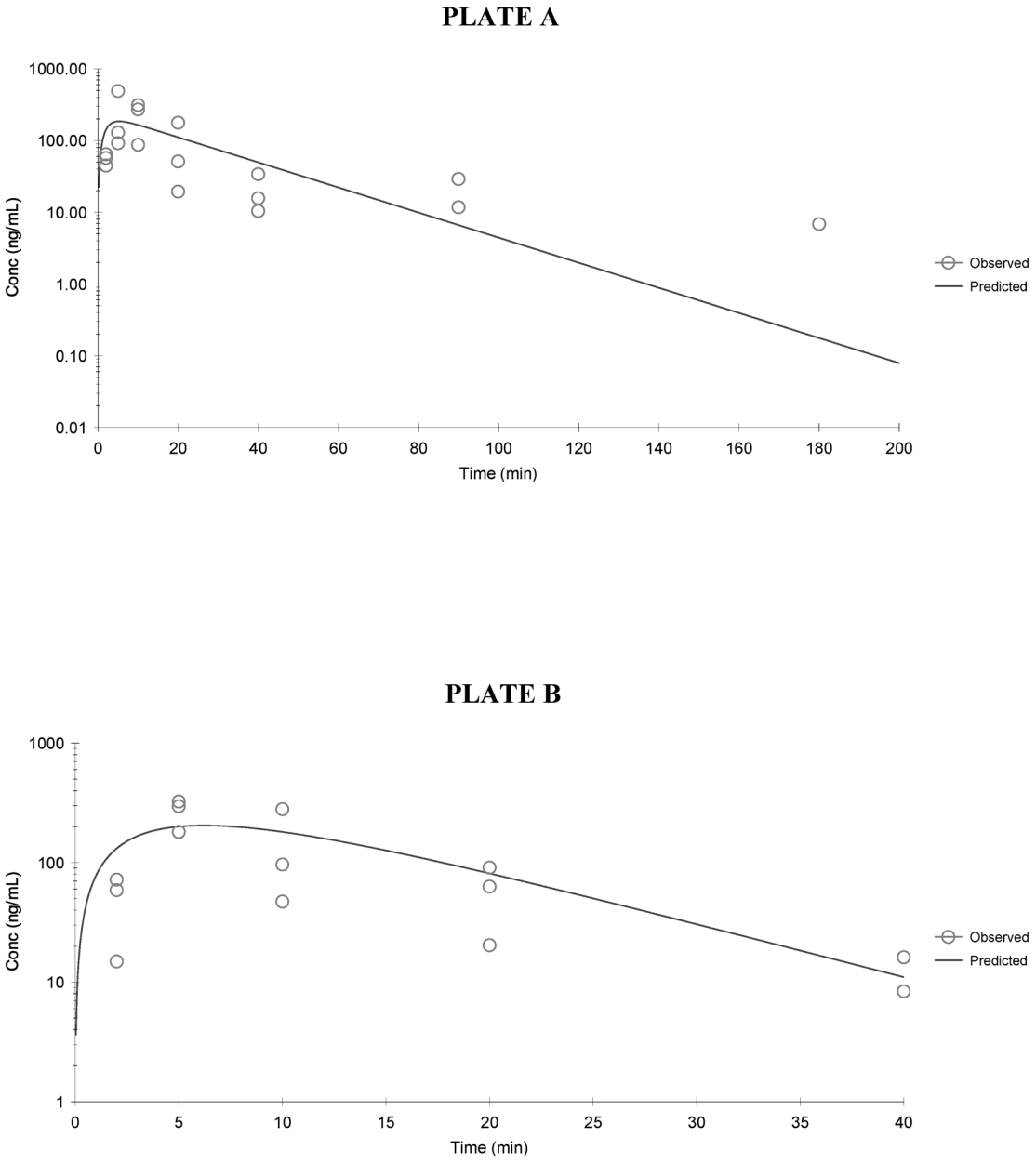


FIGURE L28
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 40 mg/kg α,β-Thujone
Data were fitted using a one-compartment model with 1/Yhat weighting.

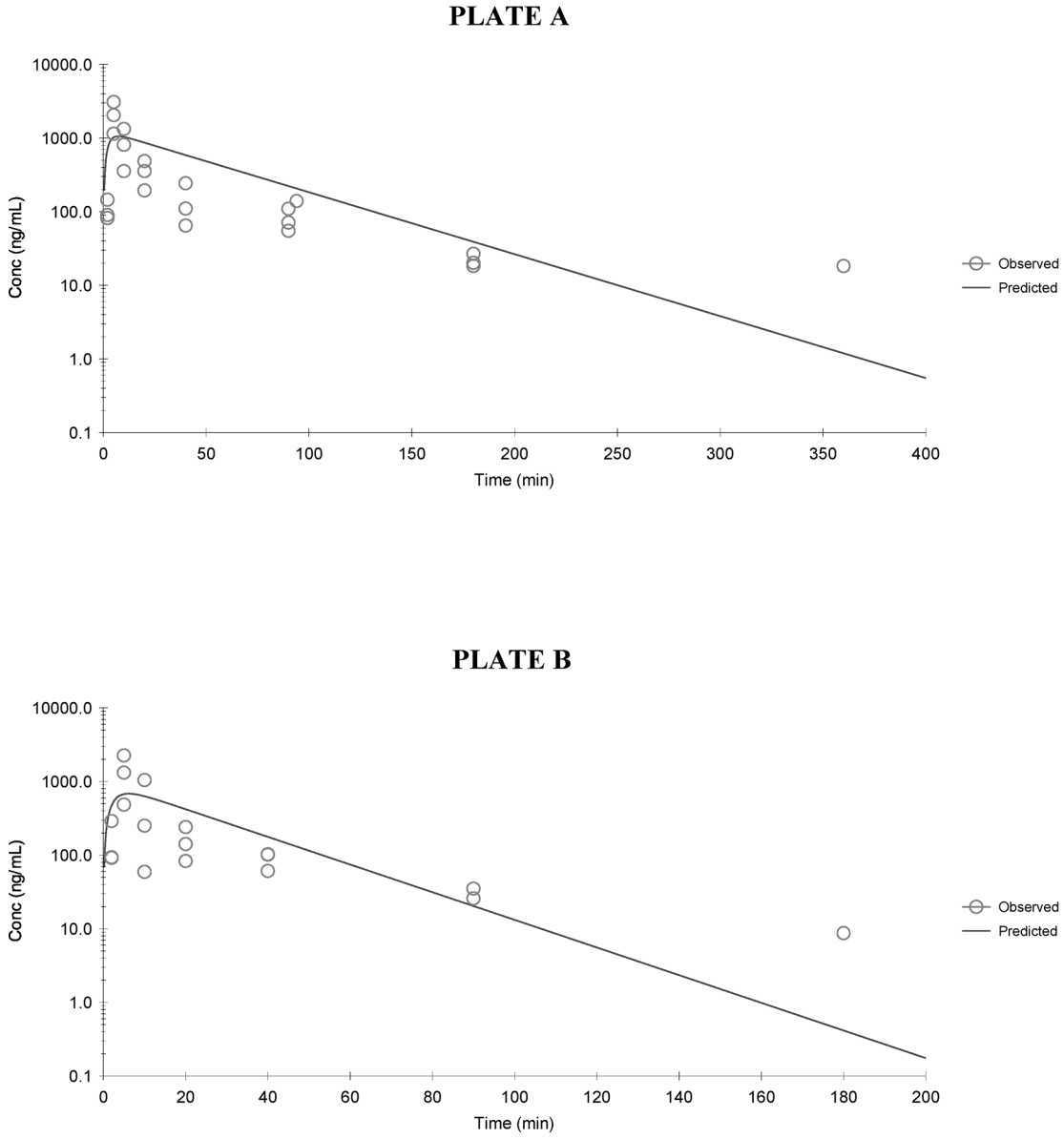


FIGURE L29
α-Thujone Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 80 mg/kg α,β-Thujone
Data were fitted using a one-compartment model with 1/Yhat weighting.

TABLE L16
Toxicokinetic Parameters for α-Thujone in the Plasma of B6C3F1 Mice Following a Single Gavage Administration of 40 or 80 mg/kg α,β-Thujone[a]

Dose (mg/kg)	Parameter	Male		Female	
		Estimate	Standard Error	Estimate	Standard Error
40	k_{01} (min^{-1})	0.522	0.590	0.237	0.239
	k_{01} $t_{1/2}$ (min)	1.33	1.50	2.92	2.95
	C_{max} (obs) (ng/mL)	238	221 (SD)	267	76 (SD)
	C_{max} (pred) (ng/mL)	185	48	204	37
	t_{max} (obs) (min)	5.00	0.00	5.00	0.00
	t_{max} (pred) (min)	5.32	3.71	6.21	1.89
	V_d_F (mL/kg)	174,000	71,000	103,000	73,000
	k_{10} (min^{-1})	0.0403	0.0230	0.103	0.079
	k_{10} $t_{1/2}$ (min)	17.2	9.8	6.72	5.13
	Cl_F (mL/min/kg)	7,020	2,570	10,700	2,400
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	7,660	ND	3,820	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	5,700	2,100	3,750	830
80	k_{01} (min^{-1})	0.448	0.413	0.407	0.483
	k_{01} $t_{1/2}$ (min)	1.55	1.43	1.70	2.02
	C_{max} (obs) (ng/mL)	2,100	1,000 (SD)	1,350	890 (SD)
	C_{max} (pred) (ng/mL)	1,060	240	683	200
	t_{max} (obs) (min)	5.00	0.00	5.00	0.00
	t_{max} (pred) (min)	7.33	4.59	6.16	4.20
	V_d_F (mL/kg)	65,600	19,900	89,700	44,900
	k_{10} (min^{-1})	0.0194	0.0081	0.0433	0.0288
	k_{10} $t_{1/2}$ (min)	35.8	14.9	16.0	10.6
	Cl_F (mL/min/kg)	1,270	390	3,880	1,550
	AUC_{last} ($ng \bullet mL^{-1} \bullet min$)	40,200	ND	13,400	ND
	AUC_{∞} ($ng \bullet mL^{-1} \bullet min$)	63,000	19,000	20,600	8,200

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation; ND=not determined.

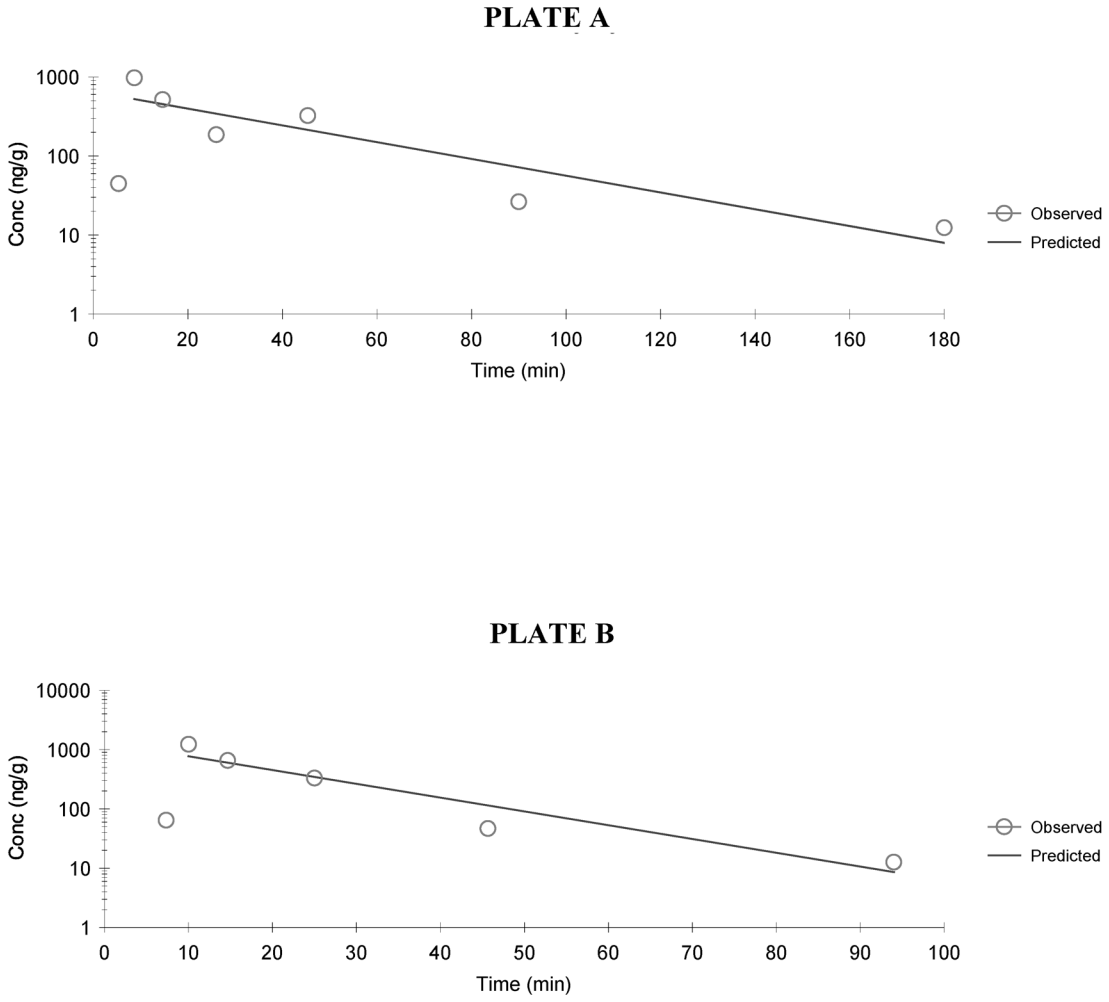


FIGURE L30
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 40 mg/kg α,β-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

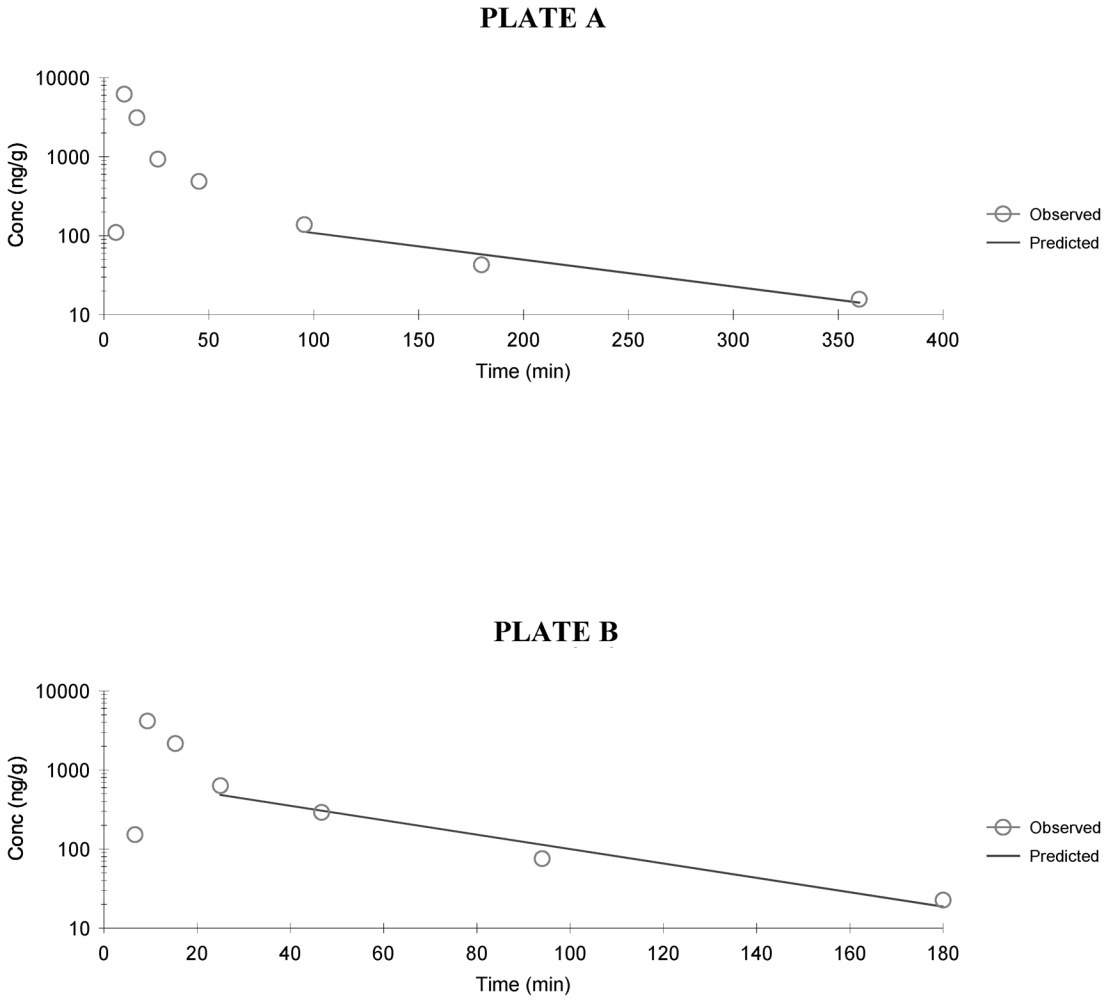
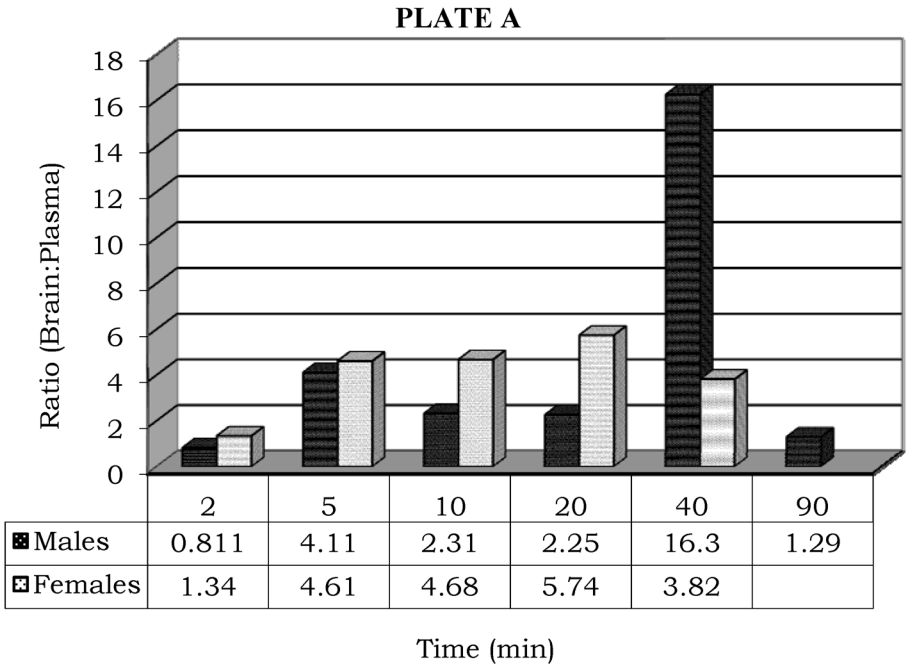


FIGURE L31
α-Thujone Brain Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice Following a Single Gavage Administration of 80 mg/kg α,β-Thujone
Data were fitted using a noncompartmental model with uniform weighting.

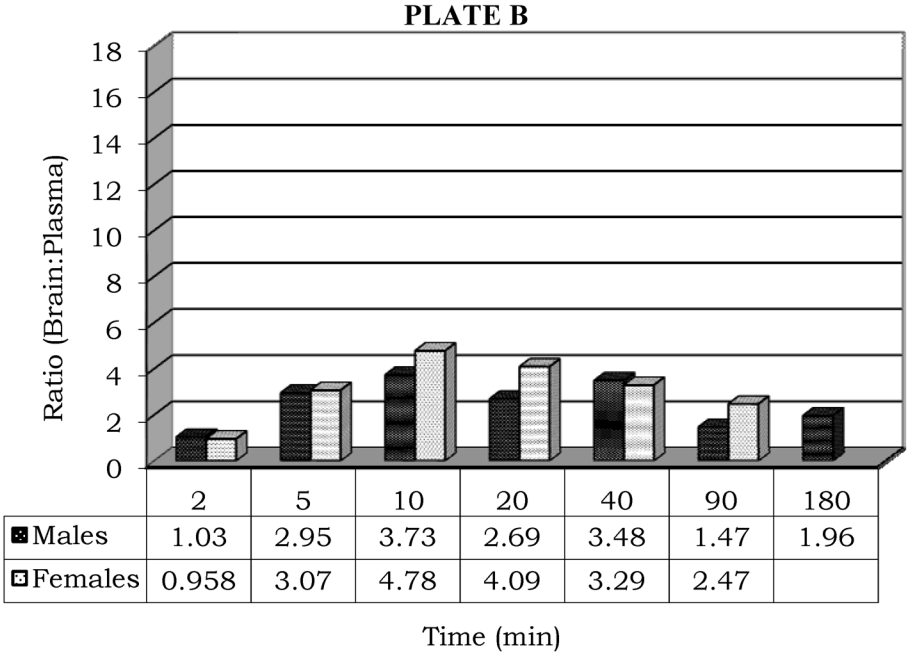
TABLE L17
Toxicokinetic Parameters for α-Thujone in the Brain of B6C3F1 Mice Following a Single Gavage Administration of 40 or 80 mg/kg α,β-Thujone[a]

Dose (mg/kg)	Parameter	Male	Female
40	C_{max} (obs) (ng/g)	976 (SD=1,080)	1,230 (SD=470)
	t_{max} (obs) (min)	8.67	10.0
	$t_{1/2}$ (min)	28.4	12.9
	AUC_{last} (ng•g^{-1}•min)	24,800	16,900
	AUC_{∞_pred} (ng•g^{-1}•min)	25,100	17,000
80	C_{max} (obs) (ng/g)	6,180 (SD=1,450)	4,160 (SD=1,150)
	t_{max} (obs) (min)	9.67	9.33
	$t_{1/2}$ (min)	88.8	33.0
	AUC_{last} (ng•g^{-1}•min)	103,000	61,600
	AUC_{∞_pred} (ng•g^{-1}•min)	105,000	62,500

[a] Toxicokinetic parameter estimates are reported to three significant figures; SD=standard deviation. The noncompartmental model did not provide estimates of variability.



	2	5	10	20	40	90
Males	0.811	4.11	2.31	2.25	16.3	1.29
Females	1.34	4.61	4.68	5.74	3.82	



	2	5	10	20	40	90	180
Males	1.03	2.95	3.73	2.69	3.48	1.47	1.96
Females	0.958	3.07	4.78	4.09	3.29	2.47	

FIGURE L32
Brain:Plasma Concentration Ratios of α-Thujone in B6C3F1 Mice Following a Single Gavage Administration of 40 (Plate A) or 80 (Plate B) mg/kg α,β-Thujone
n>1 Conc/Time Points; Target Times Used

ERRATUM:
An error was identified in the NTP Technical Report on alpha,beta-Thujone (TR-570). On pages 7 and 15, there was an error in the trade name listed for alpha,beta-Thujone. The trade name was not Esberitox® as originally stated in the report. This error has been corrected in the PDF version of this report. [March 19, 2012].

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