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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Kava Kava Extract (CAS No. 9000-38-8) in F344/N Rats and B6C3F1 Mice (Gavage Studies)

NTP TR 571

MARCH 2012

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF KAVA KAVA EXTRACT
(CAS No. 9000-38-8)

IN F344/N RATS AND B6C3F1 MICE
(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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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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CONTENTS

SUMMARY

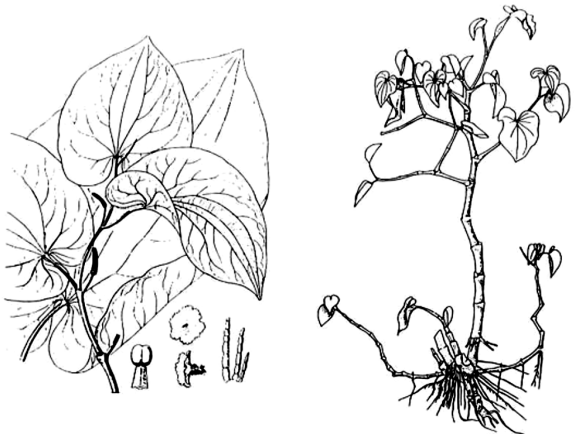
Background
Kava kava products are extracts from the root of the tropical shrub *Piper methysticum*. It was originally used for ceremonial beverages in the South Pacific and currently is used as an herbal product as an alternative to anti-anxiety drugs. We studied the effects of kava kava extract on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods
We deposited solutions containing kava kava extract in corn oil through a tube directly into the stomachs of 50 male and female rats and mice per dose group 5 days per week for 2 years. Exposed rats received either 0.1, 0.3 or 1 gram of kava kava extract per kilogram of body weight and mice received 0.25, 0.5, or 1 g/kg. Control animals received corn oil 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
There was a notable increase in uncommon malignant liver cancers (hepatoblastomas) in male mice and in hepatocellular carcinomas in female mice. In male rats receiving kava kava extract there was a slight increase in common testicular tumors. Other non-cancerous lesions that occurred in exposed animals were hepatocyte hypertrophy of the liver in male and female rats and mice and forestomach inflammation in male and female rats and female mice.

Conclusions
We conclude that kava kava extract caused cancers of the liver in male and female mice. Increased incidences of testicular tumors in male rats might have been related to kava kava exposure. Kava kava also caused increased incidences of lesions in the liver, forestomach, kidney, eye, and pancreas of male and female rats, in the liver of male and female mice, and in the forestomach of female mice.

ABSTRACT



KAVA KAVA EXTRACT

CAS No. 9000-38-8

Synonyms: Antares, ava; ava pepper; ava pepper shrub; ava root; awa; bornyl cinnamate; cavain; (+)-dihydrokawain-5-ol; Fijian kava; flavokavines A and B; 6-dihydroyangonin; gea; gi; grog; intoxicating long pepper; intoxicating pepper; kao; kava kava extract LI 140; kava kava rhizome; kava root; kavain; kavakava; kavalactones; kavapiper; kavapyrones; kavarod; kavasporal forte; kave-kave; kawa; kawa kawa; kawa pepper; Kawa Pfeffer; kew; LI150; long pepper; *Macropiper latifolium*; malohu; maluk; maori kava; meruk; 11-methoxy-5, 5-hydroxydihydrokawain; milik; olanzapine; pepe kava; piperis methystici rhizome; pipermethystine; Rauschpfeffer; rhizoma piperis methystici; rhizome di kava-kava sakaua; risperidone; sakau; tonga; WS 1490; wurzelstock; yagona; yangona; yaqona; yongona

Botanical name: *Piper methysticum*

Kava beverages, made from dried roots of the shrub *Piper methysticum*, have been used ceremonially and socially in the South Pacific and in Europe since the 1700s. The drink is reported to have pleasant mild psychoactive effects, similar to alcoholic beverages. In the United States, kava kava is an herbal product used extensively as an alternative to anti-anxiety drugs such as Xanax® and Valium®. It has also been reported as being used to help children with hyperactivity and as a skin-conditioning agent in cosmetics. Kava kava was nominated by the National Cancer Institute for study because of its increasing use as a dietary supplement in the mainstream United States market and reports of liver toxicity among humans. Male and female F344/N rats and B6C3F1 mice received kava kava extract in corn oil by gavage for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were administered kava kava extract in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg body weight, 5 days per week for 16 days. One female rat administered 2.0 g/kg kava kava extract died on day 3 of the study. Mean body weights of all dosed groups of rats were similar to those of the vehicle controls. Clinical findings included abnormal breathing, ataxia, and lethargy in the 2.0 g/kg groups of males and females and ataxia and lethargy in the 1.0 g/kg group of females. Liver weights were significantly increased in 1.0 and 2.0 g/kg males and in 0.5 g/kg or greater

females compared to the vehicle controls. Minimal hepatocellular hypertrophy occurred in all 2.0 g/kg males and in all females administered 0.25 g/kg or greater.

2-Week Study in Mice

Groups of five male and five female mice were administered kava kava extract in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg body weight, 5 days per week for 17 days. In the 2.0 g/kg group of males, one died on day 2 and one died on day 3. Mean body weights of all dosed groups of mice were similar to those of the vehicle controls. Clinical findings included abnormal breathing, ataxia, and lethargy in males and females in the 1.0 and 2.0 g/kg groups. Liver weights of 2.0 g/kg males and females were significantly increased. The incidence of hepatocellular hypertrophy in 2.0 g/kg female mice was significantly greater than that in the vehicle control group.

3-Month Study in Rats

Groups of 10 male and 10 female rats were administered kava kava extract in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 14 weeks. Deaths attributed to kava kava extract administration included three males and four females in the 2.0 g/kg groups and one female in the 1.0 g/kg group. One 0.25 g/kg male and one vehicle control female also died before the end of the study. The mean body weights of males in the 1.0 and 2.0 g/kg groups and females in the 2.0 g/kg group were significantly less than those of the vehicle controls. Ataxia and lethargy were observed in males and females in the 1.0 g/kg groups during week 1 and in the 2.0 g/kg groups throughout the study. Increased γ-glutamyltransferase activity in 1.0 g/kg females and 2.0 g/kg males and females may represent enzyme induction. However, the hepatocellular hypertrophy observed in the 2.0 g/kg females may have contributed to the increased γ-glutamyltransferase activity. The liver weights of 0.25 g/kg or greater males and 0.5 g/kg or greater females were significantly increased compared to the vehicle controls. The kidney weights of 0.5 g/kg or greater males and females were significantly increased compared to the vehicle controls. The incidence of hepatocellular hypertrophy in 2.0 g/kg females was significantly greater than that in the vehicle controls.

3-Month Study in Mice

Groups of 10 male and 10 female mice were administered kava kava extract in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 14 weeks. Four male and three female 2.0 g/kg mice died during week 1; these deaths were attributed to kava kava extract administration. One additional 2.0 g/kg female died during week 6 due to a gavage accident. The mean body weights of dosed males and females were similar to those of the vehicle controls. Ataxia and lethargy occurred in males and females in the 1.0 and 2.0 g/kg groups during week 1. The liver weights of 2.0 g/kg males and 1.0 and 2.0 g/kg females were significantly increased compared to those of the vehicle control groups. The incidences of centrilobular hypertrophy in the liver of 0.5 g/kg or greater males and 1.0 and 2.0 g/kg females were significantly greater than those in the vehicle controls.

2-Year Study in Rats

Groups of 49 or 50 male and 50 female rats were administered kava kava extract in corn oil by gavage at doses of 0, 0.1, 0.3, or 1.0 g/kg, 5 days per week for 104 (males) or 105 (females) weeks. Survival of dosed groups of males and females was similar to that of the vehicle controls. Mean body weights of males administered 1.0 g/kg were less than those of the vehicle controls after week 65, and those of the 1.0 g/kg females were less than those of the vehicle controls after week 41. Clinical findings included ataxia and lethargy that occurred in 21 males and 14 females in the 1.0 g/kg groups during the first 4 weeks of the study. After week 5, ataxia and lethargy were noted in 10 males and eight females in the 1.0 g/kg groups and these findings were observed randomly and intermittently throughout the study. At approximately 1 year into the study, twitching and seizures were observed in males and females in all dosed groups but mainly in the 1.0 g/kg groups.

There was a dose-related increase in the incidences of interstitial cell adenoma in the testis with increased incidences of bilateral neoplasms.

The incidences of hepatocellular hypertrophy in 1.0 g/kg males and females were significantly greater than those in the vehicle controls. Increased γ-glutamyltransferase activity and/or bile salt concentrations in males and females may represent a cholestatic event related to the hepatocellular hypertrophy observed in rats. Enzyme induction may have

played a role in the increased γ-glutamyltransferase activity. Significantly increased incidences of centrilobular fatty change occurred in 0.1 and 1.0 g/kg males. The incidences of inflammation, ulcer, and epithelial hyperplasia in the forestomach were significantly increased in 1.0 g/kg males and females. The severity of nephropathy was increased in 1.0 g/kg male rats, and the incidence of nephropathy was significantly increased in 1.0 g/kg females. Incidences of transitional epithelial hyperplasia of the pelvis of the kidney were significantly increased in 1.0 g/kg males and 0.3 and 1.0 g/kg females. The incidences of retinal degeneration in the eye were significantly increased in 1.0 g/kg males and females. The incidences of metaplasia of pancreatic acinar cells to a hepatocytic morphology increased in 1.0 g/kg males and females, and the increase in males was significant.

Significantly decreased incidences of pars distalis adenoma in the pituitary gland occurred in 1.0 g/kg males and in 0.1 and 1.0 g/kg females. The incidence of fibroadenoma of the mammary gland in 1.0 g/kg females was significantly less than that in the vehicle control group.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice received kava kava extract in corn oil by gavage at doses of 0, 0.25, 0.5, or 1.0 g/kg, 5 days per week for 105 weeks. Survival of dosed groups of males and females was similar to that of the vehicle controls. Mean body weights of males administered 1.0 g/kg were generally similar to those of the vehicle controls until the end of the study; however, those of 1.0 g/kg females were less than those of the vehicle controls after week 21. Clinical findings included ataxia and lethargy that occurred in 13 males and 31 females in the 1.0 g/kg groups during the first week of the study. Decreasing numbers of animals exhibited ataxia or lethargy during the remainder of the study, but these findings were observed in 1.0 g/kg females as late as week 101.

The incidences of hepatoblastoma in 0.5 and 1.0 g/kg males were significantly increased compared to the vehicle controls. The incidences of hepatocellular carcinoma or hepatoblastoma (combined) were significantly increased in 0.5 g/kg males. Incidences of hepatocellular carcinoma were increased in all dosed groups of females, and the increase was significant in the 0.25 g/kg group. The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in 0.25 and 0.5 g/kg females.

In the liver, the incidences of centrilobular hypertrophy in all dosed groups of males and females were significantly greater than those in the vehicle control groups. Significantly increased incidences of eosinophilic foci occurred in 0.5 g/kg males and in 1.0 g/kg males and females, and the incidence of angiectasis was significantly increased in the 1.0 g/kg males. The incidences of hepatocellular necrosis were significantly increased in 0.25 and 1.0 g/kg males.

In the forestomach, the incidences of chronic inflammation, epithelial hyperplasia, and erosion were significantly increased in 0.5 and 1.0 g/kg females, and the incidence of ulceration was significantly increased in 1.0 g/kg females.

GENETIC TOXICOLOGY

Kava kava extract was tested for bacterial mutagenicity over a broad range of concentrations in two independent assays using several strains of bacteria (*S. typhimurium* tester strains TA97, TA98, TA100, and TA1535 and *E. coli* strain WP2 *uvrA*/pKM101), with and without exogenous metabolic activation. No increase in mutant colonies was seen in any of the tester strains, under any activation condition. *In vivo*, no increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female B6C3F1 mice administered kava kava extract by gavage for 3 months.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity** of kava kava extract in male F344/N rats based on

marginal increases in the incidences of testicular interstitial cell adenoma. There was *no evidence of carcinogenic activity* of kava kava extract in female F344/N rats administered 0.1, 0.3, or 1.0 g/kg. There was *clear evidence of carcinogenic activity* of kava kava extract in male B6C3F1 mice based on increased incidences of hepatoblastoma. There was *some evidence of carcinogenic activity* of kava kava extract in female B6C3F1 mice based on increased incidences of hepatocellular adenoma or carcinoma (combined).

Kava kava extract administration resulted in increased incidences of nonneoplastic lesions in the liver, forestomach, kidney, eye, and pancreas of male and female rats, liver of male and female mice, and forestomach of female mice.

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

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Kava Kava Extract

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in corn oil by gavage	0, 0.1, 0.3, or 1.0 g/kg	0, 0.1, 0.3, or 1.0 g/kg	0, 0.25, 0.5, or 1.0 g/kg	0, 0.25, 0.5, or 1.0 g/kg
Body weights	1.0 g/kg group 10% less than the vehicle control group after week 65	1.0 g/kg group 10% less than the vehicle control group after week 41	Dosed groups generally similar to the vehicle control group	1.0 g/kg group 11% less than the vehicle control group after week 21
Survival rates	34/49, 35/50, 34/50, 31/50	34/50, 35/50, 24/50, 34/50	34/50, 33/50, 35/50, 36/50	38/50, 34/50, 45/50, 37/50
Nonneoplastic effects	Liver: hepatocyte, hypertrophy (0/49, 2/50, 2/50, 22/50); centrilobular, fatty change (1/49, 7/50, 4/50, 21/50) Stomach, Forestomach: inflammation (8/49, 4/50, 9/50, 22/50); ulcer (4/49, 0/50, 6/50, 13/50); epithelium, hyperplasia (6/49, 4/50, 11/50, 27/50) Kidney: severity of nephropathy (1.4, 1.2, 1.8, 3.1); pelvis, transitional epithelium, hyperplasia (0/49, 1/50, 1/50, 15/50) Eye: retina, degeneration (6/49, 6/50, 10/50, 16/50) Pancreas: acinus, metaplasia, hepatocyte (0/49, 0/50, 0/50, 6/50)	Liver: hepatocyte, hypertrophy (5/50, 2/50, 3/50, 33/50) Stomach, Forestomach: inflammation (5/49, 7/50, 7/50, 13/50); ulcer (1/49, 1/50, 3/50, 7/50); epithelium, hyperplasia (5/49, 6/50, 8/50, 19/50) Kidney: nephropathy (34/50, 35/50, 37/50, 43/50) Eye: retina, degeneration (5/50, 5/50, 5/50, 12/50) Pancreas: acinus, metaplasia, hepatocyte (0/49, 1/50, 0/50, 4/50)	Liver: centrilobular, hypertrophy (0/50, 34/50, 30/50, 39/50); eosinophilic focus (28/50, 32/50, 42/50, 43/50); angiectasis (3/50, 6/50, 7/50, 10/50); necrosis (3/50, 10/50, 7/50, 13/50)	Liver: centrilobular, hypertrophy (0/50, 20/50, 48/50, 49/50); eosinophilic focus (9/50, 7/50, 16/50, 26/50) Stomach, Forestomach: inflammation, chronic (3/50, 6/50, 21/50, 22/50); epithelium, hyperplasia (3/50, 6/50, 23/50, 24/50); erosion (0/50, 1/50, 14/50, 11/50); ulcer (0/50, 2/50, 3/50, 6/50)
Neoplastic effects	None	None	Liver: hepatoblastoma (0/50, 4/50, 9/50, 12/50)	Liver: hepatocellular carcinoma (3/50, 13/50, 8/50, 8/50); hepatocellular adenoma or carcinoma (10/50, 21/50, 20/50, 13/50)
Equivocal findings	Testes: interstitial cell, adenoma (37/49, 44/50, 49/50, 46/50)	None	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	Clear evidence	Some evidence
Genetic toxicology				
Bacterial gene mutations:	Negative in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 with and without S9; negative in *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9			
Micronucleated erythrocytes Mouse peripheral blood *in vivo*:	Negative in males and females			

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 kava kava extract 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 kava kava extract received public review by the National Toxicology Program's Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of kava kava extract by describing it as a leading dietary supplement with rapidly growing use in the United States market. Kava kava extract was nominated by the National Cancer Institute, based on widespread exposure, reports of hepatotoxicity in humans, increasing concern about its use by the U.S. Food and Drug Administration and the World Health Organization, and a lack of toxicity and carcinogenicity data. Two-week, 3-month, and 2-year gavage studies were conducted in male and female F344/N rats and B6C3F1 mice. The proposed conclusions for the 2-year gavage studies were *equivocal evidence of carcinogenic activity* of kava kava extract in male F344/N rats, *no evidence of carcinogenic activity* of kava kava extract in female F344/N rats, *clear evidence of carcinogenic activity* of kava kava extract in male B6C3F1 mice, and *some evidence of carcinogenic activity* of kava kava extract in female B6C3F1 mice.

Dr. Birt, the first primary reviewer, found the report to be well written and appreciated the opportunity to learn more about botanicals. She found the design of the experiments to be fully appropriate. She suggested several minor clarifications regarding statistical significance in the survival data; the relationship between the kavalactone component of dried kava kava and the lipid-soluble resin; the concentration of kava kava used in the studies; and inclusion of the genus, species, variety and accession information in the draft Technical Report.

Dr. Miller, the second primary reviewer, inquired about the doses used in the studies as compared with actual typical exposures. In addition, he asked about the percentage of the population that was exposed to kava kava extract and exhibited liver damage as a result, as well as any potential threshold dose at which liver damage could be anticipated. He noted the mention in the report about sedative effects during the early phases of the study that resolved upon extended use. He provided other minor editorial suggestions.

Dr. Rice, the third primary reviewer, concurred with prior comments that this was a standard bioassay that had been well conducted. He asked for clarification about how close to the actual "article of commerce" the studied extract was in terms of concentration of kavalactones, the pharmacologically active ingredient.

Dr. Chhabra said that in toxicity and carcinogenicity studies, it is typical to use doses several times the normal human dosage in order to engender toxic or carcinogenic effects, if any, while in safety studies it is more typical to dose at realistic exposure levels. He said there is very little information about human exposures to herbal products, given the fact that there has been little regulation. Per Dr. Rice's comments, Dr. Chhabra said the kava kava extract used in the current studies was comparable in its contents of kavalactones available to that from three or four different vendors.

Dr. P.C. Howard, FDA, said that prior to 2004 or 2005, the agency had limited authority over dietary supplements. He added that the FDA does take authority over a product and take action if clinical evidence of toxicity is found.

Dr. Klaunig asked about the designation of *clear evidence* in the male mice versus *some evidence* in the females, and whether it was because of the presence of hepatoblastomas in the males. Dr. Chhabra confirmed that conclusion.

Dr. M. Nagarkatti, University of South Carolina School of Medicine, echoed an earlier comment by Dr. Birt that it would be important in any report on an extract to provide detailed information about the when and where the plant was harvested. Dr. Chhabra said the NTP tries to get as much of that type of information as possible. Dr. Walker added that such characterization information is not always easy to get, particularly with commercial materials.

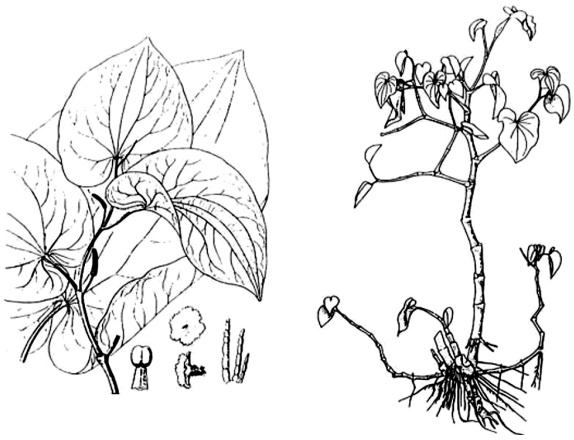
Dr. Cattley asked about prolactin levels. Dr. Chhabra explained that, after every Technical Report, NIEHS staff members hold a meeting to discuss the potential needs for follow-up studies, and that the prolactin question may be appropriate for further characterization.

Dr. Barlow said that due to the earlier onset dates in the evidence of interstitial cell/Leydig cell adenomas in male rats, a change from *equivocal evidence* to *some evidence* of carcinogenic activity should be considered. Dr. Chhabra disagreed with that suggestion. Dr. Barlow also questioned the use of "combined" terminology in the report, and Dr. Chhabra replied that that question

could be addressed when the study conclusions were considered.

Dr. Birt moved to accept the conclusions as written, and Dr. Miller seconded the motion. Dr. Cattley disagreed with the language in the conclusion regarding *clear evidence* based on increased incidence of carcinoma or hepatoblastoma, because that number was largely driven by the hepatoblastoma data. He recommended removing the language about the combined incidence and moved to amend the wording of the conclusion to state that there was *clear evidence* of carcinogenic activity of kava kava extract in male B6C3F1 mice based on increased incidences of hepatoblastoma. Dr. Klaunig seconded the motion and the panel voted unanimously with 10 votes in favor of the motion to accept the revised conclusion for male mice and accept the other conclusions as written.

INTRODUCTION



KAVA KAVA EXTRACT

CAS No. 9000-38-8

Synonyms: Antares, ava; ava pepper; ava pepper shrub; ava root; awa; bornyl cinnamate; cavain; (+)-dihydrokawain-5-ol; Fijian kava; flavokavines A and B; 6-dihydroyangonin; gea; gi; grog; intoxicating long pepper; intoxicating pepper; kao; kava kava extract LI 140; kava kava rhizome; kava root; kavain; kavakava; kavalactones; kavapiper; kavapyrones; kavarod; kavasporal forte; kave-kave; kawa; kawa kawa; kawa pepper; Kawa Pfeffer; kew; LI150; long pepper; *Macropiper latifolium*; malohu; maluk; maori kava; meruk; 11-methoxy-5, 5-hydroxydihydrokawain; milik; olanzapine; pepe kava; piperis methystici rhizome; pipermethystine; Rauschpfeffer; rhizoma piperis methystici; rhizome di kava-kava sakaua; risperidone; sakau; tonga; WS 1490; wurzelstock; yagona; yangona; yaqona; yongona

Botanical name: *Piper methysticum*

CHEMICAL AND PHYSICAL PROPERTIES

The tropical shrub *Piper methysticum* is a hardy, fairly succulent, slow-growing perennial widely cultivated in the South Pacific. Due to its traditional use as a ritual beverage known for promoting relaxation and a sense of well-being, inhabitants carried the kava kava plant widely throughout the Pacific. These cultural exchanges led to the establishment of kava kava in the three geographic regions of the Pacific: Polynesia, Melanesia, and Micronesia (Norton and Ruze, 1994).

The plant is usually harvested when it is about 2 to 2.5 meters tall. The leaves are heart-shaped, pointed, 8 to 25 centimeters long, and smooth and green on both sides. Kava kava is cultivated for its rootstock, also referred to as the stump. The stump is knotty, thick, and sometimes tuberous and often contains holes or cracks created by partial destruction of the parenchyma. A fringe of lateral roots up to 3 meters long extends from the pithy rootstock. The roots comprise a multitude of ligneous fibers and consist of more than 60% starch. Rootstock color varies from white to dark yellow, depending upon the amount of kavalactones that are contained in the lemon-yellow resin (Lebot *et al.*, 1992; Singh, 1992).

Kava kava cultivation and selection has produced numerous varieties or cultivars recognized by differences in the internodes (space between stem joints), color of stems, intensity of leaf color, and quality of the root. Different varieties are classified, named, and used for different purposes by the native people. Analysis of the composition of kava kava rootstock indicates that fresh material on average is 80% water. When dried, the rootstock consists of approximately 43% starch, 20% fibers, 12% water, 3.2% sugars, 3.6% proteins, 3.2% minerals, and 15% kavalactones, although the kavalactone component can vary between 3% to 20% of rootstock dry weight depending on the age of the plant and the cultivar. The

active principles of kava kava rootstock are mostly, if not entirely, contained in the lipid-soluble resin. The isolates of kava kava resin fall into three general categories: arylethylene-apyrones, chalcones and other flavanones, and conjugated diene ketones. The compounds of greatest pharmacological interest are the substituted α-pyrones or kava pyrones, commonly known as kavalactones. Fifteen lactones have been isolated from kava kava rootstock. The following six compounds are present in the highest concentrations and account for approximately 96% of the lipid resin: yangonin, methysticin, dihydromethysticin, kavain, dihydrokavain, and desmethoxyyangonin (Figure 1) (Shulgin, 1973; Lebot *et al.*, 1992; Dentali, 1997).

PRODUCTION, USE, AND HUMAN EXPOSURE

Kava kava is one of the most extensively used herbal products in the United States, with estimated sales of $106 million (Mirasol, 1998), and is readily available at health stores, pharmacies, and Walmart stores (ABC News, 1998). Kava kava sales generated almost EUR 100 million in 2001 and local annual sales of kava kava in Fiji have been reported in the range of $30 million, with exports amounting up to $17 million (NSM, 2010). Kava kava is used by approximately 2.2 million people in the United States as a natural alternative to anti-anxiety drugs such as Xanax® and Valium® (Gardiner *et al.*, 2007). It also has been claimed to have diuretic and antiseptic properties (Norton and Ruze, 1994; JNC Corp., 1998), it is reported to help children with hyperactivity (Symmetry, 1998), and it is often used as a skin-conditioning agent in cosmetics (Robinson *et al.*, 2009). The recommended oral dose for usage of kava as an anxiolytic is 50 to 70 mg kavalactones two to four times a day and, as a hypnotic, 150 to 210 mg in a single oral dose before bedtime (Bilia *et al.*, 2002).

The assessment of the efficacy and safety of herbal plants and herbal dietary supplements is important for human health protection (FDA, 2001, 2002; Fong, 2002; Fu *et al.*, 2002; Parkman, 2002). Although the herbal market has been rapidly growing, safety issues concerning potential side effects and toxic contamination of herbal products have not yet been adequately addressed, and toxicological data on the identification of genotoxic and tumorigenic ingredients in many herbs are lacking. Several cases of liver damage have been associated with kava kava exposure in Europe including hepatitis (Bujanda *et al.*, 2002; Humberston *et al.*, 2003; Stickel *et al.*, 2003), cirrhosis, liver failure (Escher *et al.*, 2001; Kraft *et al.*, 2001), and death (Gow *et al.*, 2003; Thomsen *et al.*, 2004). The Food and Drug Administration (FDA) has issued several warnings to consumers about the association between kava kava use and serious liver damage, but no guidelines have been established for its regulation (FDA, 2001, 2002). Hence, kava kava was nominated by the National Cancer Institute in 1998 to evaluate its potential for tumorigenicity and toxicity following chronic exposure.

REGULATORY STATUS

Kava kava sales have been suspended in several countries due to reports on its association with hepatotoxicity in humans (Russmann *et al.*, 2001, 2003; Campo *et al.*, 2002; De Smet, 2002; Parkman, 2002; Brauer *et al.*, 2003; Clough *et al.*, 2003; Humberston *et al.*, 2003; Ernst, 2006). In 2002, sales of products and preparations containing kava kava were suspended or withdrawn in Canada, Germany, Switzerland, Australia, France, and Spain (Gruenwald and Skrabal, 2003; Teschke *et al.*, 2003; Ulbricht *et al.*, 2005). In Japan, no new drug products containing kava kava were approved. In New Zealand, authorities recommended that labels contain warnings about the possible risk of liver damage. In August 2002, Health Canada banned preparations containing kava kava and 2 years later, in May 2004, the Scientific Advisory Panel on Hepatotoxicity (SAP-H) in Canada formed a special subcommittee to draft recommendations concerning "Hepatotoxicity of Health Products" after concerns were raised about four Canadian cases of liver toxicity associated with kava kava (NSM, 2010). In 2005, Germany considered making kava kava available by prescription only (NSM, 2010). In the same year, the Medicines and HealthCare products Regulatory Agency (MHRA) launched a 12-week public consultation process for interested parties to submit evidence and representations as to whether the prohibition of kava kava should continue. Based on the evidence provided by the MHRA, Germany was one of the first countries to lift its ban on kava kava. However, kava kava manufacturers still need to apply for new product registrations, even if they sold registered products in Germany before the ban.

Although kava kava sales are currently not regulated or controlled in the United States, the FDA issued a letter on December 18, 2001, stating that it was investigating whether kava kava-containing products were a health concern (FDA, 2001). The FDA noted 26 cases of liver toxicity in Germany and Switzerland, including one

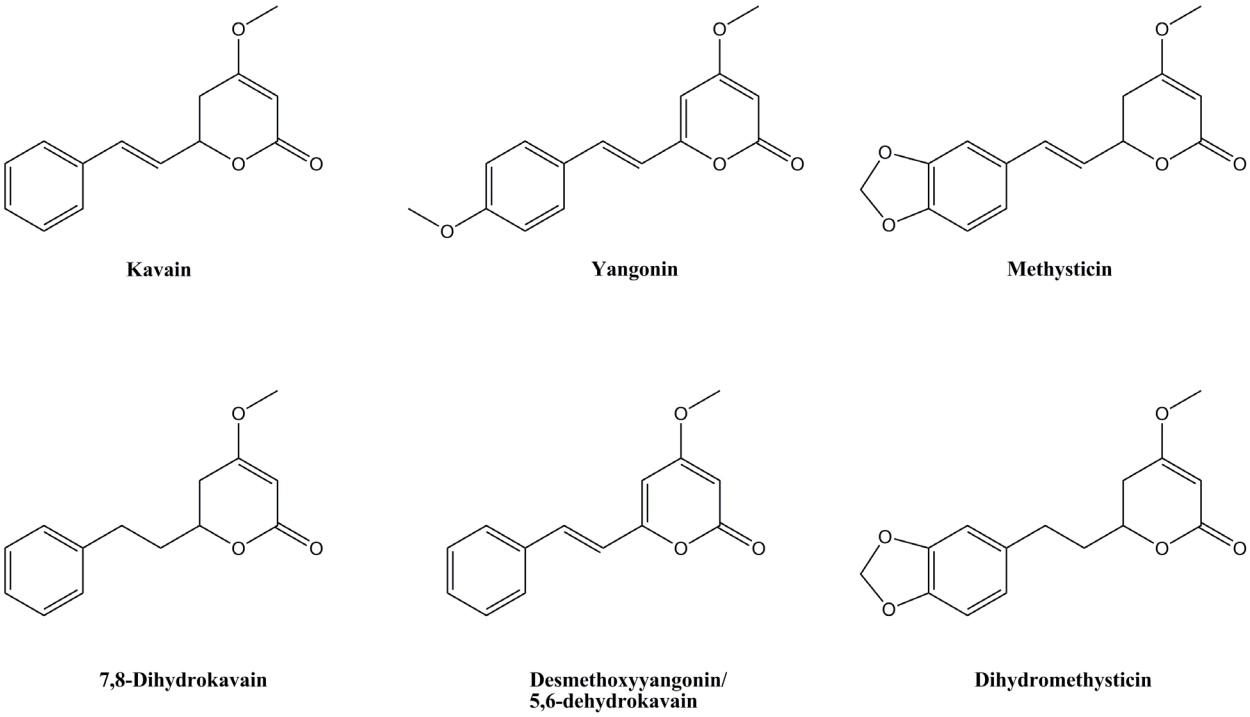


FIGURE 1
Chemical Structures of the Major Kavalactones in Kava Kava Extract

fatality and one liver transplant that were reportedly associated with kava kava products. In 2002, the United States Centers for Disease Control and Prevention (CDC) issued a report on hepatotoxicity associated with kava kava-containing products (NSM, 2010). On March 25, 2002, the FDA warned that kava kava may be associated with serious liver damage, including hepatitis, cirrhosis, and at least four cases of liver failure (FDA, 2002).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

A number of absorption, distribution, metabolism, and excretion studies, toxicokinetic studies, and gene expression studies have been conducted by the NTP (Mathews *et al.*, 2002, 2005; Guo *et al.*, 2009). The results from disposition studies on radiolabeled kavain, a primary constituent of kava kava, in male F344 rats revealed that it is rapidly absorbed and distributed to tissues following oral administration. Urinary and fecal excretion accounted for 77% and 14% of the administered dose, respectively; total radioactivity in tissues was less than 0.4% at 72 hours. Disposition of kavain in rats was similar after intravenous administration with 65% and 19% of the administered dose recovered in urine and feces, respectively (Mathews *et al.*, 2002, 2005). Based on similarities between oral and intravenous absorption data, kavain was considered to be well absorbed following oral administration in rats. In female mice, 72 hours after a single oral administration of kavalactones containing tracer levels of [^{14}C] kavain, 50% and 27% of the dose was excreted in urine and feces, respectively. Coadministration of kava extract shifted the excretion profile of kavain to 58% in urine and 26% in feces (RTI International, 2005). It is speculated that the inhibition of kavain metabolism by kava kava extract provided higher levels of kavain in hepatocytes, and that this kavain was transported into bile via canalicular Mrp2 and subsequently measured as increased levels excreted in feces. No change in disposition of orally administered kavain was observed following 7 days of pretreatment with kava kava extract (Mathews *et al.*, 2005). The estimated bioavailability of kavain in male F344/N rats based on the measurement of parent in plasma was 52%, indicating substantial first-pass metabolism following oral administration. Coadministration with kava kava extract caused a tripling of area under the kavain plasma concentration time curve and a doubling of maximum kavain plasma concentration. However, there was no effect on the toxicokinetics of kavain following a 7-day pretreatment with kava kava extract. In Balb/c mouse brain, 7,8-dihydrokavain and kavain reached maximum concentrations 5 minutes after intraperitoneal administration. By comparison, the maximum concentrations of desmethoxyyangonin and yangonin were lower although they were eliminated more slowly from the brain tissue than kavain or 7,8-dihydrokavain (Keledjian *et al.*, 1988).

In rodents, kavalactones are metabolized to numerous products including demethylation, mono- and dihydroxylation, and reduction and pyrone ring-opened products (Rasmussen *et al.*, 1979; Mathews *et al.* 2005; Fu *et al.*, 2008a,b). The extent of the metabolism and the types of metabolites depend on the structure of the kavalactone. The proposed metabolic pathways for 7,8-dihydrokavain are shown in Figure 2 (Rasmussen *et al.*, 1979).

Humans

The metabolism of kava kava in humans has been well documented. For example, Duffield *et al.* (1989) identified reduction, demethylation and monohydroxylation products in human urine following ingestion of kava kava. Unlike in rodents, dihydroxylated and pyrone ring-opened products were not among the identified metabolites. The mercapturic acid of 6-phenyl-3-hexen-2-one, a pyrone ring-opened product, was identified by Zou *et al.* (2005) in urine from two human subjects following ingestion of kava kava. Following incubation of kava kava extract with rat and human liver microsomes *in vitro*, kavain-11, 12-quinone and 7,8-dihydrokavain-11,12-quinone were identified as their corresponding glutathione conjugates, indicating the potential for the formation of dihydroxylated metabolites in humans (Johnson *et al.*, 2003). The same glutathione conjugates were observed when individual kavalactones, kavain and 7,8-dihydrokavain, were used in the system. The possible formation of dihydroxylated metabolites was further supported by the detection of glucuronide and sulfate conjugates of kavain-11,12-hydroquinone and 7,8-dihydrokavain-11,12-hydroquinone in the urine of a human volunteer administered a single dose of kava kava extract (Johnson *et al.*, 2003).

Kava extract and kavalactones (≤9 μM) modestly stimulated P-glycoprotein ATPase activities (Mathews *et al.*, 2005). Taken together, these studies raise the possibility that kava kava may have profound effects on the pharmacokinetics of many coadministrated drugs or other food supplements, subsequently leading to hepatotoxicity.

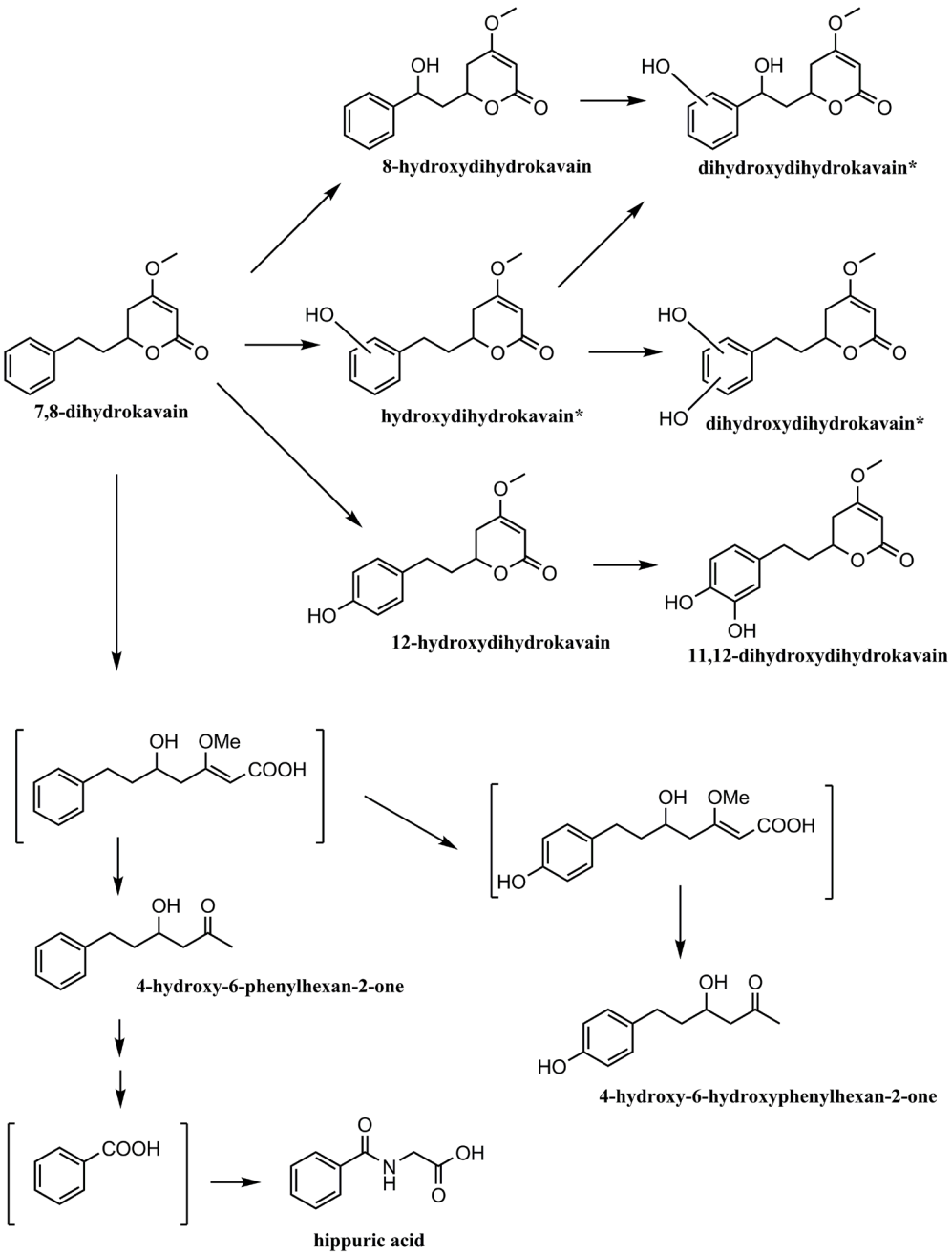


FIGURE 2
Proposed Metabolic Pathways of 7,8-Dihydrokavain Based on the Rodent Data
(adapted from Rasmussen *et al.*, 1979). Metabolites given in brackets were not identified (*indicates that the position of ring hydroxylation was not confirmed).

TOXICITY

Experimental Animals

There are a limited number of studies on the toxicity of kava extract and its constituents in experimental animals. Most of these studies have focused on the potential for clinical signs for hepatotoxicity. For example, studies by Singh and Devkota (2003) showed that Sprague-Dawley rats exposed by oral gavage to 200 or 500 mg kava kava extracts per/kg body weight per day for 2 and 4 weeks, exhibited no increases in serum markers of hepatotoxicity or malondialdehyde production. Treatment by oral gavage with kava root extract (100 mg/kg) in male F344/N rats for 2 weeks failed to elicit any significant changes in serum markers of hepatotoxicity (alanine aminotransferase and aspartate aminotransferase) or markers of hepatic lipid peroxidation and apoptosis (Lim *et al.*, 2007). Kava administration resulted in significant increases in CYP1A2 and CYP2E1 protein and decreased mRNA expression of uncoupling protein 2 (Lim *et al.*, 2007). Lim *et al*. (2007) also studied the effects of 10 mg/kg per day of kava kava leaf extracts and found increases in total hepatic GSH, TNFα, and increased activity of copper/zinc superoxide dismutase in addition to induction of CYP1A2 and CYP2E1 protein. A study by DiSilvestro *et al.* (2007) exposed Sprague-Dawley rats to kava kava extracts at oral doses ranging from 31 to 133 mg/kg and found no increases in serum markers of hepatotoxicity. Subchronic studies following oral exposure to kavain (10 to 400 mg/kg) in dogs for 91 days revealed the presence of mild toxicity with multicentric liver necrosis in one high-dose dog (Hapke *et al.*, 1971).

In vitro studies exposing HepG2 cells to kava extracts resulted in cytotoxicity at a concentration of 50 µg/mL or greater (Lüde *et al.*, 2008). The ratio of oxidized to reduced glutathione in this study was increased in HepG2 cells, whereas the cellular ATP content was maintained. Induction of apoptosis was seen at concentrations of 150 µg/mL or greater. Nerurkar *et al.* (2004) evaluated the cytotoxicity of individual kavalactones and the kava alkaloid pipermethystine in HepG2 cells. The kavalactones desmethoxyyangonin and dihydromethysticin induced cytoxicity at concentrations of 200 µM. Pipermethystine at a concentration of 50 µM induced cytotoxicity, decreased cellular ATP levels, decreased mitochondrial membrane potential, and induced apoptosis as measured by the release of caspase-3 after 24 hours of treatment.

Humans

There is conflicting evidence in the literature on kava-induced hepatotoxicity in humans. Although some reports have associated kava administration with hepatotoxicity including hepatitis, cirrhosis, and liver failure (Escher *et al.*, 2001; Campo *et al.*, 2002; Hefner, 2002; Gruenwald and Skrabal, 2003; Humberston *et al.*, 2003; Teschke *et al.*, 2003), others have shown that it is safe in most individuals at recommended doses (Denham *et al.*, 2002; Kopp, 2003). However, whether the dose or duration of use may be correlated with the risk of liver damage remains unknown. It is also unclear if the safety profile of kava is comparable to other agents used in the management of anxiety.

The toxicity of kava in humans has been partially attributed to the CYP2D6 deficiency seen in 7% to 9% of Caucasian, 5.5% of Western European, almost 1% of Asian, and less than 1% of Polynesian populations (Wanwimolruk *et al.*, 1998; Poolsup *et al.*, 2000; Ingelman-Sundberg, 2005). Reports suggest that genetic differences may constitute significant contributory factors for increased hepatotoxicity in Caucasians (Singh, 2005).

However, caution must be exercised when interpreting kava-related toxicity, because there are difficulties inherent in causality assessment when dealing with herbal hepatotoxicity. Risk factors for kava kava-related adverse reactions include daily overdose, prolonged therapy, and coingestion with up to five other herbals, dietary supplements, and synthetic drugs (Teschke *et al.*, 2008). Chronic heavy use of kava has been associated with case reports of renal dysfunction, hematologic abnormalities, pulmonary hypertension, dermopathy, and choreoathetosis (abnormal body movements) (Mathews *et al.*, 1988; Singh, 1992; Spillane *et al.*, 1997). These effects have been observed primarily in the context of heavy traditional/ceremonial use, and the causal relationship with kava is unclear due to multiple confounders. In reviews of 26 previously reported cases of kava kava-associated hepatic injury, causality was undeterminable in 16 cases and a low score from the Council for International Organizations of Medical Sciences excluded two more cases (Mathews *et al.*, 1988; Singh, 1992; Spillane *et al.*, 1997). Only one of the remaining eight cases had adhered to recommended dosing. Therefore, according to these authors' assessment, kava kava is rarely associated with hepatotoxicity, but comedication, overdose, and/or extended treatment duration may increase this risk. In addition, there have been a few reports of kava kava-induced neurotoxicity in humans and drug-herb interactions including oral and lingual dyskinesia (Schelosky *et al.*, 1995) and choreoathetosis (Spillane *et al.*, 1997).

Kava extracts and alkaloids have also been shown to inhibit cytochrome P-450s *in vitro*. Studies with human hepatocytes demonstrate that kava inhibits CYP1A2,

CYP2C9, CYP2C19, CYP3A4, CYP2D6, and CYP4A9/11, while CYP2A6, CYP2C8, and CYP2E1 activities are unaffected (Mathews *et al.*, 2002; Unger *et al.*, 2002; Anke and Ramzan, 2004a,b). Inhibition of these CYPs or a deficiency in CYP2D6 indicates that exposure to kava and other drugs and chemical agents at the same time has a high potential for causing drug interactions (Jamieson and Duffield, 1990a,b; Mathews *et al.*, 2002, 2005; Unger *et al.*, 2002; Zou *et al.*, 2002, 2004; Raucy, 2003; Teschke *et al.*, 2003; Whitton *et al.*, 2003; Anke and Ramzan, 2004a,b; Bressler, 2005; Hu *et al.*, 2005; Singh, 2005). Another study examined the cytotoxicity of kava lactones and kava extracts in MCL-5 cells, a human lymphoblastoid cell line that has been stably transfected with five human P450s (CYP1A1, CYP1A2, CYP2A6, CYP2E1, and CYP3A4), human epoxide hydrolase, and a control cell type derived from the same parental line as the MCL-5 cells (Zou *et al.*, 2004). Results revealed that the cytotoxic effects of the individual lactones and the extract were similar between the two cell lines, suggesting that metabolic activation is not involved in the cytotoxicity of these chemicals and extracts. The clinical relevance of these *in vitro* effects has not been evaluated *in vivo*.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No data on the reproductive and developmental toxicity of kava kava in experimental animals or humans were found in the literature.

CARCINOGENICITY

No data on the carcinogenicity of kava kava in experimental animals or epidemiology studies in humans were found in the literature.

GENETIC TOXICITY

Two published reports of mutagenicity data from studies with kava kava were identified. Whittaker *et al.* (2008) reported results from a gene mutation assay in mouse lymphoma L5178Y/tk$^{+/-}$ cells in which two commercial preparations (Kaviar and KavaPure) and seven purified kavalactones (D-kavain, DL-kavain, yangonin, dihydrokavain, methysticin, desmethoxy-yangonin, and dihydromethysticin) were tested in the presence of pooled human liver S9; no increases in mutation frequencies were seen with any of the kava kava extracts or preparations tested over multiple concentrations covering a cytotoxicity range of 0% to 90%. Jhoo *et al.* (2007) reported isolation of two presumptive *C*-glycoside flavonoids in *n*-butanol extracts of kava kava leaves, 2″-*O*-rhamnosylvitexin and schaftoside; these, along with six kavalactones (kavain, yangonin, dihydrokavain, methysticin, desmethoxyyangonin, and dihydromethysticin) were tested in the *umu* test for DNA damage in *Salmonella typhimurium* TA1535/pSK1002, with and without rat liver S9 for metabolic activation. The six kavalactones were not active in the *umu* test, but the two *n*-butanol leaf extracts showed dose-related increases in beta-galactosidase activity with and without S9, suggesting that they may be bacterial mutagens.

STUDY RATIONALE

Kava kava was selected by the NTP for chronic toxicity and carcinogenicity testing based on increasing human exposure and the lack of toxicity and carcinogenicity data. The NTP performed toxicity and carcinogenicity studies in F344/N rats and B6C3F1 mice using kava kava root extract formulated in corn oil, which was administered by gavage to mimic human exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Kava Kava Extract

Kava kava extract was obtained from Cosmopolitan Trading Co. (Seattle, WA) in one lot (9077SDK), which was received in several batches. Lot 9077SDK, Batch 02 was used in the 2-week studies. Prior to the start of the 3-month studies, the study laboratory (Battelle Columbus Operations, Columbus, OH) combined lot 9077SDK, Batches 02, 03, and 04 into a single batch (and assigned a lot number of 082203) that was used during the 3-month studies. Lot 9077SDK, Batch 05 was used during the 2-year studies. Identity, purity, stability, and weight loss on drying analyses were conducted by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO) (Appendix I). Reports on analyses performed in support of the kava kava extract studies are on file at the National Institute of Environmental Health Sciences.

Kava kava extract is in a medium-yellow powder form. The identity and purity of lot 9077SDK were determined using compendial methods to obtain the component profile. The bulk density of Batch 02 was determined to be 0.46396 g/mL, and that of Batch 05 was 0.4678 g/mL. Estimations of the distributions of particle sizes for Batches 02 through 05 were determined using a sieve method. To evaluate organic constituents of the test article, water, methanol, and methylene chloride extracts were prepared according to a proposed USP monograph (USP, 2000, 2002). A combination of chromatographic and spectrometric techniques was used to characterize the test article; an authentic standard of kavain was used for quantitation. Thin-layer chromatography (TLC) was performed on Batches 02 and 05 of lot 9077SDK. High-performance liquid chromatography (HPLC) with ultraviolet (UV) light detection was used to determine the composition of all four batches of the bulk chemical. Several components of Batches 02 and 05 of the test article were tentatively identified using various mass spectrometry techniques including liquid chromatography/mass spectrometry (LC/MS), gas chromatography/mass spectrometry (GC/MS), and matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS; Batch 05 only).

As determined by weight loss on drying, moisture content for lot 9077SDK was 5.42%, 5.04%, 5.35%, and 5.57% for Batches 02 through 05, respectively. TLC indicated the presence of kavain in all three extracts of Batches 02 and 05 of the test article, and methysticin in the three extracts of Batch 02. HPLC/UV profiles of methanolic (Batches 02 and 05) and aqueous (Batch 02) extracts of the test article indicated the presence of 10 components, eight of which were identified; in Batch 05, these 10 components were shown to account for all but 1.96% of the total peak area. LC/MS analyses of methanolic and aqueous extracts of Batches 02 and 05 confirmed the presence of six kavalactones quantitated in the HPLC/UV analyses of these extracts and tentatively identified seven other components. The identified kavalactones included methysticin, dihydromethysticin, kavain, dihydrokavain, yangonin, and desmethoxyyangonin. GC/MS analyses of Batches 02 and 05 performed on hexane, methylene chloride, acetone, and methanol (Batch 02 only) extracts of the bulk material confirmed the presence and identity of kavalactones in organic extracts of the test article. Additional confirmation of the presence and identity of the kavalactones was obtained from MALDI-TOF/MS analyses of aliquots of Batch 05 diluted with methanol:water (70:30, v/v). Using HPLC/UV analyses, the six major kavalactones were quantitated in methanolic extracts of all four batches of lot 9077SDK, and the results were very consistent across all the batches. Aliquots of Batches 02 and 05 were submitted for nutritional and contaminant tests using standards methods. Organochlorine and organophosphorus pesticide levels were below the detection limits for the analytical assays. Lead, mercury, and cadmium levels were less than the limits of detection, which were respectively 50, 25, and 2,000 ppb; selenium was present at 24 ppb. *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine were present at 1.4 and 31.2 ppb, respectively.

Taken together, these data indicate that all four batches of the test article were kava kava extract and their composition was consistent with the expected composition of typical kava kava extract. Results of the

nutritional and contaminant tests were deemed acceptable for use in these studies.

The bulk material was stored at room temperature, protected from light, in white, 5 gallon plastic drums and its stability was monitored using HPLC/UV analysis. No degradation was observed over the course of the studies.

Corn Oil

USP-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 2-week, 3-month, and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing kava kava extract with corn oil to give the required concentrations (Table I3). The dose formulations were stored at room temperature in clear glass bottles sealed with Teflon®-lined lids enclosed in amber plastic bags for up to 37 days (2-week studies) or 42 days (3-month and 2-year studies).

Homogeneity studies of ~200 and ~400 mg/mL dose formulations were performed by the analytical chemistry laboratory while studies of 12.5, 20, 25, 100, 200, and 400 mg/mL dose formulations were performed by the study laboratory; all of these studies used HPLC/UV analyses. Stability studies of a 12.5 mg/mL dose formulation were also performed by the analytical chemistry laboratory using HPLC/UV analysis. Homogeneity was confirmed, and stability was confirmed for at least 42 days for dose formulations stored in amber glass vials sealed with Teflon®-lined septa and crimped caps at room temperature and approximately 5° C, and for up to 3 hours under simulated animal room conditions. The study laboratory determined that a 400 mg/mL dose formulation of kava kava extract in corn oil was gavagable using a 20-gauge gavage needle.

Periodic analyses of the dose formulations of kava kava extract were conducted by the study laboratory using HPLC with UV detection. During the 2-week studies, the dose formulations were analyzed once; all six dose formulations for rats and mice were within 10% of the target concentrations (Table I4). Animal room samples of these dose formulations were also analyzed; four of five for rats and all five for mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I5). Of the dose formulations analyzed during the studies, all 18 for rats and mice were within 10% of the target concentrations; 14 of 15 animal room samples for rats and 13 of 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 11 weeks; animal room samples were also analyzed (Table I6). Of the dose formulations analyzed during the studies, 29 of 30 for rats and all 30 for mice were within 10% of the target concentrations; all 12 animal room samples for rats and all 12 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. Animals were quarantined for 12 days and were approximately 6 weeks old on the first day of the studies. Before the studies began, three male and two 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 kava kava extract in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg body weight, 5 days per week for 16 days (rats) or 17 (mice) days. Control animals received the corn oil vehicle alone. 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 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 rats and mice. The heart, right kidney, liver, lung, right testis, and thymus from each animal were weighed. Histopathologic examinations of selected tissues were performed on vehicle control and 2.0 g/kg rats and mice; these tissues were examined to a no-effect level in the remaining dosed groups. 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 kava kava extract 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 were 3 to 4 weeks old and mice were 4 to 5 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and mice were quarantined for 13 (females) or 14 (males) days. Rats and mice 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. At 1 month and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female core study rats and mice were administered kava kava extract in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 14 weeks. Clinical pathology study groups of 10 male and 10 female rats were administered the same doses 5 days per week for up to 23 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings for core study rats and mice were recorded once a week beginning on day 1 and at the end of the studies. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected via cardiac puncture from 10 male clinical pathology study rats on days 4 and 23 and from male core study rats and mice at the end of the studies for hematology and clinical chemistry (rats). Blood was collected via the retroorbital plexus (rats) or sinus (mice) of female clinical pathology study rats on days 4 and 23 and female core study rats and mice at the end of the studies for hematology (only rats at day 4) and clinical chemistry (rats). For hematology analyses, blood from each animal was collected into a tube containing EDTA. Erythrocyte, platelet, and leukocyte counts, hematocrit values, hemoglobin concentration, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration analyses, and reticulocyte counts were determined using an Advia® 120 Hematology System (Bayer Diagnostics, Tarrytown, NY) and reagents from the instrument manufacturer. For clinical chemistry analyses, blood was collected into a tube containing no anticoagulant. Clinical chemistry analyses were performed using a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Reagents were supplied by the instrument manufacturer. 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 rats and mice administered 0, 0.25, 0.5, and 1.0 g/kg; the high dose of 2.0 g/kg was not selected because of excessive toxicity. 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 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all vehicle control and 2.0 g/kg core study rats and mice and on core study animals that died early; tissues were examined to a no-effect level in the remaining core study groups. 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 49 or 50 male and 50 female rats were administered kava kava extract in corn oil by gavage at doses of 0, 0.1, 0.3, or 1.0 g/kg, and groups of 50 male and 50 female mice received kava kava extract in corn oil by gavage at doses of 0, 0.25, 0.5, or 1.0 g/kg, 5 days per week for 104 (male rats) or 105 (female rats and mice) weeks. Additional groups of 10 male and 10 female rats designated for clinical chemistry evaluations were administered the same doses for 18 months.

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. Rats were quarantined for 13 (males) or 14 days (females) and mice were quarantined for 11 (females) or 12 days (males) 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 were 6 to 7 weeks old and mice 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

All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guidelines. Male rats were housed up to three per cage, female rats were housed five per cage, male mice were house individually, and female mice were housed three to five per cage. Feed and water were available *ad libitum*. Cages and racks were rotated 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. Body weights of core study animals were recorded initially, weekly for the first 13 weeks (male rats were not weighed at week 11), monthly thereafter, and upon study termination. Clinical findings were recorded at week 5, monthly, and upon study termination.

Blood was collected from the retroorbital plexus of clinical chemistry study rats at 6, 12, and 18 months. Blood was placed in serum separator tubes, and clinical chemistry analyses were performed as described for the 3-month study. The parameters measured are listed in Table 1.

Complete necropsies and microscopic examinations were performed on all core study 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 (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 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 liver of rats and mice; the forestomach of rats and female mice; the bone marrow, eye, kidney, parathyroid gland, and pituitary gland of rats; the spleen of mice; and the tooth and Harderian gland of male 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 Kava Kava Extract

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
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		
12 days	Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 13 (males) or 14 (females) days Mice: 11 (females) or 12 (males) days
Average Age When Studies Began		
6 weeks	5 to 6 weeks	Rats: 6 to 7 weeks Mice: 5 to 6 weeks
Date of First Dose		
February 18, 2003	Rats: September 15 (males) or 16 (females), 2003 Mice: September 17 (females) or 18 (males), 2003	Rats: August 18 (males) or 19 (females), 2004 Mice: August 23 (females) or 24 (males), 2004
Duration of Dosing		
5 days per week for 16 (rats) or 17 (mice) days	5 days per week for 14 weeks	5 days per week for 104 (male rats) or 105 (female rats and mice) weeks
Date of Last Dose		
Rats: March 5, 2003 Mice: March 6, 2003	Core study rats: December 15 (males) or 16 (females), 2003 Mice: December 17 (females) or 18 (males), 2003	Rats: August 15 (males) or 17 (females), 2004 Mice: August 22 (females) or 24 (males), 2004
Necropsy Dates		
Rats: March 6, 2003 Mice: March 7, 2003	Rats: December 16 (males) or 17 (females), 2003 Mice: December 18 (females) or 19 (males), 2003	Rats: August 14 to 16 (males) or 16 to 18 (females), 2006 Mice: August 21 to 23 (females) or 23 to 25 (males), 2006
Average Age at Necropsy		
8 to 9 weeks	18 to 19 weeks	109 to 111 weeks

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Kava Kava Extract

2-Week Studies	3-Month Studies	2-Year Studies
Size of Study Groups Five males and five females	Core study: 10 males and 10 females Clinical pathology study: 10 male and 10 female rats	Core study: 49 or 50 males and 50 females Clinical chemistry study: 10 male and 10 female rats
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: 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 3 to 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet NTP-2000 irradiated meal feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed twice weekly	NTP-2000 irradiated wafer feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 3-month studies
Water Tap water (Columbus municipal supply) via glass bottles with Teflon®-lined stopper tubes, available *ad libitum,* change twice weekly	Same as 2-week studies, except via automatic watering system (Edstrom Industries, Waterford, WI)	Same as 3-month studies
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice weekly	Polycarbonate (Lab Products, Inc., Seaford, DE), changed once (male mice) or twice (rats and female mice) weekly	Same as 3-month studies
Bedding Irradiated Sani-Chips® (P.J. Murphy Forest Products, Corp., Montville, NJ), changed twice weekly	Irradiated Sani-Chips® (P.J. Murphy Forest Products, Corp., Montville, NJ), changed once (male mice) or twice (rats and female mice) weekly	Same as 3-month studies
Cage Filters Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed once	Same as 2-week studies, except changed ever 2 weeks	Same as 3-month studies
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed once	Same as 2-week studies, except changed every 2 weeks	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
Doses 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg in corn oil by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice)	0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg in corn oil by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice)	Rats: 0, 0.1, 0.3, or 1.0 g/kg in corn oil by gavage (dosing volume 5 mL/kg) Mice: 0, 0.25, 0.5, or 1.0 g/kg in corn oil by gavage (dosing volume 10 mL/kg)

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Kava Kava Extract

2-Week Studies	3-Month Studies	2-Year Studies
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; animals were weighed initially, weekly, and at the end of the studies; clinical findings for core study animals were recorded on day 1, weekly, and at the end of the studies.	Observed twice daily; core study animals were weighed initially, weekly for the first 13 weeks (males rats were not weighed at week 11), monthly thereafter, and upon study termination; clinical findings for core study animals were recorded at week 5, monthly, and at the end of the studies.
Method of Sacrifice		
Carbon dioxide asphyxiation	Exsanguination (males) with carbon dioxide anesthesia; carbon dioxide asphyxiation (females)	Same as 2-week studies
Necropsy		
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus from all rats and mice.	Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus of core study rats and mice.	Necropsies were performed on core study animals.
Clinical Pathology		
None	Blood was collected via cardiac puncture from 10 male clinical pathology study rats on days 4 and 23 and from male core study rats and mice at the end of the studies for hematology and clinical chemistry (rats). Blood was collected via the retroorbital sinus of female clinical pathology study rats on days 4 and 23 and female core study rats and mice at the end of the studies for hematology (only rats at day 4) and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, gamma glutamyl transpeptidase, and bile acids	Blood was collected from the retroorbital plexus of clinical chemistry study rats at 6, 12, and 18 months. ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, gamma glutamyl transpeptidase, and bile acids

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Kava Kava Extract

2-Week Studies	3-Month Studies	2-Year Studies
Histopathology Histopathology was performed on vehicle control and 2.0 g/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: brain, kidney, liver, and lung.	Complete histopathology was performed on core study animals that died early, vehicle controls, and 2.0 g/kg rats and mice: In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lungs, 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.	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 only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lungs, 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 studies, spermatid and sperm samples were collected from male animals in the vehicle control and 0.25, 0.5, and 1.0 g/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 females dosed at the vehicle control and at 0.25, 0.5, and 1.0 g/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, B4, C1, C4, D1, and D4 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, clinical chemistry, and 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 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 proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as 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 kava kava extract was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. 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

One female rat administered 2.0 g/kg kava kava extract died on day 3 of the study (Table 2); the cause of death was not determined. The final mean body weights and mean body weight gains of all dosed groups of rats were similar to those of the vehicle controls. Clinical findings included abnormal breathing, ataxia, and lethargy in 2.0 g/kg males and females and ataxia and lethargy in 1.0 g/kg females. No gross lesions related to kava kava extract administration were observed.

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

Dose (g/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	104 ± 3	187 ± 5	83 ± 2	
0.125	5/5	103 ± 4	187 ± 7	84 ± 3	100
0.25	5/5	103 ± 3	185 ± 6	83 ± 4	99
0.5	5/5	102 ± 3	184 ± 5	82 ± 2	98
1.0	5/5	103 ± 3	186 ± 6	83 ± 4	100
2.0	5/5	104 ± 2	176 ± 4	72 ± 2	94
Female					
0	5/5	96 ± 2	134 ± 1	38 ± 1	
0.125	5/5	97 ± 2	137 ± 2	41 ± 1	102
0.25	5/5	96 ± 2	137 ± 2	41 ± 1	102
0.5	5/5	97 ± 3	135 ± 3	39 ± 1	101
1.0	5/5	97 ± 2	137 ± 2	40 ± 1	102
2.0	4/5[c]	97 ± 2	132 ± 3	34 ± 2	98

[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 in weights and weight changes are not significant by Dunnett's test.

[b] Number of animals surviving at 16 days/number initially in group

[c] Day of death: 3

Absolute liver weights were significantly increased compared to the vehicle controls in males receiving 1.0 and 2.0 g/kg and in females receiving 0.5 g/kg or greater; relative liver weights were significantly increased in males receiving 0.5 g/kg or greater and in females receiving 0.25 g/kg or greater (Table G1).

The incidences of minimal hepatocellular hypertrophy were significantly increased in males receiving 2.0 g/kg and in females receiving 0.25 g/kg or greater (Table 3).

Dose Selection Rationale: The 2-week studies did not show any effects on survival or body weights. The histopathologic changes were minimal. Therefore, doses of 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg were selected for the 3-month gavage study in rats.

TABLE 3
Incidences of Nonneoplastic Lesions of the Liver in Rats in the 2-Week Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
Number Examined Microscopically	5	0	0	0	5	5
Hepatocyte, Hypertrophy[a]	0				0	5** (1.0)[b]
Female						
Number Examined Microscopically	5	5	5	5	5	5
Hepatocyte, Hypertrophy	0	0	5** (1.0)	5** (1.0)	5** (1.0)	5** (1.0)

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

3-MONTH STUDY

Deaths attributed to kava kava extract administration included three males and four females in the 2.0 g/kg groups and one female in the 1.0 g/kg group (Table 4). One 0.25 g/kg male and one vehicle control female also died before the end of the study. The final mean body weights and mean body weight gains of males in the 1.0 and 2.0 g/kg groups and females in the 2.0 g/kg group were significantly less than those of the vehicle controls (Table 4 and Figure 3). Ataxia and lethargy were observed in males and females in the 1.0 g/kg groups during week 1 and in the 2.0 g/kg groups throughout the study. No chemical-related gross lesions were seen in early death or terminal sacrifice rats.

TABLE 4
Survival and Body Weights of Rats in the 3-Month Gavage Study of Kava Kava Extract[a]

Dose (g/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	86 ± 1	346 ± 6	260 ± 5	
0.125	10/10	85 ± 1	334 ± 6	249 ± 5	97
0.25	9/10[c]	84 ± 1	334 ± 5	250 ± 5	97
0.5	10/10	85 ± 2	339 ± 4	254 ± 4	98
1.0	10/10	84 ± 1	316 ± 7**	232 ± 6**	91
2.0	7/10[d]	85 ± 1	286 ± 6**	202 ± 6**	83
Female					
0	9/10[e]	90 ± 2	197 ± 2	107 ± 3	
0.125	10/10	90 ± 2	196 ± 2	106 ± 1	100
0.25	10/10	90 ± 2	193 ± 2	104 ± 4	98
0.5	10/10	90 ± 2	196 ± 4	106 ± 4	99
1.0	9/10[f]	90 ± 2	190 ± 4	101 ± 3	96
2.0	6/10[g]	89 ± 2	178 ± 4**	88 ± 3**	90

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' 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] Week of death: 4

[d] Weeks of death: 1, 12, 12

[e] Week of death: 3

[f] Week of death: 5

[g] Weeks of death: 1, 1, 1, 7

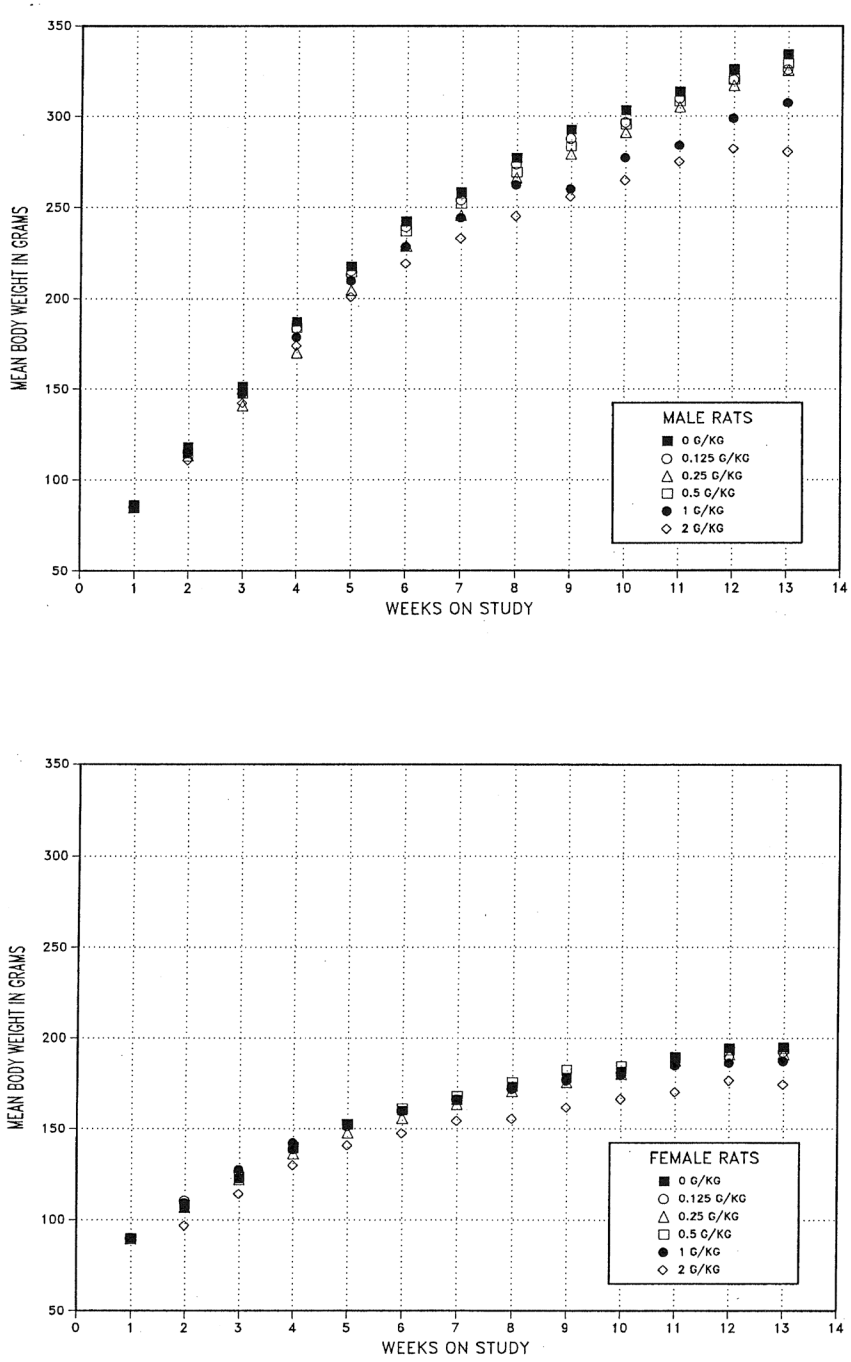


FIGURE 3
Growth Curves for Rats Administered Kava Kava Extract by Gavage for 3 Months

The hematology and clinical chemistry data for rats in the 3-month study are presented in Tables 5 and F1. There were decreases in serum alanine aminotransferase (ALT), alkaline phosphatase (ALP) and sorbitol dehydrogenase (SDH) activities in dosed male rats at week 14; females were unaffected. At all three time points, the high dose (2.0 g/kg) male and female rats demonstrated multiple-fold increases in serum γ-glutamyltransferase (GGT) activity; the 1.0 g/kg females were also affected at week 14. Increases in serum GGT activity are used as a marker of cholestasis. Other markers of cholestasis were decreased (ALP activity) or unaffected (bile salts concentration) and did not support the increased GGT activities. Dose-related increases in cholesterol concentrations occurred in the top three male and female dose groups (0.5, 1.0, and 2.0 g/kg) at all time points. Lower dose groups (0.125 and 0.25 g/kg) also demonstrated this alteration, but less consistently. Triglyceride concentration, another marker of lipid metabolism, was unaffected. Total protein and albumin (and by extrapolation, globulin) concentrations were minimally increased (<15%) in the top four female dose groups (0.25, 0.5, 1.0, and 2.0 g/kg) on day 23. By week 14, all female and the top three male dose groups (0.5, 1.0, and 2.0 g/kg) demonstrated this change in serum proteins. The increase for the different protein fractions was proportional. There were no changes in the hematology results considered attributable to kava kava extract administration (Table F1).

The absolute and relative liver weights of 0.25 g/kg or greater males and 0.5 g/kg or greater females were significantly increased compared to the vehicle controls (Table G2). In females, the increased liver weights were dose-related and consistent with the hepatocellular hypertrophy observed microscopically. The absolute and relative kidney weights of 0.5 g/kg or greater males and females were significantly increased compared to the vehicle controls.

There were no significant differences in sperm parameters of male rats or the estrous cyclicity of female rats administered 0.25, 0.5, or 1.0 g/kg kava kava extract when compared to the vehicle controls (Tables H1 and H2).

TABLE 5
Selected Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
n						
Day 4	10	10	10	10	10	8
Day 23	10	10	10	10	10	8
Week 14	10	10	9	10	10	7
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	5.9 ± 0.1
Day 23	6.9 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	6.9 ± 0.1
Week 14	6.8 ± 0.1	6.7 ± 0.0	6.8 ± 0.1	7.1 ± 0.1**	7.4 ± 0.1**	7.9 ± 0.3**
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.4 ± 0.1	4.3 ± 0.1	4.2 ± 0.1
Day 23	4.8 ± 0.1	4.7 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.9 ± 0.1	4.8 ± 0.0
Week 14	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.0**	5.0 ± 0.1**	5.3 ± 0.1**
Cholesterol (mg/dL)						
Day 4	95 ± 1	100 ± 1**	99 ± 2*	114 ± 2**	124 ± 3**	132 ± 6**
Day 23	87 ± 1	87 ± 2	91 ± 2	97 ± 2**	97 ± 3**	94 ± 3**
Week 14	77 ± 1	75 ± 1	79 ± 1	83 ± 2*	86 ± 2**	92 ± 4**
Triglycerides (mg/dL)						
Day 4	66 ± 4	74 ± 7	72 ± 4	83 ± 7	88 ± 8	89 ± 10
Day 23	173 ± 16	141 ± 16	150 ± 10	146 ± 14	111 ± 9**	114 ± 8**
Week 14	113 ± 11	104 ± 9	118 ± 9	115 ± 11	88 ± 8	94 ± 13
Alanine aminotransferase (IU/L)						
Day 4	54 ± 2	57 ± 1	53 ± 2	53 ± 1	55 ± 2	73 ± 5*
Day 23	49 ± 2	47 ± 1	48 ± 1	47 ± 2	44 ± 1	54 ± 2
Week 14	71 ± 5	53 ± 2**	47 ± 1**	40 ± 1**	45 ± 2**	63 ± 4**
Alkaline phosphatase (IU/L)						
Day 4	656 ± 20	677 ± 10	659 ± 13	652 ± 12	662 ± 15	685 ± 30
Day 23	492 ± 9	472 ± 6	481 ± 6	457 ± 12*	424 ± 10**	442 ± 13**
Week 14	225 ± 6	221 ± 5	215 ± 6	209 ± 5	202 ± 5**	209 ± 9*
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	15 ± 0	16 ± 1	16 ± 1	16 ± 1	20 ± 2
Day 23	21 ± 1	19 ± 1	20 ± 1	20 ± 1	18 ± 1*	20 ± 1
Week 14	18 ± 1	16 ± 1	13 ± 1**	13 ± 1**	14 ± 1**	16 ± 3*
γ-Glutamyltransferase (IU/L)						
Day 4	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.3	0.3 ± 0.2	0.1 ± 0.1	2.8 ± 0.6**
Day 23	0.0 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.9 ± 0.2**
Week 14	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.1**
Bile salts (μmol/L)						
Day 4	11.2 ± 2.0	10.1 ± 1.7	7.2 ± 1.0	6.5 ± 0.9	7.9 ± 1.5	13.3 ± 2.7
Day 23	10.6 ± 2.2	12.9 ± 2.1	8.7 ± 2.3	7.9 ± 1.6	7.7 ± 1.5	8.4 ± 2.2
Week 14	8.6 ± 1.8	6.0 ± 1.1	5.9 ± 1.2	7.2 ± 1.3	6.1 ± 1.1	9.1 ± 1.1

TABLE 5
Selected Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Female						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	10	10	9	6
Total protein (g/dL)						
Day 4	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.0	6.2 ± 0.0*	6.1 ± 0.1	5.9 ± 0.1
Day 23	5.8 ± 0.1	6.0 ± 0.1	6.1 ± 0.1*	6.2 ± 0.0**	6.2 ± 0.1**	6.3 ± 0.1**
Week 14	6.4 ± 0.1	6.7 ± 0.1**	6.5 ± 0.1*	6.8 ± 0.1**	6.8 ± 0.1**	7.2 ± 0.1**
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	4.3 ± 0.0
Day 23	4.4 ± 0.0	4.5 ± 0.1	4.5 ± 0.0*	4.6 ± 0.0**	4.6 ± 0.0**	4.6 ± 0.1**
Week 14	4.6 ± 0.0	4.8 ± 0.0**	4.8 ± 0.0*	4.9 ± 0.1**	4.9 ± 0.0**	5.1 ± 0.0**
Cholesterol (mg/dL)						
Day 4	85 ± 3	92 ± 3	89 ± 2	109 ± 2**	112 ± 2**	128 ± 5**
Day 23	73 ± 1	78 ± 2	83 ± 1**	90 ± 1**	100 ± 2**	107 ± 3**
Week 14	69 ± 2	83 ± 2**	75 ± 2*	92 ± 2**	99 ± 2**	105 ± 4**
Triglycerides (mg/dL)						
Day 4	76 ± 6	78 ± 6	66 ± 2	78 ± 4	68 ± 6	74 ± 4
Day 23	47 ± 6	51 ± 4	51 ± 5	53 ± 4	55 ± 5	64 ± 6
Week 14	56 ± 4	62 ± 7	52 ± 5	53 ± 4	57 ± 5	55 ± 8
Alanine aminotransferase (IU/L)						
Day 4	43 ± 1	43 ± 2	40 ± 1	42 ± 1	43 ± 2	57 ± 2**
Day 23	37 ± 1	37 ± 1	35 ± 1	35 ± 1	37 ± 1	55 ± 4*
Week 14	45 ± 2	50 ± 4	43 ± 2	38 ± 1	43 ± 2	62 ± 4
Alkaline phosphatase (IU/L)						
Day 4	531 ± 10	542 ± 9	515 ± 7	515 ± 13	508 ± 10	558 ± 18
Day 23	320 ± 5	333 ± 10	305 ± 10	303 ± 6	276 ± 6**	331 ± 11
Week 14	197 ± 7	176 ± 4	178 ± 4	163 ± 4**	169 ± 5*	214 ± 15
Sorbitol dehydrogenase (IU/L)						
Day 4	14 ± 1	14 ± 1	15 ± 0	14 ± 1	14 ± 1	14 ± 1
Day 23	13 ± 1	14 ± 1	14 ± 1	15 ± 1	15 ± 1	14 ± 0
Week 14	13 ± 1	15 ± 1	12 ± 1	12 ± 1	12 ± 1	12 ± 1
γ-Glutamyltransferase (IU/L)						
Day 4	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.9 ± 0.2	4.3 ± 0.7**
Day 23	0.5 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	1.0 ± 0.1	3.9 ± 0.4**
Week 14	1.3 ± 0.3	1.5 ± 0.3	1.4 ± 0.2	1.7 ± 0.3	3.1 ± 0.3**	15.3 ± 1.0**
Bile salts (μmol/L)						
Day 4	7.3 ± 1.2	9.2 ± 1.9	8.8 ± 1.3	6.3 ± 1.1	7.3 ± 1.6	11.2 ± 1.4
Day 23	12.2 ± 2.4	10.4 ± 1.8	10.0 ± 1.4	5.7 ± 0.8	8.6 ± 1.3	10.8 ± 1.6
Week 14	9.6 ± 2.3	13.5 ± 2.6	11.3 ± 1.5	9.0 ± 1.4	9.8 ± 1.2	12.2 ± 1.9

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

The incidence of hepatocellular hypertrophy in 2.0 g/kg females was significantly greater than that in the vehicle controls (Table 6). Three 0.5 g/kg females and three 1.0 g/kg females also had hepatocellular hypertrophy, and the severity of this lesion increased with increasing dose. One 1.0 g/kg male also had hepatocellular hypertrophy. Hepatocellular hypertrophy was minimal to mild in severity and consisted of a diffuse increase in cell size associated with glycogen depletion and amphophilic cytoplasm of the hepatocytes.

Atrophy of the periarteriolar lymphoid sheaths in the spleen, consisting of a reduced number of lymphocytes, was observed in the three 2.0 g/kg males and in three of the four 2.0 g/kg females that died before the end of the study and was considered to be stress related.

Thymic necrosis was noted in one of the three 2.0 g/kg males and in three of the four 2.0 g/kg females that died before the end of the study and was considered to be stress related. Thymic necrosis consisted of cortical necrosis of lymphocytes, characterized by increased numbers of deeply basophilic pyknotic or karyorrhectic nuclear fragments (compared to normal background) within the thymic cortex.

Dose Selection Rationale: In the 3-month study, there were decreases in the survival and body weights of 2.0 g/kg males and females. The increases in liver weights and incidences of hepatocellular hypertrophy in 1.0 g/kg males and females were considered to be minimal. Therefore, 1.0 g/kg was selected as the highest dose for the 2-year gavage study in rats.

TABLE 6
Incidences of Nonneoplastic Lesions of the Liver in Rats in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy[a]	0	0	0	0	1 (1.0)[b]	0
Female						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	0	0	3 (1.0)	3 (1.3)	10** (1.7)

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

2-YEAR STUDY

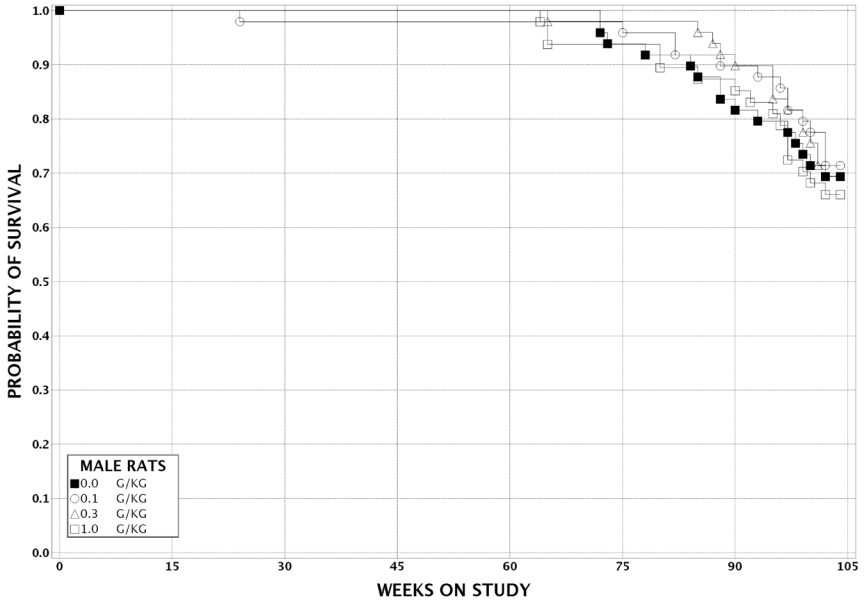
Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 7 and in the Kaplan-Meier survival curves (Figure 4). Survival of dosed groups of males and females was similar to that of the vehicle controls.

TABLE 7
Survival of Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Male				
Animals initially in study	49	50	50	50
Accidental deaths[a]	0	1	1	3
Moribund	10	10	13	13
Natural deaths	5	4	2	3
Animals surviving to study termination	34	35	34[e]	31
Percent probability of survival at end of study[b]	69	71	69	66
Mean survival (days)[c]	692	682	701	658
Survival analysis[d]	P=0.652	P=0.880N	P=1.000	P=0.879
Female				
Animals initially in study	50	50	50	50
Accidental deaths	1	1	2	2
Moribund	12	12	13	6
Natural deaths	3	2	11	8
Animals surviving to study termination	34	35[e]	24	34
Percent probability of survival at end of study	70	69	50	71
Mean survival (days)	683	680	663	654
Survival analysis	P=0.931N	P=1.000	P=0.077	P=1.000

[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. A negative trend or lower mortality in a dose group is indicated by **N**.
[e] Includes one animal that died during the last week of the study



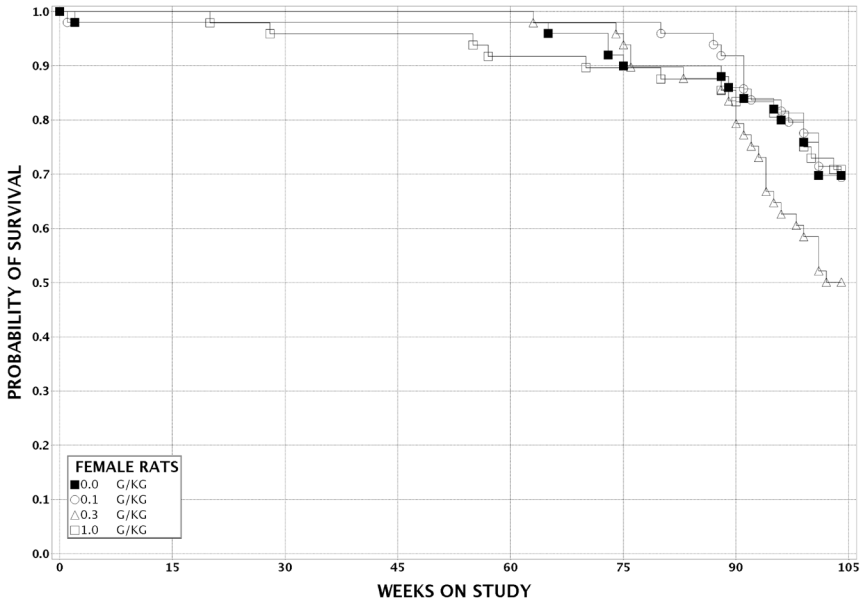


FIGURE 4
Kaplan-Meier Survival Curves for Rats Administered Kava Kava Extract by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of males administered 1.0 g/kg were less than those of the vehicle controls after week 65, and those of the 1.0 g/kg females were less than those of the vehicle controls after week 41 (Tables 8 and 9 and Figure 5). Clinical findings included ataxia and lethargy that occurred in 21 males and 14 females in the 1.0 g/kg groups during the first 4 weeks of the study. After week 5, ataxia and lethargy were noted in 10 males and eight females in the 1.0 g/kg groups and these findings were observed randomly and intermittently throughout the study. At approximately 1 year into the study, twitching and seizures were noted mostly in the high-dose group of 1.0 g/kg and to a lesser extent in the lower dosed groups. These signs were of transient nature and occurred immediately postdosing. Ruffled fur and thinness were observed in several male rats in each dosed group after month 20.

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control		0.1 g/kg			0.3 g/kg			1.0 g/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	110	49	109	99	50	109	99	50	109	100	50
8	139	49	138	100	50	138	100	50	135	97	49
15	168	49	168	100	50	169	101	50	166	99	49
22	197	49	196	100	50	198	100	50	194	98	49
29	221	49	221	100	50	221	100	50	215	98	49
36	241	49	241	100	49	241	100	50	234	97	48
43	256	49	257	100	49	255	99	50	247	97	48
50	272	49	273	100	49	271	100	50	262	96	48
57	285	49	286	100	49	285	100	50	276	97	48
64	297	49	295	99	49	295	99	50	286	96	48
78	319	49	318	100	49	317	99	50	306	96	48
85	331	49	328	99	49	328	99	50	317	96	48
113	353	49	352	100	49	349	99	50	335	95	48
141	376	49	376	100	49	378	101	50	354	94	48
169	394	49	392	99	48	395	100	50	369	94	48
197	412	49	410	100	48	410	100	50	386	94	48
225	429	49	427	100	48	430	100	50	401	94	48
253	441	49	436	99	48	444	101	50	414	94	48
281	451	49	445	99	48	454	101	50	422	93	48
309	460	49	458	100	48	466	101	50	430	94	48
337	472	49	466	99	48	477	101	50	439	93	48
365	477	49	469	98	48	481	101	50	443	93	48
393	484	49	475	98	48	489	101	50	447	92	48
421	492	49	482	98	48	496	101	50	451	92	48
449	498	49	490	98	48	504	101	50	457	92	47
477	504	49	495	98	48	509	101	49	456	90	45
505	505	47	498	99	48	511	101	49	454	90	45
533	506	46	499	99	47	513	102	49	450	89	44
561	509	45	503	99	47	520	102	49	452	89	42
589	505	44	498	99	45	515	102	49	445	88	41
617	506	41	495	98	44	518	102	45	446	88	41
645	510	40	498	98	44	528	104	44	451	89	39
673	504	39	502	100	41	512	101	40	444	88	35
701	515	35	508	99	38	512	99	36	443	86	32
Mean for weeks											
1-13	236		236	100		236	100		229	97	
14-52	421		418	99		423	100		394	94	
53-101	501		493	98		508	101		449	90	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control		0.1 g/kg			0.3 g/kg			1.0 g/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	97	50	99	102	50	98	101	50	97	100	50
8	113	50	114	101	49	114	100	50	113	100	50
15	129	49	131	101	49	128	99	50	129	100	50
22	142	49	145	102	49	142	100	50	142	100	50
29	153	49	156	102	49	153	100	50	151	99	50
36	160	49	164	103	49	161	101	50	159	99	50
43	168	49	171	102	49	168	100	50	165	99	50
50	174	49	177	102	49	175	101	50	172	99	50
57	181	49	179	99	48	178	99	50	177	98	50
64	185	49	184	100	48	182	99	50	181	98	50
71	190	49	189	99	48	187	98	50	184	97	50
78	194	49	194	100	48	191	98	50	189	97	50
85	198	49	196	99	48	194	98	50	191	97	50
113	209	49	206	99	48	202	97	49	195	94	49
141	220	49	213	97	48	211	96	49	202	92	48
169	228	49	224	98	48	220	97	49	213	93	48
197	233	49	233	100	48	229	98	49	216	93	47
225	244	49	244	100	48	241	99	49	225	92	47
253	253	49	252	100	48	249	98	49	232	92	47
281	261	49	259	100	48	256	98	49	237	91	46
309	269	49	269	100	48	266	99	49	243	90	46
337	277	49	276	100	48	274	99	49	248	89	46
365	286	49	283	99	48	281	98	49	252	88	46
393	294	49	292	99	48	287	98	49	258	88	45
421	303	49	301	99	48	298	98	49	262	87	44
449	312	49	310	99	48	308	99	48	268	86	44
477	323	48	321	100	48	316	98	48	276	86	44
505	325	48	326	100	48	323	99	48	281	86	43
533	333	45	332	100	48	329	99	44	285	85	43
561	339	45	340	100	47	335	99	43	289	85	42
589	341	45	341	100	47	337	99	42	290	85	42
617	345	44	348	101	45	341	99	41	298	86	41
645	349	42	348	100	41	346	99	36	303	87	40
673	352	40	350	100	40	348	99	30	304	86	39
701	354	36	359	101	37	349	98	28	305	86	35
Mean for weeks											
1-13	160		161	101		159	99		158	99	
14-52	244		242	99		239	98		223	91	
53-101	327		327	100		323	99		282	86	

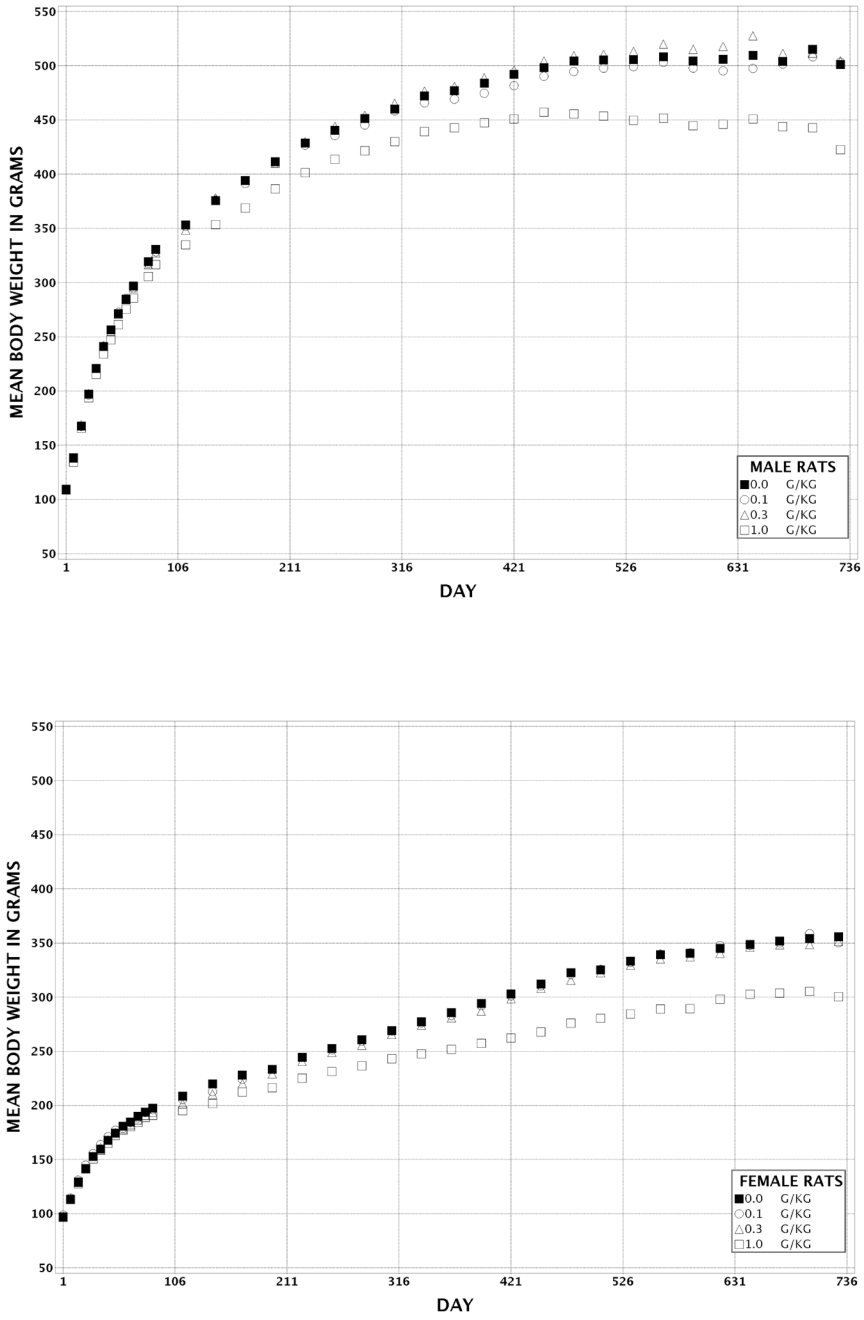


FIGURE 5
Growth Curves for Rats Administered Kava Kava Extract by Gavage for 2 Years

Clinical Pathology

The clinical chemistry data for rats in the 2-year study are presented in Tables 10 and F2. Similar to the 3-month study, changes in clinical chemistry data occurred. There were decreases in serum ALT, ALP and SDH activities in primarily the 0.3 and 1.0 g/kg male and female groups. The 1.0 g/kg male and female rats demonstrated multiple-fold increases in serum GGT activities. Additionally, bile salts, another marker of cholestasis, demonstrated increases. At all time points, there were minimal increases (<10%) in total protein and albumin (and by extrapolation, globulin) concentrations in the 1.0 g/kg male group; 1.0 g/kg females were less consistently affected. The dose-related increases in cholesterol concentrations that occurred in the 3-month study were reduced to an apparent transient effect here. The 0.3 and 1.0 g/kg males demonstrated a minimal increase in cholesterol at month 6 that disappeared by month 12. In the females, increased cholesterol concentrations occurred in all dosed groups at month 6, but by month 18 was increased in only the 1.0 g/kg group.

TABLE 10
Selected Clinical Chemistry Data for Rats in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Male				
n				
Month 6	10	10	10	10
Month 12	10	10	10	10
Month 18	10	9	10	10
Total protein (g/dL)				
Month 6	7.2 ± 0.1	7.3 ± 0.1	7.4 ± 0.1*	7.8 ± 0.1**
Month 12	7.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1	8.0 ± 0.1**
Month 18	7.2 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.6 ± 0.1**
Albumin (g/dL)				
Month 6	4.8 ± 0.0	4.9 ± 0.0	5.0 ± 0.1*	5.3 ± 0.1**
Month 12	5.1 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	5.4 ± 0.0**
Month 18	4.7 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.1**
Cholesterol (mg/dL)				
Month 6	94 ± 2	95 ± 2	102 ± 2*	108 ± 2**
Month 12	122 ± 3	118 ± 3	117 ± 4	128 ± 5
Month 18	121 ± 4	121 ± 5	125 ± 7	137 ± 7
Alanine aminotransferase (IU/L)				
Month 6	102 ± 7	81 ± 3*	67 ± 6**	55 ± 1**
Month 12	113 ± 8	88 ± 3*	79 ± 4**	72 ± 4**
Month 18	108 ± 8	103 ± 9	66 ± 4**	60 ± 2**
Alkaline phosphatase (IU/L)				
Month 6	234 ± 7	225 ± 5	212 ± 3*	203 ± 5**
Month 12	198 ± 7	204 ± 4	178 ± 4	187 ± 6
Month 18	194 ± 6	195 ± 5	174 ± 4*	145 ± 5**
Sorbitol dehydrogenase (IU/L)				
Month 6	25 ± 1	24 ± 1	20 ± 2**	15 ± 1**
Month 12	66 ± 5	60 ± 5	66 ± 9	50 ± 4
Month 18	35 ± 2	29 ± 3	26 ± 2**	26 ± 1**
γ-Glutamyltransferase (IU/L)				
Month 6	0.5 ± 0.2	0.0 ± 0.0*	0.1 ± 0.1	0.6 ± 0.2
Month 12	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.2
Month 18	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	1.1 ± 0.2**
Bile salts (μmol/L)				
Month 6	6.2 ± 1.2	7.4 ± 1.5	5.6 ± 1.2	5.6 ± 0.7
Month 12	3.8 ± 0.5	8.0 ± 1.6	4.5 ± 0.7	9.9 ± 1.6**
Month 18	9.4 ± 1.9	10.1 ± 2.4	9.5 ± 1.5	16.4 ± 3.5

TABLE 10
Selected Clinical Chemistry Data for Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Female				
n				
Month 6	10	10	10	10
Month 12	10	10	10	10
Month 18	10	10	10	8
Total protein (g/dL)				
Month 6	6.8 ± 0.1	7.1 ± 0.1	7.3 ± 0.1**	7.3 ± 0.1**
Month 12	7.3 ± 0.1	7.4 ± 0.1	7.7 ± 0.1*	7.5 ± 0.1
Month 18	7.6 ± 0.1	7.7 ± 0.3	7.8 ± 0.1	8.0 ± 0.1
Albumin (g/dL)				
Month 6	4.9 ± 0.1	5.1 ± 0.1	5.3 ± 0.1**	5.3 ± 0.1**
Month 12	5.1 ± 0.1	5.2 ± 0.1	5.5 ± 0.1*	5.4 ± 0.0
Month 18	5.2 ± 0.1	5.3 ± 0.2	5.5 ± 0.1	5.7 ± 0.1**
Cholesterol (mg/dL)				
Month 6	90 ± 3	99 ± 3*	106 ± 3**	128 ± 3**
Month 12	105 ± 2	112 ± 3	128 ± 5**	149 ± 3**
Month 18	111 ± 4	121 ± 5	120 ± 3	166 ± 6**
Alanine aminotransferase (IU/L)				
Month 6	66 ± 5	61 ± 5	48 ± 2**	45 ± 2**
Month 12	65 ± 2	58 ± 3*	54 ± 2**	50 ± 2**
Month 18	67 ± 2	66 ± 6	59 ± 2*	55 ± 2**
Alkaline phosphatase (IU/L)				
Month 6	231 ± 9	215 ± 8	197 ± 7*	184 ± 7**
Month 12	209 ± 5	186 ± 7	196 ± 6	136 ± 7**
Month 18	230 ± 7	232 ± 25	220 ± 8	149 ± 7**
Sorbitol dehydrogenase (IU/L)				
Month 6	15 ± 1	15 ± 1	13 ± 1	14 ± 1
Month 12	32 ± 2	25 ± 2*	28 ± 2	24 ± 2*
Month 18	23 ± 1	21 ± 1	21 ± 2	20 ± 1
γ-Glutamyltransferase (IU/L)				
Month 6	1.7 ± 0.2	2.2 ± 0.3	1.6 ± 0.3	4.3 ± 0.3**
Month 12	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.2	3.5 ± 0.6**
Month 18	0.5 ± 0.2	0.4 ± 0.3	0.5 ± 0.2	4.4 ± 0.7**
Bile salts (μmol/L)				
Month 6	7.1 ± 1.3	5.2 ± 0.9	4.1 ± 0.4	11.3 ± 1.8
Month 12	4.9 ± 0.5	6.6 ± 0.9	7.8 ± 0.7*	7.3 ± 0.9
Month 18	5.5 ± 0.8	10.9 ± 2.5*	11.6 ± 1.3**	15.4 ± 2.0**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the testis, liver, forestomach, kidney, eye, pancreas, parathyroid gland, bone marrow, pituitary gland, and mammary gland. 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.

Testis: In male rats, there were significant increases in the incidences of testicular interstitial (Leydig) cell adenoma in the 0.3 and 1.0 g/kg groups with significantly increased incidences of bilateral interstitial cell adenoma in the 0.3 and 1.0 g/kg groups compared to those in the vehicle controls (Tables 11, A1, and A2). The incidence of interstitial cell adenoma in 0.3 g/kg males exceeded the historical control range for corn oil gavage studies but not for all routes combined (Tables11 and A3a).

Interstitial cell adenoma was diagnosed when the diameter of an interstitial cell aggregate was equal to or exceeded the diameter of one seminiferous tubule and caused compression of the surrounding seminiferous tubules. Many adenomas were large, multilobular neoplasms that caused considerable compression and atrophy of the surrounding testicular tissue.

A significantly decreased incidence of interstitial cell hyperplasia occurred in 1.0 g/kg males compared to that in the vehicle controls; however, although the incidences decreased, the severities increased with increasing dose (Tables 11 and A4). As there is known continuum between interstitial cell hyperplasia and interstitial (Leydig) cell adenoma, hence, decrease in hyperplasia may be due to conversion into tumors in the testes. Interstitial cell hyperplasia was diagnosed when an interstitial cell aggregate was present but was less than the diameter of one seminiferous tubule. Severity scores were subjectively based on the size and number of the interstitial cell aggregates.

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Testis in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Number Examined Microscopically	49	50	50	50
Interstitial Cell, Hyperplasia[a]	17 (1.9)[b]	15 (2.1)	10 (2.4)	4**(2.5)
Bilateral Interstitial Cell Adenoma	29	32	40*	43**
Interstitial Cell Adenoma (includes bilateral)[c]				
Overall rate[d]	37/49 (76%)	44/50 (88%)	49/50 (98%)	46/50 (92%)
Adjusted rate[e]	80.2%	92.9%	98.3%	99.0%
Terminal rate[f]	28/34 (82%)	32/35 (91%)	34/34 (100%)	31/31 (100%)
First incidence (days)	545	571	454	454
Poly-3 test[g]	P=0.003	P=0.056	P=0.002	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 176/199 (88.4% ± 8.6%), range 76%-94%; all routes: 1,053/1,298 (81.1% ± 13.4%), range 54%-98%
[d] Number of animals with neoplasm per number of animals with testis examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] 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 differential mortality in animals that do not reach terminal sacrifice.

Liver: The incidences of hepatocellular hypertrophy in 1.0 g/kg males and females were significantly greater than those in the vehicle controls (Tables 12, A4, and B4). Significantly increased incidences of centrilobular fatty change occurred in 0.1 and 1.0 g/kg males, and two incidences of centrilobular fatty change occurred in 1.0 g/kg females. Cystic degeneration was observed in all dosed groups of males, and the incidence in 1.0 g/kg males was significantly increased. One 1.0 g/kg female had cystic degeneration.

Microscopically, hypertrophy consisted of an irregular increase in the size of hepatocytes, usually in a centrilobular distribution (Plates 1 and 2). Centrilobular fatty change consisted of poorly demarcated areas of hepatocytes with large, clear cytoplasmic vacuoles, usually in the centrilobular and midzonal regions. Cystic degeneration consisted of multilocular cystic areas containing a finely granular or flocculent eosinophilic material, apparently resulting from the distention and occasional rupture of adjacent hepatocytes.

Forestomach: The incidences of inflammation, ulcer, and epithelial hyperplasia were significantly increased in 1.0 g/kg males and females, and the incidences tended to increase in a dose-related manner (Tables 12, A4, and B4). Erosion was observed in one 0.3 g/kg male and four 1.0 g/kg males.

Microscopically, erosion consisted of loss of the mucosal epithelial cells, with intact muscularis mucosa. Ulcer consisted of a loss of the stratified squamous epithelium and exposure of the lamina muscularis. The ulcers were usually accompanied by peripheral hyperplasia and underlying inflammation (Plate 3). Hyperplasia of the forestomach epithelium consisted of a focal, poorly demarcated thickening of the normal stratified squamous epithelium due to an increase in the number of epithelial cells present; the mucosa was occasionally thrown into small convolutions. Inflammation of the forestomach consisted of a thickening of the submucosa by an admixture of inflammatory cells (predominantly lymphocytes and macrophages, with fewer plasma cells and neutrophils) and lightly proteinaceous fluid.

Kidney: The severity of nephropathy generally increased with increasing dose in male rats, and the incidence in 1.0 g/kg females was significantly greater than that in the vehicle control group (Tables 12, A4, and B4). Incidences of transitional epithelial hyperplasia of the renal pelvis were significantly increased in 1.0 g/kg males and 0.3 and 1.0 g/kg females.

Nephropathy consisted of focal to multifocal regenerative renal tubules surrounded by a thickened basement membrane, glomerular thickening, tubular protein casts and chronic inflammatory infiltrates with fibrosis (Plate 4). Increased severity of nephropathy is usually associated with focal to diffuse thickening of the urothelium (Montgomery and Seely, 1990). Transitional epithelial hyperplasia may be focal or diffuse, is several cell layers thick in the transitional epithelium, and is located at the junction of the papilla and the pelvis. This change is commonly seen in the renal pelvis with severe nephropathy (Montgomery and Seely, 1990).

Eye: The incidences of retinal degeneration were significantly increased in 1.0 g/kg males and females (Tables 12, A4, and B4). The degeneration consisted of a thinning and loss of the external retinal layers, such as the photoreceptors and external nuclear layers, with a decreased cellularity and disorganization of the remaining retinal layers.

Pancreas: The incidences of metaplasia of pancreatic acinar cells to a hepatocytic morphology increased in 1.0 g/kg males and females, and the increase in males was significant (Tables 12, A4, and B4). Microscopically, this lesion was characterized by the presence of small clusters of apparently normal hepatocytes adjacent to islets of Langerhans (Plate 5). This lesion has been noted as a spontaneous background change, as well as due to methyl-deficient diets, and is thought to be related to trans-differentiation of pancreatic acinar cells due to hypomethylation of DNA (Hoover and Poirer, 1986).

Parathyroid Gland: The incidence of hyperplasia was significantly increased in 1.0 g/kg males (Tables 12 and A4). Microscopically, hyperplasia consisted of a poorly demarcated focal area of increased cellularity without significant adjacent compression. This lesion is likely secondary to nephropathy (Montgomery and Seely, 1990).

Bone Marrow: The incidence of hyperplasia of the bone marrow was significantly increased in 1.0 g/kg males (vehicle control, 16/49; 0.1 g/kg, 17/50; 0.3 g/kg, 24/50; 1.0 g/kg, 29/50; Table A4). Hyperplasia consisted of an increase in the normal active hematopoietic tissue, filling the marrow cavity to the exclusion of adipose tissue. Both myeloid and erythroid cell series were affected; megakaryocytes were often increased as well.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control		0.1 g/kg		0.3 g/kg		1.0 g/kg	
Male								
Liver[a]	49		50		50		50	
Hepatocyte, Hypertrophy[b]	0		2	(1.0)[c]	2	(1.0)	22**	(1.4)
Centrilobular, Fatty Change	1	(2.0)	7*	(1.3)	4	(1.3)	21**	(1.7)
Degeneration, Cystic	0		4	(1.0)	3	(1.3)	5*	(1.2)
Stomach, Forestomach	49		50		50		50	
Inflammation	8	(2.3)	4	(1.5)	9	(2.8)	22**	(2.0)
Ulcer	4	(2.5)	0		6	(3.0)	13*	(1.6)
Epithelium, Hyperplasia	6	(2.2)	4	(1.5)	11	(2.5)	27**	(2.3)
Erosion	0		0		1	(2.0)	4	(1.0)
Kidney	49		50		50		50	
Nephropathy	46	(1.4)	47	(1.2)	48	(1.8)	48	(3.1)
Pelvis, Transitional Epithelium, Hyperplasia	0		1	(1.0)	1	(2.0)	15**	(1.3)
Eye	49		50		50		50	
Retina, Degeneration	6	(1.5)	6	(1.0)	10	(1.5)	16**	(1.6)
Pancreas	49		50		50		50	
Acinus, Metaplasia, Hepatocyte	0		0		0		6*	(1.5)
Parathyroid Gland	49		50		48		50	
Hyperplasia	1	(2.0)	0		1	(1.0)	10**	(1.8)
Female								
Liver	50		50		50		50	
Hepatocyte, Hypertrophy	5	(1.6)	2	(1.0)	3	(1.3)	33**	(1.8)
Centrilobular, Fatty Change	0		0		0		2	(2.0)
Degeneration, Cystic	0		0		0		1	(1.0)
Stomach, Forestomach	49		50		50		50	
Inflammation	5	(2.0)	7	(1.7)	7	(2.1)	13*	(1.8)
Ulcer	1	(3.0)	1	(4.0)	3	(2.3)	7*	(2.3)
Epithelium, Hyperplasia	5	(2.2)	6	(2.3)	8	(1.8)	19**	(1.9)
Kidney	50		50		50		50	
Nephropathy	34	(1.0)	35	(1.0)	37	(1.1)	43**	(1.2)
Pelvis, Transitional Epithelium, Hyperplasia	0		0		4*	(1.5)	6*	(1.2)
Eye	50		50		50		50	
Retina, Degeneration	5	(2.6)	5	(3.0)	5	(2.4)	12*	(1.7)
Pancreas	49		50		50		50	
Acinus, Metaplasia, Hepatocyte	0		1	(1.0)	0		4	(1.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[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

Pituitary Gland (Pars Distalis): The incidences of adenoma occurred with negative trends in males and females, and the incidences in 1.0 g/kg males and 0.1 and 1.0 g/kg females were significantly less than in the vehicle controls (Tables 13, A1, A2, B1, and B2). These decreases are greater than can be expected from reduced body weight and survival (Haseman *et al.*, 1998). The incidences in all groups of males were within the historical control ranges for corn oil gavage studies and for all routes combined, the incidences in all dosed groups of females were less than the historical control range for corn oil gavage studies, and the incidence in 1.0 g/kg females was below the historical range for all routes of study (Tables 13, A3b, and B3a). The adenoma consisted of a focal area of increased cellularity by a homogeneous population, with peripheral compression and possible mitotic figures or cellular atypia.

Mammary Gland: There was a negative trend in the incidences of fibroadenoma in females, and the incidence in the 1.0 g/kg group was significantly less than that in the vehicle control group (Tables 14, B1, and B2). The decreased incidences of fibroadenoma are greater than can be expected from reduced body weight and survival (Haseman *et al.*, 1998). The incidences in the 0.3 and 1.0 g/kg groups were below the historical control range for corn oil gavage studies, and the incidence in the 1.0 g/kg group was below the range for all routes combined (Tables 14 and B3b). Fibroadenomas are the most common benign neoplasm of the mammary gland in F344/N rats and it consists of both ductular and/or alveolar epithelium and fibrous connective tissue. The epithelium generally is uniform and single-layered. The alveoli and ductules are surrounded by prominent mature connective tissue.

TABLE 13
Incidences of Adenoma of the Pituitary Gland (Pars Distalis) in Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Male				
Adenoma[a]				
Overall rate[b]	21/49 (43%)	20/50 (40%)	15/50 (30%)	8/50 (16%)
Adjusted rate[c]	44.9%	44.2%	32.2%	18.6%
Terminal rate[d]	14/34 (41%)	18/35 (51%)	9/34 (27%)	4/31 (13%)
First incidence (days)	504	525	590	447
Poly-3 test[e]	P=0.003N	P=0.557N	P=0.146N	P=0.006N
Female				
Adenoma[f]				
Overall rate	29/50 (58%)	20/50 (40%)	24/50 (48%)	8/50 (16%)
Adjusted rate	63.0%	43.7%	54.5%	19.1%
Terminal rate	20/34 (59%)	15/34 (44%)	12/24 (50%)	6/34 (18%)
First incidence (days)	615	558	518	664
Poly-3 test	P<0.001N	P=0.046N	P=0.267N	P<0.001N

[a] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 57/199 (28.7% ± 14.3%), range 12%-43%; all routes: 604/1,293 (46.8% ± 20.6%), range 12%-74%

[b] Number of animals with neoplasm per number of animals with pituitary gland examined microscopically

[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 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] Historical incidence for corn oil gavage studies: 106/200 (53.0% ± 3.8%), range 50%-58%; all routes: 682/1,247 (54.7% ± 11.3%), range 32%-73%

TABLE 14
Incidences of Fibroadenoma of the Mammary Gland in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Number Necropsied	50	50	50	50
Fibroadenoma, Multiple[a]	10	7	2*	0**
Fibroadenoma (includes multiple)[b]				
Overall rate[c]	24/50 (48%)	24/50 (48%)	15/50 (30%)	4/50 (8%)
Adjusted rate[d]	53.0%	52.6%	35.7%	9.6%
Terminal rate[e]	20/34 (59%)	19/34 (56%)	8/24 (33%)	3/34 (9%)
First incidence (days)	450	615	615	688
Poly-3 test[f]	P<0.001N	P=0.570N	P=0.075N	P<0.001N

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 88/200 (44.0% ± 7.8%), range 34%-52%; all routes: 655/1,250 (52.4% ± 14.0%), range 28%-86%
[c] Number of animals with neoplasm per number of animals necropsied
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] 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 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**.

MICE
2-WEEK STUDY

In the 2.0 g/kg group of males, one mouse died on day 2 and one died on day 3 (Table 15); the cause of death was not determined. The final mean body weights and mean body weight gains of all dosed groups of mice were similar to those of the vehicle controls. Clinical findings included abnormal breathing, ataxia, and lethargy in males and females in the 1.0 and 2.0 g/kg groups. No gross lesions were observed.

Absolute liver weights were significantly increased in 2.0 g/kg males and females compared to the vehicle controls; relative liver weights were significantly increased in the 1.0 and 2.0 g/kg males and in 0.25 g/kg or greater females (Table G3).

The incidence of hepatocellular hypertrophy in 2.0 g/kg female mice was significantly greater than that in the vehicle control group (vehicle control, 0/5; 0.125 g/kg, 0/0; 0.25 g/kg, 0/0; 0.5 g/kg, 0/0; 1.0 g/kg, 0/5; 2.0 g/kg, 5/5).

Dose Selection Rationale: There were two deaths in male mice during first 3 days of the 2-week study, most likely due to gavaging errors. In the female mice, there were no adverse effects on survival. The mean body weights of dosed groups were comparable to the vehicle controls. The effects on liver weights and histology were considered minimal. Therefore, doses of 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg were selected for the 3-month gavage study in mice.

TABLE 15
Survival and Body Weights of Mice in the 2-Week Gavage Study of Kava Kava Extract[a]

Dose (g/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	21.5 ± 0.4	23.1 ± 0.6	1.7 ± 0.2	
0.125	5/5	21.2 ± 0.2	22.4 ± 0.8	1.3 ± 0.5	97
0.25	5/5	21.1 ± 0.4	23.1 ± 0.6	2.0 ± 0.2	100
0.5	5/5	21.1 ± 0.5	22.2 ± 0.6	1.1 ± 0.2	96
1.0	5/5	21.1 ± 0.4	22.9 ± 0.4	1.8 ± 0.1	99
2.0	3/5[c]	21.3 ± 0.5	22.4 ± 0.4	1.6 ± 0.2	97
Female					
0	5/5	18.0 ± 0.3	20.3 ± 0.3	2.3 ± 0.1	
0.125	5/5	18.1 ± 0.5	19.5 ± 0.7	1.4 ± 0.4	96
0.25	5/5	17.9 ± 0.5	19.2 ± 0.8	1.3 ± 0.4	94
0.5	5/5	17.7 ± 0.5	19.5 ± 0.6	1.8 ± 0.3	96
1.0	5/5	17.7 ± 0.3	20.0 ± 0.4	2.3 ± 0.2	99
2.0	5/5	18.2 ± 0.3	19.6 ± 0.5	1.4 ± 0.6	96

[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 in weights and weight changes are not significant by Dunnett's test.
[b] Number of animals surviving at 17 days/number initially in group
[c] Days of death: 2, 3

3-MONTH STUDY

Four male and three female 2.0 g/kg mice died during week 1; these deaths were attributed to kava kava extract administration (Table 16). One additional female died during week 6 due to a gavage accident. The final mean body weights and mean body weight gains of dosed males and females were similar to those of the vehicle controls (Table 16 and Figure 6). Ataxia and lethargy were observed in males and females in the 1.0 and 2.0 g/kg groups during week 1. No chemical-related gross lesions were seen in early death or terminal sacrifice mice.

TABLE 16
Survival and Body Weights of Mice in the 3-Month Gavage Study of Kava Kava Extract[a]

Dose (g/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	23.3 ± 0.4	34.4 ± 0.9	11.2 ± 0.6	
0.125	10/10	23.4 ± 0.3	35.5 ± 1.0	12.1 ± 0.8	103
0.25	10/10	23.3 ± 0.4	35.4 ± 0.9	12.1 ± 0.9	103
0.5	10/10	23.0 ± 0.4	36.0 ± 1.0	13.0 ± 0.8	105
1.0	10/10	23.3 ± 0.4	35.3 ± 0.9	12.1 ± 0.7	103
2.0	6/10[c]	23.5 ± 0.3	32.4 ± 0.4	9.0 ± 0.5	94
Female					
0	10/10	19.0 ± 0.3	28.1 ± 0.9	9.2 ± 0.7	
0.125	10/10	18.8 ± 0.3	28.5 ± 0.4	9.7 ± 0.5	101
0.25	10/10	18.5 ± 0.3	27.7 ± 0.8	9.2 ± 0.6	99
0.5	10/10	18.9 ± 0.3	29.1 ± 0.3	10.2 ± 0.3	104
1.0	10/10	19.0 ± 0.2	27.1 ± 0.8	8.1 ± 0.7	96
2.0	6/10[d]	18.8 ± 0.2	27.0 ± 0.5	8.0 ± 0.3	96

[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 in weights and weight changes are not significant by Dunnett's test.
[b] Number of animals surviving at 3 months/number initially in group
[c] Weeks of death: 1, 1, 1, 1
[d] Weeks of death: 1, 1, 1, 6

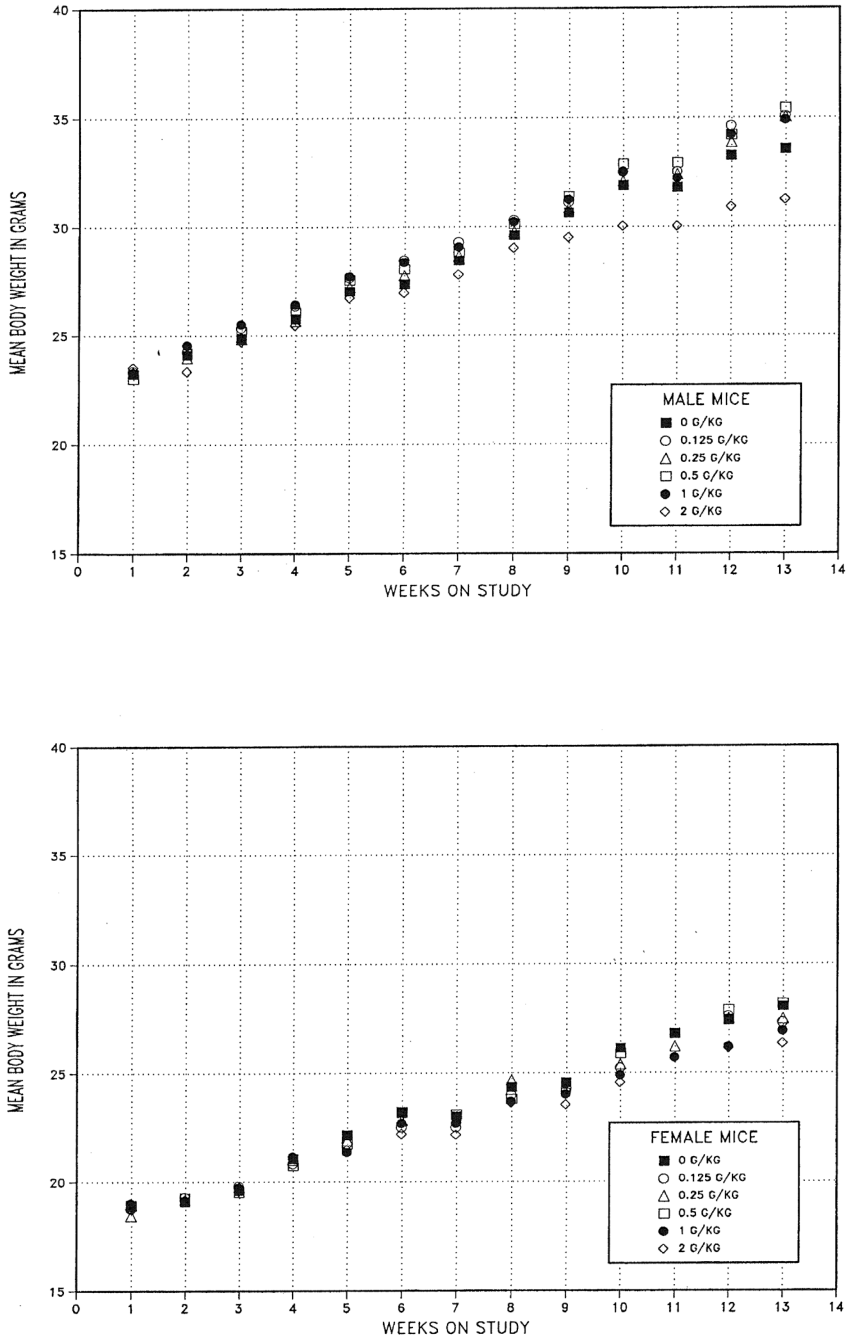


FIGURE 6
Growth Curves for Mice Administered Kava Kava Extract by Gavage for 3 Months

There were no changes in the hematology data of mice that were considered attributable to kava kava extract administration (Table F3).

The absolute liver weights of 2.0 g/kg males and 0.5 g/kg or greater females and the relative liver weights of 1.0 and 2.0 g/kg males and females were significantly increased compared to those of the vehicle control groups (Table G4). These increased liver weights were consistent with the hepatocellular hypertrophy observed microscopically. The absolute and relative kidney weights of 2.0 g/kg females were also significantly increased; however, no corresponding microscopic change was found.

There were no significant differences in sperm parameters of male mice or the estrous cyclicity of female mice administered 0.25, 0.5, or 1.0 g/kg kava kava extract when compared to the vehicle controls (Tables H3 and H4).

The incidences of centrilobular hypertrophy in the liver of 0.5 g/kg or greater males and 1.0 and 2.0 g/kg females were significantly greater than those in the vehicle controls (Table 17). The no-observed-effect level for this lesion was 0.25 g/kg in males and females. The severity of centrilobular hypertrophy was minimal to moderate. The lesion consisted of enlarged hepatocytes, primarily located in the centrilobular regions, and was characterized by increased hepatocellular size and ground-glass cytoplasmic eosinophilia and decreased cytoplasmic glycogen content.

Thymic necrosis was noted in three of the four 2.0 g/kg males and in two of the four 2.0 g/kg females that died before the end of the study; these incidences were considered to be related to stress.

Dose Selection Rationale: In the 3-month study, there was decreased survival in 2.0 g/kg male and female mice. The final mean body weights of dosed mice were similar to those of the vehicle controls. Mild liver hypertrophy in the 1.0 g/kg groups was not considered to be dose-limiting. Therefore, doses of 0.25, 0.5, and 1.0 g/kg were selected for the 2-year gavage study in mice.

TABLE 17
Incidences of Nonneoplastic Lesions of the Liver in Mice in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Centrilobular, Hypertrophy[a]	0	0	0	9** (1.0)[b]	10** (2.0)	7** (2.6)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Centrilobular, Hypertrophy	0	0	0	2 (1.0)	5* (1.0)	4* (1.5)

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

2-YEAR STUDY

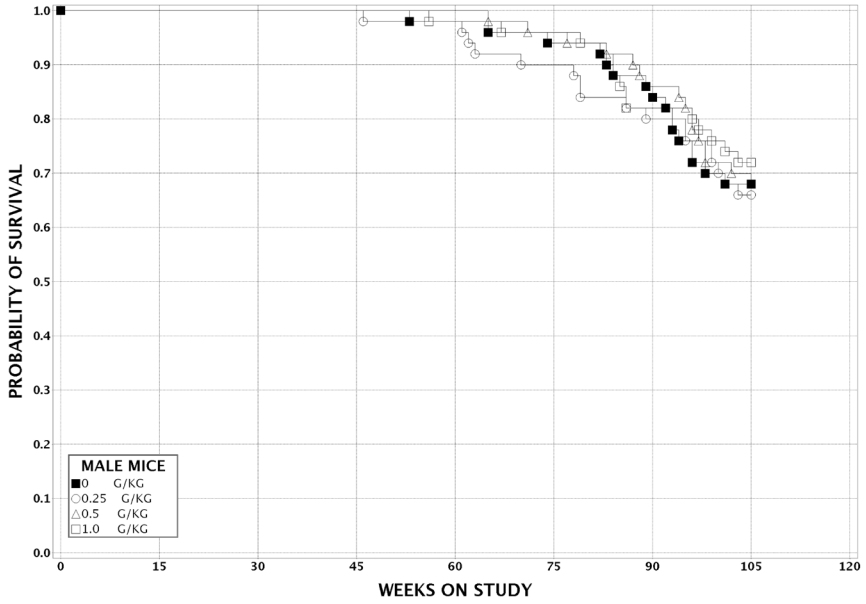
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 18 and in the Kaplan-Meier survival curves (Figure 7). Survival of dosed groups of males and females was similar to that of the vehicle controls.

TABLE 18
Survival of Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Male				
Animals initially in study	50	50	50	50
Moribund	3	10	10	7
Natural deaths	13	7	5	7
Animals surviving to study termination	34[a]	33	35[a]	36
Percent probability of survival at end of study[b]	68	66	68	72
Mean survival (days)[c]	689	678	697	693
Survival analysis[d]	P=0.629N	P=0.956	P=1.000	P=0.798N
Female				
Animals initially in study	50	50	50	50
Accidental deaths[e]	1	0	0	1
Moribund	4	4	1	7
Natural deaths	7	12	4	5
Animals surviving to study termination	38	34	45	37
Percent probability of survival at end of study	78	68	90	76
Mean survival (days)	695	704	716	695
Survival analysis	P=0.796N	P=0.481	P=0.159N	P=1.000

[a] Includes one animal that died during the last week of the study
[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. A negative trend or lower mortality in a dose group is indicated by **N**.
[e] Censored from survival analyses



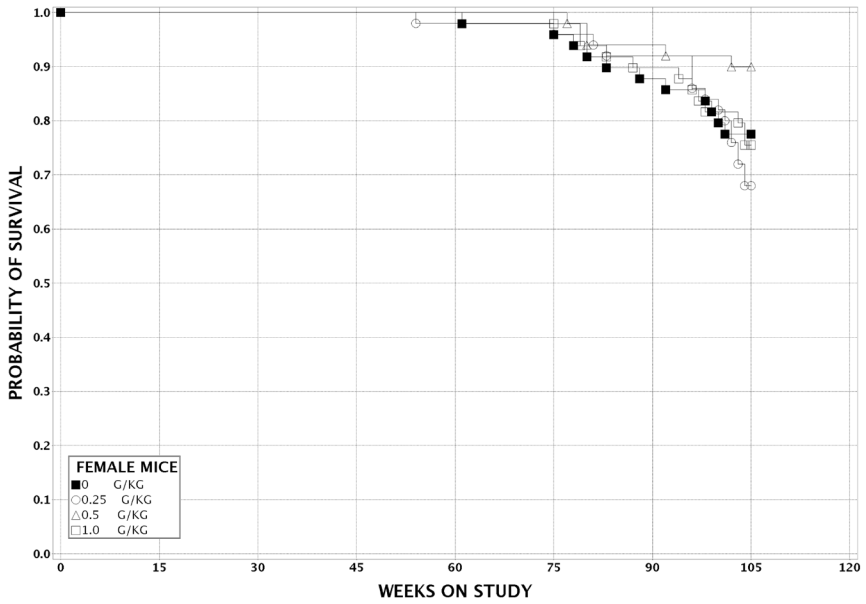
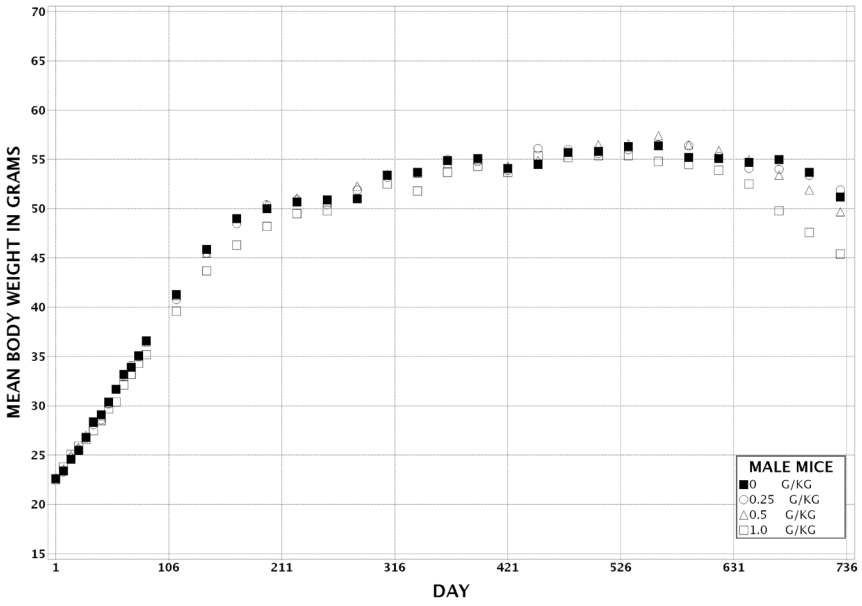


FIGURE 7
Kaplan-Meier Survival Curves for Mice Administered Kava Kava Extract by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of males administered 1.0 g/kg were generally similar to those of the vehicle controls until the end of the study; those of 1.0 g/kg females were less than those of the vehicle controls after week 21 (Figure 8 and Tables 19 and 20). Clinical findings included ataxia and lethargy that occurred in 13 males and 31 females in the 1.0 g/kg groups during the first week of the study. Fewer animals exhibited ataxia or lethargy during the remainder of the study, but these findings were observed in 1.0 g/kg females as late as week 101.



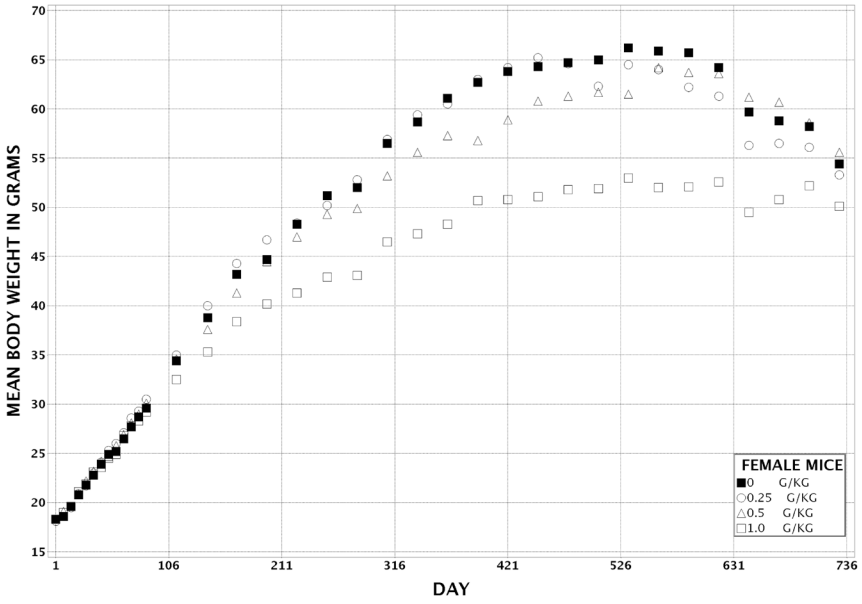


FIGURE 8
Growth Curves for Mice Administered Kava Kava Extract by Gavage for 2 Years

TABLE 19
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control		0.25 g/kg			0.5 g/kg			1.0 g/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	22.6	50	22.5	100	50	22.7	101	50	22.5	100	50
8	23.4	50	23.3	100	50	23.6	101	50	23.8	102	50
15	24.6	50	24.7	100	50	25.0	102	50	25.1	102	50
22	25.5	50	25.6	101	50	25.8	101	50	25.9	102	50
29	26.8	50	26.7	100	50	27.0	101	50	26.6	99	50
36	28.4	50	28.1	99	50	28.3	100	50	27.5	97	50
43	29.1	50	28.6	98	50	29.0	100	50	28.5	98	50
50	30.4	50	30.2	100	50	30.3	100	50	29.7	98	50
57	31.7	50	31.7	100	50	31.7	100	50	30.4	96	50
64	33.2	50	33.1	100	50	33.0	99	50	32.1	97	50
71	33.9	50	34.1	101	50	34.0	100	50	33.2	98	50
78	35.1	50	35.0	100	50	35.0	100	50	34.3	98	50
85	36.6	50	36.5	100	50	36.5	100	50	35.2	96	50
113	41.3	50	40.8	99	50	41.2	100	50	39.6	96	50
141	45.9	50	45.5	99	50	45.5	99	50	43.7	95	50
169	49.0	50	48.5	99	50	49.0	100	50	46.3	94	50
197	50.0	50	50.4	101	50	50.4	101	50	48.2	96	50
225	50.7	50	50.9	101	50	51.1	101	50	49.5	98	50
253	50.9	50	50.4	99	50	50.8	100	50	49.8	98	50
281	51.0	50	51.9	102	50	52.3	103	50	51.0	100	50
309	53.4	50	53.2	100	50	53.5	100	50	52.5	98	50
337	53.7	50	53.6	100	49	53.6	100	50	51.8	96	50
365	54.9	50	55.0	100	49	54.6	100	50	53.7	98	50
393	55.1	49	54.8	100	49	55.0	100	50	54.3	99	49
421	54.1	49	53.9	100	49	54.3	100	50	53.7	99	49
449	54.5	49	56.1	103	46	54.9	101	50	55.4	102	49
477	55.7	48	56.0	101	46	55.8	100	49	55.2	99	48
505	55.8	48	55.6	100	45	56.5	101	48	55.4	99	48
533	56.3	47	56.0	100	45	56.6	101	48	55.4	99	48
561	56.4	47	56.5	100	42	57.4	102	47	54.8	97	47
589	55.2	44	56.4	102	42	56.5	102	46	54.5	99	44
617	55.1	43	55.3	100	41	55.9	101	44	53.9	98	41
645	54.7	41	54.1	99	40	55.0	101	43	52.5	96	41
673	55.0	36	54.0	98	38	53.4	97	39	49.8	91	39
701	53.7	35	53.4	99	35	51.9	97	36	47.6	89	38
Mean for weeks											
1-13	29.3		29.2	100		29.4	100		28.8	98	
14-52	49.5		49.5	100		49.7	100		48.0	97	
53-101	55.1		55.2	100		55.2	100		53.6	97	

TABLE 20
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control		0.25 g/kg			0.5 g/kg			1.0 g/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	18.3	50	18.1	99	50	18.3	100	50	18.3	100	50
8	18.6	50	18.6	100	50	19.1	103	50	19.0	102	50
15	19.6	50	19.5	100	50	19.6	100	50	19.6	100	50
22	20.8	50	20.9	100	50	20.9	100	50	21.1	101	50
29	21.8	50	21.7	100	50	22.2	102	50	21.9	101	50
36	22.8	50	23.0	101	50	23.2	102	50	23.1	101	50
43	23.9	50	24.1	101	50	24.2	101	50	23.6	99	50
50	24.9	50	25.3	102	50	24.7	100	50	24.5	99	50
57	25.2	50	26.0	103	50	25.8	102	50	24.9	99	50
64	26.5	50	27.1	102	50	26.9	102	50	26.8	101	50
71	27.7	50	28.6	103	50	28.1	102	50	27.8	101	50
78	28.7	50	29.3	102	50	29.0	101	50	28.3	99	50
85	29.6	50	30.5	103	50	30.1	102	50	29.2	99	50
113	34.4	50	35.0	102	50	34.6	101	50	32.5	94	50
142	38.8	50	40.0	103	50	37.6	97	50	35.3	91	50
169	43.2	50	44.3	103	50	41.3	96	50	38.4	89	49
197	44.7	50	46.7	104	50	44.5	99	50	40.2	90	49
225	48.3	50	48.4	100	50	47.0	98	50	41.3	86	49
253	51.2	50	50.2	98	50	49.3	96	50	42.9	84	49
281	52.0	50	52.8	102	50	49.9	96	50	43.1	83	49
309	56.5	50	56.9	101	50	53.2	94	50	46.5	82	49
337	58.7	50	59.4	101	50	55.6	95	50	47.3	81	49
365	61.1	50	60.5	99	50	57.3	94	50	48.3	79	49
393	62.7	50	63.0	101	49	56.8	91	50	50.7	81	49
421	63.8	48	64.2	101	49	58.9	92	50	50.8	80	49
449	64.3	48	65.2	101	49	60.8	95	50	51.1	80	49
477	64.7	48	64.6	100	49	61.3	95	50	51.8	80	49
505	65.0	48	62.3	96	49	61.7	95	50	51.9	80	49
533	66.2	47	64.5	98	48	61.5	93	50	53.0	80	48
561	65.9	45	64.0	97	48	64.2	97	47	52.0	79	46
589	65.7	44	62.2	95	46	63.7	97	47	52.1	79	45
617	64.2	43	61.3	96	46	63.6	99	47	52.6	82	44
645	59.7	42	56.3	94	46	61.2	103	46	49.5	83	44
673	58.8	42	56.5	96	43	60.7	103	46	50.8	86	42
701	58.2	38	56.1	96	40	58.6	101	46	52.2	90	40
Mean for weeks											
1-13	23.7		24.1	102		24.0	101		23.7	100	
14-52	47.5		48.2	101		45.9	97		40.8	86	
53-101	63.1		61.6	98		60.8	96		51.3	81	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms and/or nonneoplastic lesions of the liver and forestomach. 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 C for male mice and Appendix D for female mice.

Liver: The incidences of hepatocellular adenoma (multiple) were significantly increased in 1.0 g/kg males and 0.5 g/kg females compared to those in the vehicle controls (Tables 21, C1, and D1). The incidences of hepatoblastoma in 0.5 and 1.0 g/kg males were significantly increased compared to the vehicle controls (Tables 21, C1, and C2). The incidences of hepatoblastoma in 0.5 and 1.0 g/kg males and hepatocellular adenoma in all dosed groups of males exceeded the historical control ranges for corn oil gavage studies but not for all routes combined (Tables 21 and C3a). Incidences of hepatocellular carcinoma were increased in all dosed groups of females, and the increase was significant in the 0.25 g/kg group (Tables 21, D1, and D2). The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in 0.25 and 0.5 g/kg females. The incidences of hepatocellular carcinoma in all dosed groups of females and hepatocellular adenoma or carcinoma (combined) in 0.25 and 0.5 g/kg females exceeded the historical range for corn oil gavage studies but not for all routes combined (Tables 21 and D3). The incidence of hepatocellular carcinoma or hepatoblastoma (combined) was significantly increased in 0.5 g/kg males compared to the vehicle controls (Tables 21 and C2); incidences of these combined neoplasms exceeded the historical ranges for corn oil gavage studies for all dosed groups of males and the incidence in the 0.5 g/kg group exceeded the historical range for all routes combined (Tables 21 and C3a). One female in the 0.5 g/kg group had a hepatoblastoma (Tables 21 and D1).

Increased incidences of metastatic hepatocellular carcinoma to the lungs were observed in all dosed groups of males compared to the vehicle controls with the greatest increase in the 1.0 g/kg group (vehicle control, 5/50; 0.25 g/kg, 7/50; 0.5 g/kg, 7/50; 1.0 g/kg, 10/50; Table C1). Increased incidences of metastatic hepatocellular carcinoma to the lungs were also observed in 0.25 and 0.5 g/kg females (1/50, 4/50, 2/50, 1/50; Table D1).

The incidences of centrilobular hypertrophy in all dosed groups of males and females were significantly increased, and the severities of the lesion tended to increase with increasing dose (Tables 21, C4, and D4). Significantly increased incidences of eosinophilic focus occurred in 0.5 g/kg males and in 1.0 g/kg males and females. The incidences of angiectasis increased in a dose-related manner in males, and the increase in the 1.0 g/kg group was significant. The incidences of hepatocellular necrosis were significantly increased in 0.25 and 1.0 g/kg males.

Histologically, hepatocellular adenomas were variably sized, nodular lesions composed of well-differentiated, neoplastic hepatocytes that typically compressed the adjacent hepatic parenchyma (Plate 6). Portal areas and central veins were typically absent, and mild cellular atypia was often present. Hepatocellular carcinomas were variably well demarcated from the surrounding hepatic parenchyma and were composed of neoplastic hepatocytes that displayed mild to marked cellular and nuclear pleomorphism and mitoses. The predominant pattern displayed by most neoplasms in this study was trabecular, although focal areas had glandular or solid patterns of growth (Plates 7 and 8). Necrosis was occasionally quite extensive, and metastasis to the lung was frequently observed. Hepatoblastomas tended to arise within hepatocellular adenomas or carcinomas and were composed of small, basophilic fusiform cells with a high nucleus to cytoplasm ratio (Plates 9 and 10). Mitoses, large, irregularly shaped cystic areas filled with blood, and areas of necrosis were common.

Centrilobular hypertrophy was characterized by enlargement of centrilobular hepatocytes with increased amounts of eosinophilic cytoplasm and enlarged nuclei (Plates 11 and 12). This lesion was often variable in its presence and severity between lobes and within regions of the same lobe. Eosinophilic foci consisted of well-differentiated hepatocytes containing increased amounts of eosinophilic cytoplasm. Portal areas and central veins were often present, and minimal compression of the adjacent parenchyma occurred occasionally. Hepatocellular necrosis was characterized by focal, widely scattered, randomly distributed areas of necrosis of hepatocytes often infiltrated by small numbers of mixed inflammatory cells. Necrosis was not diagnosed when it was deemed to be secondary to neoplasia. Angiectasis was characterized by variably sized dilatations of the hepatic sinusoids, typically occurring in small clusters randomly arranged throughout the hepatic parenchyma and without a sub-anatomic orientation. The sinusoids were lined by an attenuated to unapparent endothelium.

TABLE 21
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Male				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hypertrophy[a]	0	34** (1.0)[b]	30** (2.0)	39** (2.0)
Eosinophilic Focus	28	32	42**	43**
Angiectasis	3 (1.0)	6 (1.0)	7 (1.1)	10* (1.7)
Necrosis	3 (1.7)	10* (2.0)	7 (2.0)	13** (2.0)
Hepatocellular Adenoma, Multiple	13	19	19	23*
Hepatocellular Adenoma (includes multiple)[c]	27	32	29	35
Hepatocellular Carcinoma, Multiple	4	3	7	5
Hepatocellular Carcinoma (includes multiple)[d]	20	18	26	20
Hepatoblastoma, Multiple	0	0	2	3
Hepatoblastoma (includes multiple)[e]				
Overall rate[f]	0/50 (0%)	4/50 (8%)	9/50 (18%)	12/50 (24%)
Adjusted rate[g]	0.0%	9.4%	20.1%	26.4%
Terminal rate[h]	0/34 (0%)	2/33 (6%)	8/34 (24%)	8/36 (22%)
First incidence (days)	—[i]	687	679	582
Poly-3 test[j]	P<0.001	P=0.057	P=0.002	P<0.001
Hepatocellular Carcinoma or Hepatoblastoma (includes multiple)[k]				
Overall rate	20/50 (40%)	21/50 (42%)	30/50 (60%)	25/50 (50%)
Adjusted rate	42.7%	46.8%	61.7%	53.3%
Terminal rate	11/34 (32%)	14/33 (42%)	19/34 (56%)	16/36 (44%)
First incidence (days)	455	431	455	574
Poly-3 test	P=0.136	P=0.426	P=0.046	P=0.205

TABLE 21
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Female				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hypertrophy	0	20** (1.0)	48** (1.9)	49** (2.0)
Eosinophilic Focus	9	7	16	26**
Hepatocellular Adenoma, Multiple	0	4	6*	1
Hepatocellular Adenoma (includes multiple)[l]	8	11	14	5
Hepatocellular Carcinoma, Multiple	1	1	1	0
Hepatocellular Carcinoma (includes multiple)[m]				
Overall rate	3/50 (6%)	13/50 (26%)	8/50 (16%)	8/50 (16%)
Adjusted rate	6.7%	28.1%	16.5%	17.2%
Terminal rate	3/38 (8%)	9/34 (27%)	6/45 (13%)	3/37 (8%)
First incidence (days)	729 (T)	701	534	604
Poly-3 test	P=0.337	P=0.007	P=0.126	P=0.109
Hepatocellular Adenoma or Carcinoma (includes multiple)[n]				
Overall rate	10/50 (20%)	21/50 (42%)	20/50 (40%)	13/50 (26%)
Adjusted rate	22.1%	45.1%	41.2%	28.0%
Terminal rate	9/38 (24%)	16/34 (47%)	18/45 (40%)	8/37 (22%)
First incidence (days)	560	669	534	604
Poly-3 test	P=0.542	P=0.015	P=0.036	P=0.338
Hepatoblastoma	0	0	1	0

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 121/250 (48.4% ± 4.6%), range 44%-54%; all routes: 684/1,248 (54.8% ± 11.7%), range 24%-72%
[d] Historical incidence for corn oil gavage studies: 72/250 (28.8% ± 9.0%), range 16%-40%; all routes: 389/1,248 (31.2% ± 10.3%), range 16%-56%
[e] Historical incidence for corn oil gavage studies: 10/250 (4.0% ± 3.2%), range 0%-8%; all routes: 50/1,248 (4.0% ± 6.7%), range 0%-34%
[f] Number of animals with neoplasm per number of animals with liver examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] Not applicable; no neoplasms in animal group
[j] 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 differential mortality in animals that do not reach terminal sacrifice.
[k] Historical incidence for corn oil gavage studies: 79/250 (31.6% ± 7.7%), range 22%-40%; all routes: 421/1,248 (33.7% ± 10.8%), range 18%-58%
[l] Historical incidence for corn oil gavage studies: 41/247 (16.6% ± 8.1%), range 6%-27%; all routes: 353/1,296 (27.2% ± 16.5%), range 2%-62%
[m] Historical incidence for corn oil gavage studies: 14/247 (5.7% ± 3.0%), range 2%-10%; all routes: 136/1,296 (10.5% ± 9.7%), range 0%-46%
[n] Historical incidence for corn oil gavage studies: 51/247 (20.7% ± 10.4%), range 8%-35%; all routes: 428/1,296 (33.0% ± 18.8%), range 6%-76%

Forestomach: The incidences of chronic inflammation were significantly increased in 0.5 and 1.0 g/kg females compared to the vehicle controls; severity also increased in these groups (Tables 22 and D4). Incidences of epithelial hyperplasia were significantly increased in 0.5 and 1.0 g/kg females and slightly increased in all dosed groups of males (Tables 22, C4, and D4). Significantly increased incidences of erosion in 0.5 and 1.0 g/kg females and ulceration in 1.0 g/kg females also occurred.

Epithelial hyperplasia was characterized by a widespread thickening of the squamous mucosa, often with the development of rete epithelial pegs of hyperplastic epithelium in the moderate and marked examples (Plate 13). Chronic inflammation was composed predominately of mononuclear infiltrates, particularly in the submucosa deep to the areas of unulcerated, hyperplastic mucosa. Neutrophilic infiltrates were also present and predominated in areas of erosion and ulceration. The neutrophilic infiltrates were occasionally quite marked along the eroded and ulcerated surface and extended into the superficial keratin layer. In moderate to marked examples, large nodules of mononuclear inflammatory cells, predominately lymphocytes, were present in the submucosa and along the serosal surface and were occasionally visible grossly. Erosion or ulceration typically occurred in the areas of hyperplasia, with neutrophilic infiltrates the predominant type of inflammatory response deep to the ulcers. Erosion consisted of epithelial loss that did not extend to the epithelial basement membrane; when it did, ulcer was diagnosed.

Malignant Lymphoma: An increased incidence of malignant lymphoma occurred in 1.0 g/kg males (vehicle control, 2/50; 0.25 g/kg, 3/50; 0.5 g/kg, 1/50; 1.0 g/kg, 6/50; Tables C1 and C2). Although the increase was not statistically significant, the incidence in 1.0 g/kg males exceeded the historical control range for corn oil gavage studies [5/250 (2.0% ± 1.4%), range 0%-4%] and was at the high end of the range for all routes combined [48/1,250 (3.8% ± 3.0%), range 0%-12%] (Table C3b). The spleen was the most frequently affected organ in five out of the six cases seen in the 1.0 g/kg group. Other organs, only sporadically involved, were the lymph nodes, kidney, liver, adrenal gland, salivary gland, stomach, heart, thyroid gland, prostate gland, and thymus.

Histologically, the characteristics of malignant lymphoma in male mice varied and represented examples of "early" lymphoma, characterized by an inappropriate focal to regional proliferation of lymphocytes that distorted the preexisting architecture but did not fully efface the red and white pulp, as well as more classic examples of advanced lymphoma, characterized by multi-organ involvement and effacement of normal splenic and other organ architecture. Cytologically, mild to moderate pleomorphism with variable numbers of mitoses was present in each case.

GENETIC TOXICOLOGY

Kava kava extract was tested for bacterial mutagenicity in two independent assays using several strains of bacteria (*Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 and *Escherichia coli* strain WP2 *uvrA*/pKM101), with and without exogenous metabolic activation supplied by induced rat or hamster liver S9. Concentrations of kava kava extract ranged from 33 to 10,000 µg/plate. No increase in mutant colonies was seen in any of the tester strains, under any activation condition (Tables E1 and E2).

Peripheral blood samples from the mice in the 3-month study were evaluated for frequency of micronucleated erythrocytes; kava kava extract was administered via gavage, and doses ranged from 0.125 to 2.0 g/kg per day. No increases in the frequencies of micronucleated erythrocytes were observed in either male or female mice, and no significant changes in the percentage of reticulocytes in peripheral blood were observed (Table E3). These results indicate that treatment with kava kava extract did not produce chromosomal damage or bone marrow toxicity in male or female B6C3F1 mice.

TABLE 22
Incidences of Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Male				
Stomach, Forestomach[a]	50	50	50	50
Inflammation, Chronic[b]	19 (1.7)[c]	22 (1.6)	24 (1.6)	24 (1.5)
Epithelium, Hyperplasia	18 (2.3)	22 (1.6)	25 (2.0)	22 (1.8)
Female				
Stomach, Forestomach	50	50	50	50
Inflammation, Chronic	3 (1.7)	6 (1.5)	21** (2.6)	22** (2.5)
Epithelium, Hyperplasia	3 (1.3)	6 (1.3)	23** (2.8)	24** (2.3)
Erosion	0	1 (2.0)	14** (3.1)	11** (2.7)
Ulcer	0	2 (2.5)	3 (3.0)	6* (2.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with forestomach 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

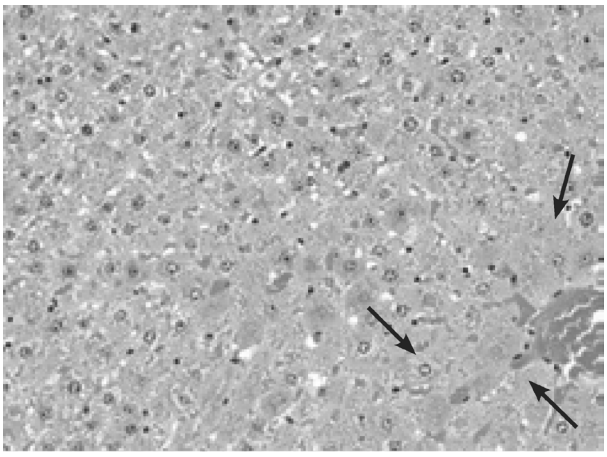


Plate 1
Mild (grade 2) centrilobular hypertrophy (arrows) in the liver of a female rat administered 1.0 g kava kava extract/kg body weight by gavage for 2 years. Compare to Plate 2. H&E

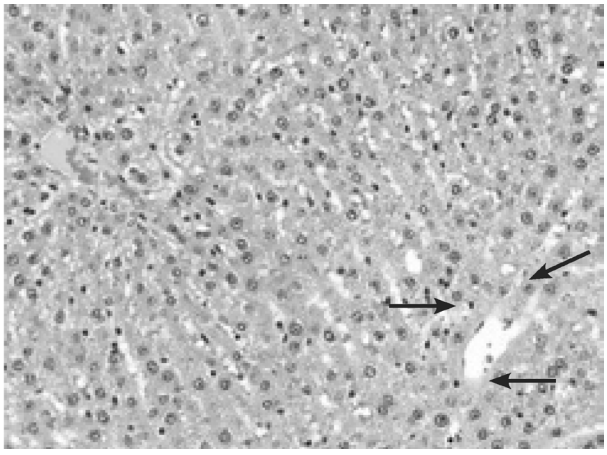


Plate 2
Liver of a vehicle control male rat from the 2-year kava kava extract study. Note (arrows) the normal aspect of the centrilobular hepatocytes. Compare to Plate 1. H&E

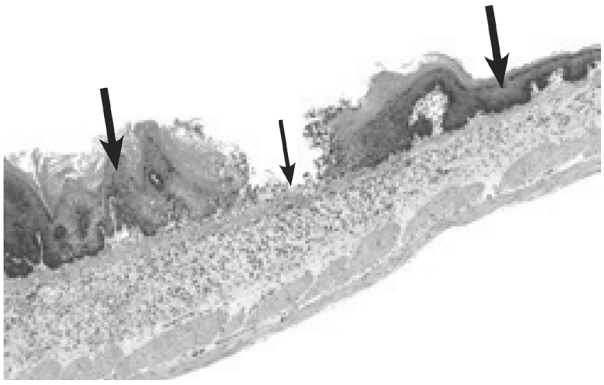


Plate 3
Moderate (grade 3) hyperplasia (thick arrow) and mild (grade 2) ulceration (thin arrow) in the forestomach of a male rat administered 1.0 g kava kava extract/kg body weight by gavage for 2 years. H&E

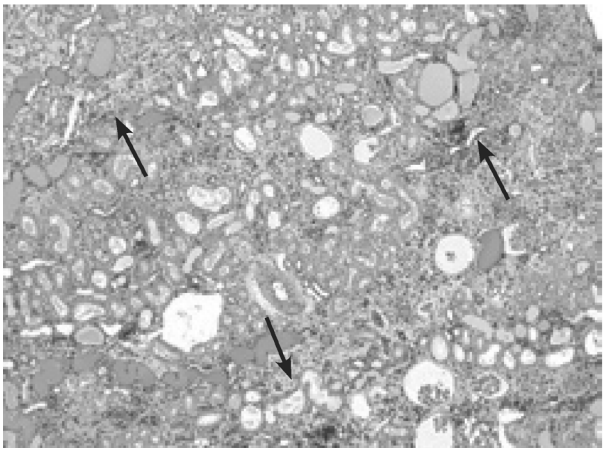


Plate 4
Marked (grade 4) nephropathy (arrows) in the kidney of a male rat administered 1.0 g kava kava extract/kg body weight by gavage for 2 years. H&E

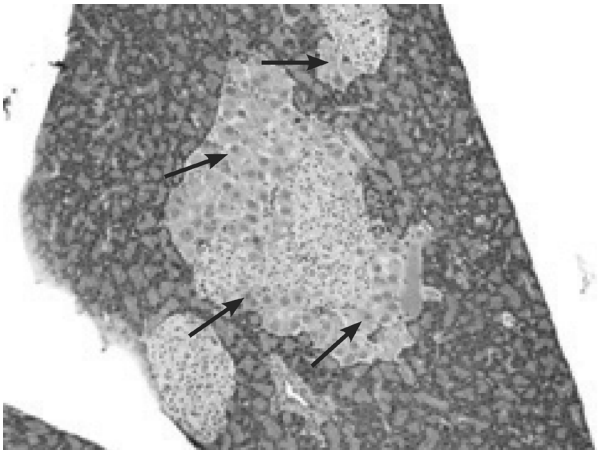


Plate 5
Minimal (grade 1) metaplasia of acinar cells to a hepatocytic morphology in the pancreas of a female rat administered 1.0 g kava kava extract/kg body weight by gavage for 2 years. The lesion is characterized by the presence of small clusters of apparently normal hepatocytes adjacent to islets of Langerhans (arrows). H&E

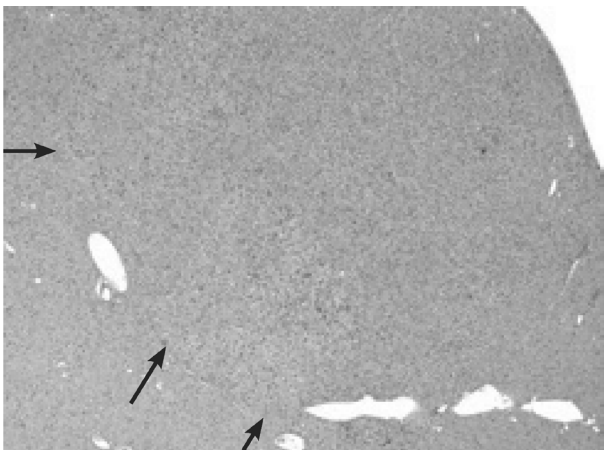


Plate 6
Hepatocellular adenoma from a male mouse administered 0.5 g kava kava extract/kg body weight by gavage for 2 years. Note (arrows) margins of the adenoma compressing the adjacent normal tissue. H&E

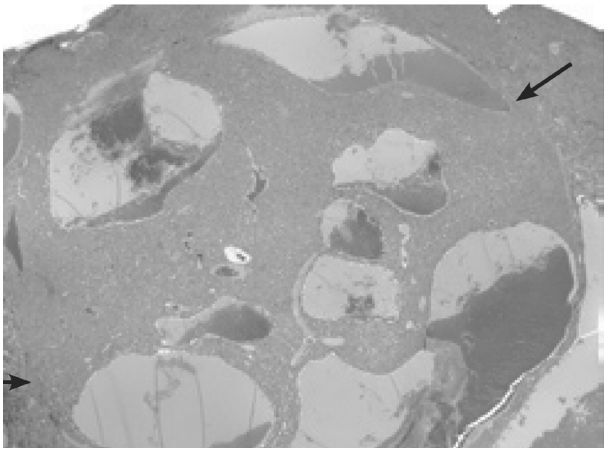


Plate 7
Hepatocellular carcinoma (arrows) from a male mouse administered 0.25 g kava kava extract/kg body weight by gavage for 2 years. H&E

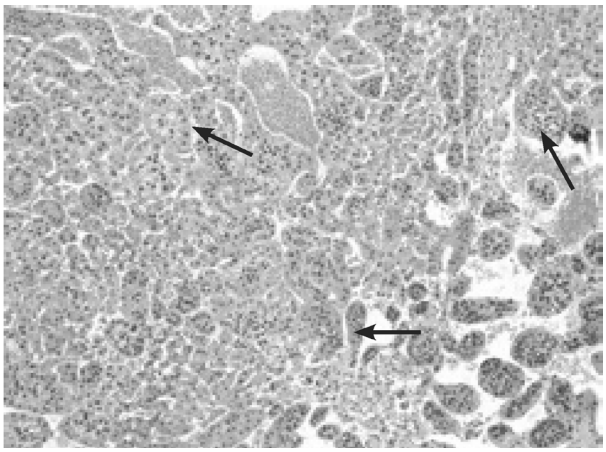


Plate 8
Higher magnification of Plate 7. Note (arrows) the trabecular architecture displayed by the neoplastic cells. H&E

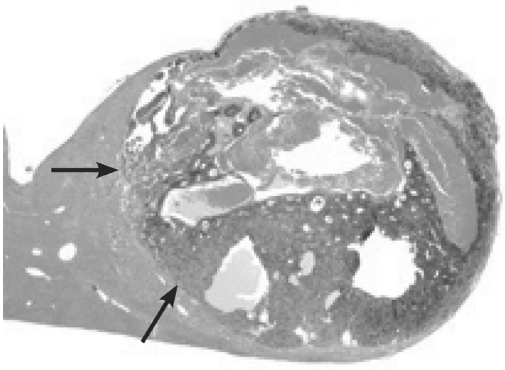


Plate 9
Hepatoblastoma (arrows) from a male mouse administered 1.0 g kava kava extract/kg body weight by gavage for 2 years (arrows). H&E

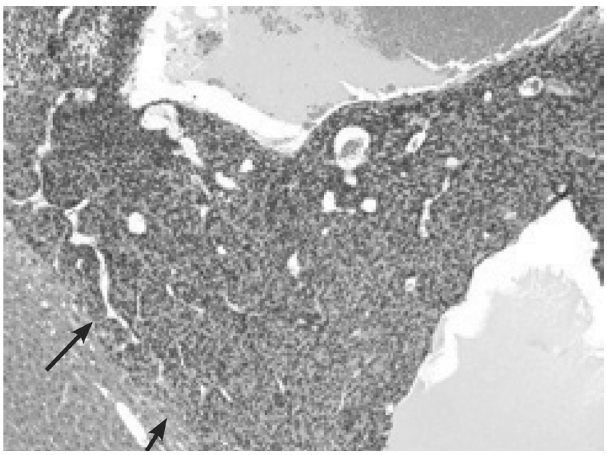


Plate 10
Higher magnification of Plate 9. Note (arrows) the hyperbasophilic appearance of the mass due to the presence of basophilic fusiform cells with a high nucleus to cytoplasm ratio. H&E

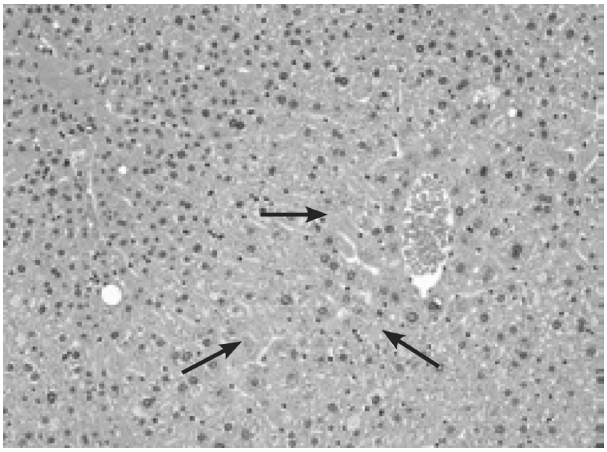


Plate 11
Mild (grade 2) centrilobular hypertrophy (arrows) in the liver of a male mouse administered 1.0 g kava kava extract/kg body weight by gavage for 2 years. Compare to Plate 12. H&E

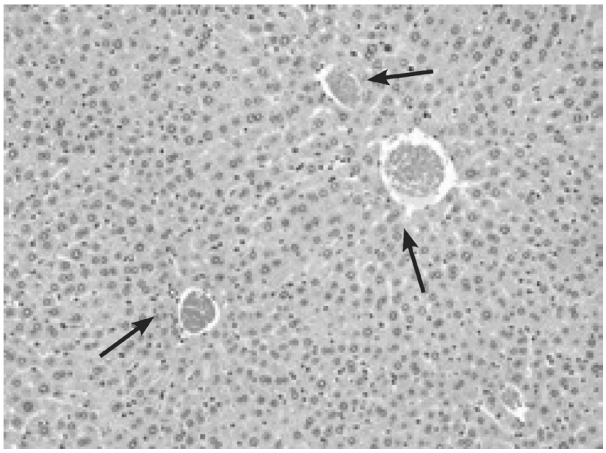


Plate 12
Liver of a vehicle control male mouse from the 2-year kava kava extract study. Note (arrows) the normal aspect of the centrilobular hepatocytes. Compare to Plate 11. H&E

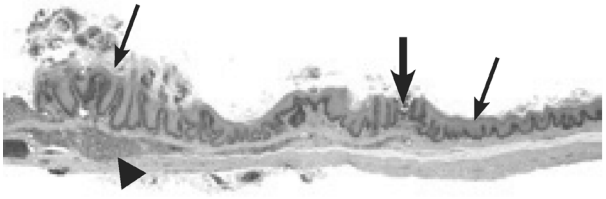


Plate 13
Moderate (grade 3) hyperplasia (thin arrows), mild (grade 2) erosion (thick arrow), and mild (grade 2) submucosal inflammation (arrowhead) in the forestomach of a female mouse administered 1.0 g kava kava extract/kg body weight by gavage for 2 years. H&E

DISCUSSION AND CONCLUSIONS

Kava kava has been used to treat anxiety in humans, with effects observed after as few as one to two doses with progressive improvements over 1 to 4 weeks (Pittler and Ernst, 2000, 2002, 2003; Basch *et al.*, 2002). Although commercial preparations of kava kava were used world-wide as anxiolytics, they have been withdrawn in several European and Canadian markets due to safety concerns (Stafford, 2001; Ernst, 2002a,b; Boon and Wong, 2003; Mills *et al.*, 2003; Schulze *et al.*, 2003). Several cases of liver damage have been associated with kava kava exposure in Europe including hepatitis (Bujanda *et al.*, 2002; Humberston *et al.*, 2003; Stickel *et al.*, 2003), cirrhosis and liver failure (Escher *et al.*, 2001; Kraft *et al.*, 2001), and death (Gow *et al.*, 2003; Thomsen *et al.*, 2004). However, since the correlation of dose and duration of kava kava use with the risk of liver damage is unclear, the assessment of its safety profile compared with other agents used in the management of anxiety remains an area of controversy, and it continues to be used widely in the United States. Hence, kava kava was nominated for study to the NTP due to the lack of sufficient information in the literature on its toxicity characterization. The NTP conducted 2-week, 3-month, and 2-year toxicity and carcinogenicity studies in F344/N rats and B6C3F1 mice.

The liver was consistently seen as the major target organ of toxicity in both rats and mice in the 3-month studies as indicated by liver enlargement and persistent hepatocellular hypertrophy. Supporting evidence from clinical chemistry showed several-fold increases in serum GGT activities in rats in the 3-month and 2-year studies. The increased GGT activities and bile salt concentrations (observed in the 2-year studies) may have been related to the hepatocellular hypertrophy through an intrahepatic cholestatic mechanism. Since increased GGT activity is shown to be induced by steroids and other drugs (Hall, 2007), enzyme induction may also have played a role in the GGT activity increases.

To identify a potential mechanism for liver toxicity, microarray, real-time polymerase chain reaction (RT PCR) and immunohistochemistry studies were conducted on livers of the 3-month rats and mice and are published elsewhere (Clayton *et al.*, 2007; Guo *et al.*, 2009, 2010). Results from the microarray data revealed a dose-dependent induction in several drug-metabolizing enzymes belonging to the cytochrome P450 family, notably CYP1A1. These findings were confirmed using RT PCR (Guo *et al.*, 2009, 2010) and immunohistochemical analysis (Clayton *et al.*, 2007). In addition, dose-related alterations were noted in other Phase I and Phase II drug metabolizing enzymes as well as transporters (Guo *et al.*, 2009, 2010). Based on these studies, kava kava appears to induce liver toxicity in rats and mice via alteration of hepatic metabolizing enzymes. These findings raise the possibility that kava kava consumption as a dietary supplement may result in hepatic toxicity due to its enzyme modulating effects. Furthermore, kava kava may have profound effects on the pharmacokinetics of many coadministrated drugs or other food supplements, potentially exacerbating hepatotoxicity. However, these studies were not designed to assess herb-drug interactions in humans.

In the 2-year studies, survival of the dosed groups of rats and mice was comparable to that of the vehicle controls. Mean body weights were generally decreased (10% or more) in 1.0 g/kg male and female rats and female mice at the end of the study. The liver was the major site of carcinogenic activity in mice with increased incidences of eosinophilic foci, which are often viewed as histopathologic precursors to hepatocellular neoplasms, in the 0.5 and 1.0 g/kg males and females. In male mice, there were dose-related increases in the incidences and multiplicities of hepatoblastoma and hepatocellular adenoma as well as an increase in the combined incidence of hepatocellular carcinoma or hepatoblastoma. Hepatoblastomas are believed to arise *de novo* or from within hepatocellular adenomas or carcinomas, and approximately 25% are believed to metastasize (Allen *et al.*, 2004). Since hepatoblastomas are malignant, have a low spontaneous background incidence in mice, and the incidences in the current study lie outside those of historical controls, it was concluded that there was clear evidence of carcinogenic activity in male mice.

In female mice, there were treatment-related increases in the incidences of hepatocellular carcinoma and statistically significant increases in the combined incidences of hepatocellular adenoma or carcinoma in the 0.25 and 0.5 g/kg groups. The incidences exceeded the historical control range for corn oil gavage studies. However, there were no significant increases in the

incidences of liver neoplasms in the 1.0 g/kg group. To assess whether the lack of effect in the high-dose group of 1.0 g/kg could be attributed to decreases in body weight, a statistical model was applied to adjust for body weight, average survival time, housing, and route of administration. The predicted incidence of liver neoplasms in female mice based on body weight in the absence of a chemical effect was not significantly different from that in the 1.0 g/kg females suggesting little evidence for a chemical-driven effect. Therefore, the significant increases in the combined incidences and multiplicities of hepatocellular adenoma or carcinoma in 0.25 and 0.5 g/kg females, but not in 1.0 g/kg females, led to the conclusion of some evidence of carcinogenic activity in female mice.

In male rats, there were marginal but statistically significant increases in the incidences of testicular interstitial (Leydig) cell adenoma, with increased bilateral incidences, in the 0.3 and 1.0 g/kg groups relative to the concurrent vehicle controls. The incidence of adenoma in the testis of vehicle control male rats in the current study is relatively low compared to those in the historical controls in recent corn oil gavage studies [176/199 (mean of 88%) (88% ± 9%), range of 76% to 94%] or for all studies combined [1,053/1,298 (mean of 81%) (81% ± 13%), range of 54% to 98%]. The broad range of incidences in the historical control data together with the lower than expected incidence of neoplasms in the concurrent vehicle controls, poses uncertainty in determining whether the testicular adenomas were treatment related. Hence, the increased incidences of unilateral and bilateral Leydig cell adenoma were considered equivocal evidence of carcinogenic activity in male rats, which is in congruence with a previous NTP study of malachite green chloride and leucomalachite green (NTP, 2005). In female rats, there were no statistically significant increases in the incidences of any neoplasms; therefore, there was no evidence of carcinogenicity in female rats.

In male rats, there were dose-related decreases in the incidences of pituitary gland adenoma. Although the current studies are not designed to evaluate potential mechanisms related to decreased neoplasm incidences, increases in the incidences of testicular adenoma in conjunction with decreases in the incidences of pituitary gland neoplasms have been noted in other NTP studies, malachite green chloride and leucomalachite green and dibromoacetic acid (NTP, 2005, 2007). Furthermore, associations between decreases in the incidences of interstitial cell neoplasms of the testis and elevated rates of pituitary gland neoplasms in male F344/N rats have been reported in the literature (Haseman *et al.*, 1997). However, reasons for the opposite association of increased incidences of testis adenoma and decreased incidences of pituitary gland adenoma as seen in the current study remain unclear.

In female rats, there were decreases in the incidences of spontaneous mammary gland fibroadenoma and pituitary gland adenoma. Similar decreases in the incidences of mammary gland fibroadenoma have been noted in the NTP studies on ginseng, milk thistle extract, and pulegone (NTP, 2011a,b,c). Findings from the current study can be explained, in part, with studies in the literature that demonstrate that pituitary gland adenomas produce multiple hormones, including prolactin, which is implicated in the development of mammary gland neoplasms (McComb *et al.*, 1984; Attia, 1985). Pituitary gland adenomas immunoreactive for prolactin are known to commonly occur in F344/N and Sprague-Dawley rats (McComb *et al.*, 1984; Sandusky *et al.*, 1988). Consequently, it has been shown that inhibition of prolactin secretion can decrease the development of mammary gland adenomas (Welsch *et al.*, 1981).

Nonneoplastic lesions associated with kava kava extract administration in the current studies included hepatocellular hypertrophy in the liver and inflammation, ulcer, and epithelial hyperplasia in the forestomach of both rats and mice; nephropathy, retinal degeneration of the eye, and acinus metaplasia in the pancreas of male and female rats; centrilobular fatty change in the liver of male rats; and eosinophilic foci in the liver of mice.

There were species differences in the sensitivity of liver neoplasm induction in animals exposed to kava kava extract in the current studies. Even though liver toxicity was observed in both species in the 3-month and 2-year studies, kava kava extract did not induce liver neoplasms in rats. This pattern is corroborated with findings in the literature that demonstrate that mice appear to be more sensitive to the induction of liver neoplasms than rats (NTP, 1993; Bucher *et al.*, 1998). The results of the NTP bacterial mutagenicity and *in vivo* micronucleus studies (Appendix E) as well as the available published information (Jhoo *et al.*, 2007; Whittaker *et al.*, 2008) suggest that kava kava extract is not mutagenic, therefore, the carcinogenic activity in mice is most likely mediated through nongenotoxic mechanisms involving enzyme activation and/or free radical generation (Guo *et al.*, 2009, 2010).

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity** of kava kava extract in male F344/N rats based on marginal increases in the incidences of testicular interstitial cell adenoma. There was *no evidence of carcinogenic activity* of kava kava extract in female F344/N rats administered 0.1, 0.3, or 1.0 g/kg. There was *clear evidence of carcinogenic activity* of kava kava extract in male B6C3F1 mice based on increased incidences of hepatoblastoma. There was *some evidence of carcinogenic activity* of kava kava extract in female B6C3F1 mice based on increased incidences of hepatocellular adenoma or carcinoma (combined).

Kava kava extract administration resulted in increased incidences of nonneoplastic lesions in the liver, forestomach, kidney, eye, and pancreas of male and female rats, liver of male and female mice, and forestomach of female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Peer Review Panel's 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 KAVA KAVA EXTRACT

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	49	50	50	50
Early deaths				
Accidental deaths		1	1	3
Moribund	10	10	13	13
Natural deaths	5	4	2	3
Survivors				
Died last week of study			1	
Terminal sacrifice	34	35	33	31
Animals examined microscopically	49	50	50	50
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(50)	(50)
Intestine large, colon	(49)	(50)	(50)	(50)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Intestine small, ileum	(49)	(50)	(50)	(50)
Liver	(49)	(50)	(50)	(50)
Cholangioma		1 (2%)		
Fibrosarcoma, metastatic, stomach, glandular			1 (2%)	
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)			1 (2%)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hepatocellular adenoma	1 (2%)	1 (2%)		1 (2%)
Mesentery	(6)	(8)	(7)	(6)
Oral mucosa	(1)	(1)	(0)	(1)
Squamous cell carcinoma				1 (100%)
Squamous cell papilloma	1 (100%)			
Pancreas	(49)	(50)	(50)	(50)
Mast cell tumor malignant		1 (2%)		
Acinus, adenoma	1 (2%)		1 (2%)	
Salivary glands	(49)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Sarcoma				1 (2%)
Schwannoma malignant			2 (4%)	
Stomach, forestomach	(49)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(50)
Carcinoid tumor malignant			1 (2%)	
Muscularis, fibrosarcoma			1 (2%)	
Tooth	(10)	(6)	(9)	(11)
Cardiovascular System				
Blood vessel	(49)	(50)	(50)	(50)
Heart	(49)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle				1 (2%)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Endocrine System (continued)				
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	4 (8%)	8 (16%)	2 (4%)	2 (4%)
Pheochromocytoma malignant		2 (4%)		
Bilateral, pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	4 (8%)	2 (4%)	1 (2%)	3 (6%)
Adenoma, multiple			1 (2%)	
Parathyroid gland	(49)	(50)	(48)	(50)
Adenoma	1 (2%)	1 (2%)		
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, adenoma	21 (43%)	20 (40%)	15 (30%)	8 (16%)
Thyroid gland	(49)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)	2 (4%)	
C-cell, adenoma	8 (16%)	8 (16%)	6 (12%)	2 (4%)
C-cell, carcinoma			2 (4%)	
Follicular cell, adenoma	1 (2%)		1 (2%)	
Follicular cell, carcinoma			1 (2%)	
General Body System				
Peritoneum	(0)	(0)	(1)	(0)
Paraganglioma			1 (100%)	
Tissue NOS	(0)	(1)	(0)	(0)
Rhabdomyosarcoma		1 (100%)		
Genital System				
Coagulating gland	(2)	(2)	(0)	(0)
Epididymis	(49)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Preputial gland	(49)	(50)	(49)	(50)
Adenoma				1 (2%)
Carcinoma	1 (2%)	1 (2%)		
Schwannoma malignant		1 (2%)		
Prostate	(49)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Seminal vesicle	(49)	(50)	(50)	(50)
Testes	(49)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	29 (59%)	32 (64%)	40 (80%)	43 (86%)
Interstitial cell, adenoma	8 (16%)	12 (24%)	9 (18%)	3 (6%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Mast cell tumor malignant		1 (2%)		
Lymph node	(4)	(3)	(2)	(5)
Deep cervical, fibrous histiocytoma, metastatic, skeletal muscle				1 (20%)
Pancreatic, mast cell tumor malignant		1 (33%)		
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle				1 (2%)
Mast cell tumor malignant		1 (2%)		
Spleen	(49)	(50)	(50)	(50)
Mast cell tumor malignant		1 (2%)		
Thymus	(48)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Fibroadenoma		1 (2%)	2 (4%)	3 (6%)
Skin	(49)	(50)	(50)	(50)
Basal cell adenoma			2 (4%)	1 (2%)
Dermis, fibroma	1 (2%)			1 (2%)
Epidermis, basal cell adenoma	2 (4%)			
Epidermis, keratoacanthoma			1 (2%)	
Epidermis, squamous cell carcinoma			1 (2%)	
Epidermis, trichoepithelioma			1 (2%)	
Subcutaneous tissue, fibroma	6 (12%)	5 (10%)	7 (14%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma			1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)		1 (2%)	
Subcutaneous tissue, liposarcoma	1 (2%)			
Subcutaneous tissue, osteosarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, schwannoma malignant, metastatic, salivary glands			1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)		
Skeletal muscle	(1)	(0)	(1)	(1)
Fibrous histiocytoma	1 (100%)			1 (100%)
Rhabdomyosarcoma			1 (100%)	
Nervous System				
Brain	(49)	(50)	(50)	(50)
Meningioma malignant		1 (2%)		
Spinal cord	(2)	(0)	(0)	(0)
Glioma malignant	1 (50%)			
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)		2 (4%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)		
Carcinoma, metastatic, salivary glands		1 (2%)		
Carcinoma, metastatic, thyroid gland			1 (2%)	
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)			1 (2%)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Neural crest tumor, metastatic, ear		1 (2%)	1 (2%)	
Osteosarcoma, metastatic, skin		1 (2%)	1 (2%)	
Schwannoma malignant, metastatic, salivary glands			1 (2%)	
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Mediastinum, osteosarcoma, metastatic, bone	1 (2%)			
Nose	(49)	(50)	(50)	(50)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Special Senses System				
Ear	(0)	(2)	(1)	(0)
Neural crest tumor		2 (100%)	1 (100%)	
Eye	(49)	(50)	(50)	(50)
Harderian gland	(49)	(50)	(50)	(50)
Carcinoma, metastatic, oral mucosa				1 (2%)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Nephroblastoma		1 (2%)		
Ureter	(1)	(1)	(0)	(0)
Polyp		1 (100%)		
Urethra	(1)	(0)	(0)	(0)
Urinary bladder	(49)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(49)	(50)	(50)	(50)
Leukemia mononuclear	7 (14%)	8 (16%)	7 (14%)	11 (22%)
Lymphoma malignant	1 (2%)		1 (2%)	1 (2%)
Mesothelioma malignant	3 (6%)	4 (8%)	1 (2%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	49	50	48
Total primary neoplasms	110	125	118	91
Total animals with benign neoplasms	49	48	50	47
Total benign neoplasms	92	94	96	73
Total animals with malignant neoplasms	17	23	20	17
Total malignant neoplasms	18	29	21	18
Total animals with metastatic neoplasms	2	4	6	2
Total metastatic neoplasms	4	7	7	7
Total animals with uncertain neoplasms-benign or malignant		2	1	
Total uncertain neoplasms		2	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 Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	5/49 (10%)	8/50 (16%)	2/50 (4%)	2/50 (4%)
Adjusted rate[b]	11.5%	17.9%	4.4%	4.8%
Terminal rate[c]	4/34 (12%)	8/35 (23%)	2/34 (6%)	2/31 (7%)
First incidence (days)	709	727 (T)	727 (T)	727 (T)
Poly-3 test[d]	P=0.086N	P=0.292	P=0.196N	P=0.234N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	5/49 (10%)	10/50 (20%)	2/50 (4%)	2/50 (4%)
Adjusted rate	11.5%	22.3%	4.4%	4.8%
Terminal rate	4/34 (12%)	9/35 (26%)	2/34 (6%)	2/31 (7%)
First incidence (days)	709	666	727 (T)	727 (T)
Poly-3 test	P=0.053N	P=0.142	P=0.196N	P=0.234N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/49 (4%)	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.6%	0.0%	4.4%	9.7%
Terminal rate	2/34 (6%)	0/35 (0%)	1/34 (3%)	4/31 (13%)
First incidence (days)	727 (T)	—[e]	608	727 (T)
Poly-3 test	P=0.078	P=0.231N	P=0.673N	P=0.316
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/49 (4%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.6%	2.2%	4.4%	9.7%
Terminal rate	2/34 (6%)	1/35 (3%)	1/34 (3%)	4/31 (13%)
First incidence (days)	727 (T)	727 (T)	608	727 (T)
Poly-3 test	P=0.130	P=0.490N	P=0.673N	P=0.316
Mammary Gland: Fibroadenoma				
Overall rate	0/49 (0%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	0.0%	2.2%	4.4%	7.2%
Terminal rate	0/34 (0%)	0/35 (0%)	2/34 (6%)	3/31 (10%)
First incidence (days)	—	709	727 (T)	727 (T)
Poly-3 test	P=0.080	P=0.506	P=0.248	P=0.111
Pancreatic Islets: Adenoma				
Overall rate	4/49 (8%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	9.2%	4.5%	4.4%	7.1%
Terminal rate	3/34 (9%)	2/35 (6%)	0/34 (0%)	2/31 (7%)
First incidence (days)	651	727 (T)	694	556
Poly-3 test	P=0.600	P=0.327N	P=0.315N	P=0.521N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	21/49 (43%)	20/50 (40%)	15/50 (30%)	8/50 (16%)
Adjusted rate	44.9%	44.2%	32.2%	18.6%
Terminal rate	14/34 (41%)	18/35 (51%)	9/34 (27%)	4/31 (13%)
First incidence (days)	504	525	590	447
Poly-3 test	P=0.003N	P=0.557N	P=0.146N	P=0.006N
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	2/49 (4%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.6%	0.0%	6.6%	2.4%
Terminal rate	2/34 (6%)	0/35 (0%)	2/34 (6%)	0/31 (0%)
First incidence (days)	727 (T)	—	664	589
Poly-3 test	P=0.585N	P=0.231N	P=0.525	P=0.512N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Skin: Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	2/49 (4%)	0/50 (0%)	5/50 (10%)	1/50 (2%)
Adjusted rate	4.6%	0.0%	10.9%	2.4%
Terminal rate	2/34 (6%)	0/35 (0%)	4/34 (12%)	0/31 (0%)
First incidence (days)	727 (T)	—	664	589
Poly-3 test	P=0.549N	P=0.231N	P=0.239	P=0.512N
Skin: Fibroma				
Overall rate	7/49 (14%)	5/50 (10%)	7/50 (14%)	2/50 (4%)
Adjusted rate	16.1%	11.2%	15.1%	4.7%
Terminal rate	6/34 (18%)	4/35 (11%)	4/34 (12%)	0/31 (0%)
First incidence (days)	709	700	608	454
Poly-3 test	P=0.089N	P=0.358N	P=0.561N	P=0.083N
Skin: Fibroma or Fibrous Histiocytoma				
Overall rate	7/49 (14%)	6/50 (12%)	7/50 (14%)	2/50 (4%)
Adjusted rate	16.1%	13.3%	15.1%	4.7%
Terminal rate	6/34 (18%)	4/35 (11%)	4/34 (12%)	0/31 (0%)
First incidence (days)	709	571	608	454
Poly-3 test	P=0.073N	P=0.468N	P=0.561N	P=0.083N
Testes: Adenoma				
Overall rate	37/49 (76%)	44/50 (88%)	49/50 (98%)	46/50 (92%)
Adjusted rate	80.2%	92.9%	98.3%	99.0%
Terminal rate	28/34 (82%)	32/35 (91%)	34/34 (100%)	31/31 (100%)
First incidence (days)	545	571	454	454
Poly-3 test	P=0.003	P=0.056	P=0.002	P<0.001
Thyroid Gland (C-Cell): Adenoma				
Overall rate	8/49 (16%)	9/50 (18%)	8/50 (16%)	2/50 (4%)
Adjusted rate	18.1%	20.1%	17.5%	4.8%
Terminal rate	6/34 (18%)	7/35 (20%)	7/34 (21%)	2/31 (7%)
First incidence (days)	545	673	694	727 (T)
Poly-3 test	P=0.030N	P=0.514	P=0.580N	P=0.055N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	8/49 (16%)	9/50 (18%)	9/50 (18%)	2/50 (4%)
Adjusted rate	18.1%	20.1%	19.7%	4.8%
Terminal rate	6/34 (18%)	7/35 (20%)	7/34 (21%)	2/31 (7%)
First incidence (days)	545	673	694	727 (T)
Poly-3 test	P=0.031N	P=0.514	P=0.531	P=0.055N
All Organs: Mononuclear Cell Leukemia				
Overall rate	7/49 (14%)	8/50 (16%)	7/50 (14%)	11/50 (22%)
Adjusted rate	15.8%	17.7%	15.2%	26.2%
Terminal rate	4/34 (12%)	5/35 (14%)	5/34 (15%)	8/31 (26%)
First incidence (days)	610	615	590	665
Poly-3 test	P=0.125	P=0.522	P=0.580N	P=0.179
All Organs: Malignant Mesothelioma				
Overall rate	3/49 (6%)	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rate	6.9%	8.8%	2.2%	7.2%
Terminal rate	2/34 (6%)	1/35 (3%)	1/34 (3%)	3/31 (10%)
First incidence (days)	675	571	727 (T)	727 (T)
Poly-3 test	P=0.591N	P=0.523	P=0.289N	P=0.640

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
All Organs: Benign Neoplasms				
Overall rate	49/49 (100%)	48/50 (96%)	50/50 (100%)	47/50 (94%)
Adjusted rate	100.0%	100.0%	100.0%	99.5%
Terminal rate	34/34 (100%)	35/35 (100%)	34/34 (100%)	31/31 (100%)
First incidence (days)	504	525	454	447
Poly-3 test	P=0.980N	P=1.000	—[f]	P=0.999N
All Organs: Malignant Neoplasms				
Overall rate	17/49 (35%)	23/50 (46%)	20/50 (40%)	17/50 (34%)
Adjusted rate	37.0%	47.2%	41.8%	38.9%
Terminal rate	8/34 (24%)	10/35 (29%)	11/34 (32%)	10/31 (32%)
First incidence (days)	545	168	590	451
Poly-3 test	P=0.442N	P=0.212	P=0.398	P=0.512
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/49 (100%)	49/50 (98%)	50/50 (100%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	34/34 (100%)	35/35 (100%)	34/34 (100%)	31/31 (100%)
First incidence (days)	504	168	454	447
Poly-3 test	P=1.000	P=1.000	—	P=1.000

(T) Terminal sacrifice

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

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

[c] Observed incidence at terminal kill

[d] 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**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Adenoma of the Testis in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Isoeugenol (April 2002)	46/50
Kava Kava Extract (August 2004)	37/49
β-Myrcene (March 2002)	47/50
Pulegone (April 2003)	46/50
Total (%)	176/199 (88.4%)
Mean ± standard deviation	88.4% ± 8.6%
Range	76%-94%
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 A3b
Historical Incidence of Adenoma of the Pituitary Gland (Pars Distalis) in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Isoeugenol (April 2002)	19/50
Kava Kava Extract (August 2004)	21/49
β-Myrcene (March 2002)	6/50
Pulegone (April 2003)	11/50
Total (%)	57/199 (28.6%)
Mean ± standard deviation	28.7% ± 14.3%
Range	12%-43%
Overall Historical Incidence: All Routes	
Total (%)	604/1,293 (46.7%)
Mean ± standard deviation	46.8% ± 20.6%
Range	12%-74%

[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 Kava Kava Extract[a]

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	49	50	50	50
Early deaths				
Accidental deaths		1	1	3
Moribund	10	10	13	13
Natural deaths	5	4	2	3
Survivors				
Died last week of study			1	
Terminal sacrifice	34	35	33	31
Animals examined microscopically	49	50	50	50
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Epithelium, hyperplasia	1 (2%)			
Epithelium, necrosis	1 (2%)			
Muscularis, degeneration	1 (2%)			
Intestine large, cecum	(49)	(50)	(50)	(50)
Inflammation		1 (2%)		
Intestine large, colon	(49)	(50)	(50)	(50)
Parasite metazoan	7 (14%)	2 (4%)	5 (10%)	2 (4%)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Ectopic tissue	1 (2%)			
Intestine small, ileum	(49)	(50)	(50)	(50)
Necrosis	1 (2%)			
Liver	(49)	(50)	(50)	(50)
Angiectasis		1 (2%)	2 (4%)	1 (2%)
Basophilic focus	21 (43%)	30 (60%)	18 (36%)	13 (26%)
Clear cell focus	18 (37%)	25 (50%)	25 (50%)	11 (22%)
Degeneration, cystic		4 (8%)	3 (6%)	5 (10%)
Eosinophilic focus	15 (31%)	18 (36%)	14 (28%)	13 (26%)
Fatty change, diffuse	11 (22%)	8 (16%)	12 (24%)	8 (16%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	3 (6%)	3 (6%)	1 (2%)	
Hepatodiaphragmatic nodule	2 (4%)	7 (14%)	4 (8%)	1 (2%)
Inflammation, chronic active	36 (73%)	39 (78%)	32 (64%)	34 (68%)
Mixed cell focus	4 (8%)	5 (10%)	4 (8%)	
Necrosis	1 (2%)	2 (4%)		1 (2%)
Pigmentation, hemosiderin	1 (2%)		2 (4%)	2 (4%)
Tension lipidosis				1 (2%)
Bile duct, cyst		1 (2%)		
Bile duct, hyperplasia	32 (65%)	39 (78%)	40 (80%)	34 (68%)
Centrilobular, degeneration			1 (2%)	
Centrilobular, fatty change	1 (2%)	7 (14%)	4 (8%)	21 (42%)
Centrilobular, necrosis		2 (4%)		1 (2%)
Hepatocyte, hyperplasia			1 (2%)	
Hepatocyte, hypertrophy		2 (4%)	2 (4%)	22 (44%)
Oval cell, hyperplasia		2 (4%)	1 (2%)	1 (2%)
Mesentery	(6)	(8)	(7)	(6)
Hemorrhage	1 (17%)			
Thrombosis			1 (14%)	
Fat, necrosis	4 (67%)	7 (88%)	6 (86%)	5 (83%)
Oral mucosa	(1)	(1)	(0)	(1)
Hyperplasia, squamous		1 (100%)		

[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 Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Alimentary System (continued)				
Pancreas	(49)	(50)	(50)	(50)
Atrophy	16 (33%)	13 (26%)	18 (36%)	18 (36%)
Basophilic focus		1 (2%)		
Inflammation			2 (4%)	1 (2%)
Acinus, hyperplasia	6 (12%)	9 (18%)	9 (18%)	2 (4%)
Acinus, metaplasia, hepatocyte				6 (12%)
Salivary glands	(49)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	
Stomach, forestomach	(49)	(50)	(50)	(50)
Erosion			1 (2%)	4 (8%)
Inflammation	8 (16%)	4 (8%)	9 (18%)	22 (44%)
Mineralization		2 (4%)	1 (2%)	3 (6%)
Ulcer	4 (8%)		6 (12%)	13 (26%)
Epithelium, dysplasia	1 (2%)		2 (4%)	1 (2%)
Epithelium, hyperplasia	6 (12%)	4 (8%)	11 (22%)	27 (54%)
Stomach, glandular	(49)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation	2 (4%)	3 (6%)	3 (6%)	3 (6%)
Epithelium, necrosis	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Tooth	(10)	(6)	(9)	(11)
Malformation	10 (100%)	6 (100%)	9 (100%)	11 (100%)
Cardiovascular System				
Blood vessel	(49)	(50)	(50)	(50)
Mineralization				1 (2%)
Pulmonary artery, inflammation, chronic active			1 (2%)	
Heart	(49)	(50)	(50)	(50)
Cardiomyopathy	48 (98%)	49 (98%)	48 (96%)	46 (92%)
Inflammation, suppurative				1 (2%)
Mineralization			1 (2%)	
Atrium, thrombosis	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Coronary artery, degeneration			1 (2%)	
Epicardium, inflammation			1 (2%)	
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Degeneration, cystic	1 (2%)			
Hematopoietic cell proliferation		1 (2%)	1 (2%)	
Hypertrophy	2 (4%)	5 (10%)	3 (6%)	3 (6%)
Necrosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Vacuolization cytoplasmic	1 (2%)			
Zona fasciculata, hyperplasia	8 (16%)	6 (12%)	9 (18%)	13 (26%)
Adrenal medulla	(49)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Hyperplasia	9 (18%)	11 (22%)	10 (20%)	10 (20%)
Thrombosis				1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Parathyroid gland	(49)	(50)	(48)	(50)
Hyperplasia	1 (2%)		1 (2%)	10 (20%)
Pituitary gland	(49)	(50)	(50)	(50)
Hemorrhage	1 (2%)		1 (2%)	
Pars distalis, angiectasis			1 (2%)	
Pars distalis, hyperplasia	17 (35%)	20 (40%)	21 (42%)	11 (22%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Endocrine System (continued)				
Thyroid gland	(49)	(50)	(50)	(50)
Infiltration cellular, lymphocyte		1 (2%)		
C-cell, hyperplasia	8 (16%)	12 (24%)	12 (24%)	7 (14%)
Follicular cell, hyperplasia		2 (4%)		1 (2%)
Follicular cell, hypertrophy				1 (2%)
General Body System				
Peritoneum	(0)	(0)	(1)	(0)
Tissue NOS	(0)	(1)	(0)	(0)
Genital System				
Coagulating gland	(2)	(2)	(0)	(0)
Fibrosis	1 (50%)			
Inflammation	1 (50%)	2 (100%)		
Epididymis	(49)	(50)	(50)	(50)
Inflammation	1 (2%)	4 (8%)	3 (6%)	2 (4%)
Preputial gland	(49)	(50)	(49)	(50)
Inflammation			1 (2%)	2 (4%)
Duct, hyperplasia, atypical				1 (2%)
Prostate	(49)	(50)	(50)	(50)
Atrophy				1 (2%)
Inflammation	28 (57%)	24 (48%)	21 (42%)	16 (32%)
Epithelium, hyperplasia	9 (18%)	8 (16%)	2 (4%)	3 (6%)
Seminal vesicle	(49)	(50)	(50)	(50)
Atrophy				1 (2%)
Inflammation			1 (2%)	2 (4%)
Testes	(49)	(50)	(50)	(50)
Cyst				1 (2%)
Arteriole, necrosis	1 (2%)			
Germinal epithelium, degeneration	3 (6%)			
Interstitial cell, hyperplasia	17 (35%)	15 (30%)	10 (20%)	4 (8%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Hyperplasia	16 (33%)	17 (34%)	24 (48%)	29 (58%)
Necrosis		1 (2%)		1 (2%)
Lymph node	(4)	(3)	(2)	(5)
Mediastinal, ectasia			1 (50%)	
Mediastinal, hyperplasia, lymphoid	3 (75%)			1 (20%)
Mediastinal, infiltration cellular, plasma cell		1 (33%)	1 (50%)	
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Atrophy	2 (4%)	2 (4%)		5 (10%)
Spleen	(49)	(50)	(50)	(50)
Accessory spleen	1 (2%)			1 (2%)
Amyloid deposition		1 (2%)		
Fibrosis				2 (4%)
Hematopoietic cell proliferation	10 (20%)	10 (20%)	7 (14%)	5 (10%)
Hemorrhage	1 (2%)			
Hyperplasia, histiocytic				1 (2%)
Inflammation	1 (2%)			
Necrosis	1 (2%)			
Pigmentation, hemosiderin	31 (63%)	32 (64%)	35 (70%)	32 (64%)
Thrombosis				2 (4%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Hematopoietic System (continued)				
Spleen (continued)	(49)	(50)	(50)	(50)
Lymphoid follicle, atrophy	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Lymphoid follicle, hyperplasia			1 (2%)	
Thymus	(48)	(50)	(50)	(50)
Atrophy	47 (98%)	49 (98%)	49 (98%)	46 (92%)
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Skin	(49)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		1 (2%)
Inflammation		2 (4%)		1 (2%)
Epidermis, hyperplasia, basal cell			1 (2%)	
Subcutaneous tissue, fibrosis	1 (2%)			
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Osteopetrosis				1 (2%)
Ligament, mineralization				1 (2%)
Skeletal muscle	(1)	(0)	(1)	(1)
Nervous System				
Brain	(49)	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)		2 (4%)
Hydrocephalus		1 (2%)		1 (2%)
Necrosis	2 (4%)		2 (4%)	2 (4%)
Choroid plexus, infiltration cellular, lymphocyte			1 (2%)	
Spinal cord	(2)	(0)	(0)	(0)
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Congestion		1 (2%)	1 (2%)	1 (2%)
Foreign body				1 (2%)
Hemorrhage				1 (2%)
Inflammation, granulomatous				3 (6%)
Inflammation, chronic active	6 (12%)	3 (6%)	9 (18%)	9 (18%)
Necrosis	2 (4%)			
Thrombosis	1 (2%)		1 (2%)	
Alveolar epithelium, hyperplasia	19 (39%)	13 (26%)	20 (40%)	15 (30%)
Alveolar epithelium, hypertrophy	2 (4%)		2 (4%)	1 (2%)
Alveolar epithelium, metaplasia			1 (2%)	
Alveolar epithelium, metaplasia, squamous	1 (2%)	1 (2%)	1 (2%)	
Alveolus, infiltration cellular, histiocyte	8 (16%)	3 (6%)	11 (22%)	9 (18%)
Arteriole, hyperplasia				1 (2%)
Serosa, inflammation, acute				1 (2%)
Nose	(49)	(50)	(50)	(50)
Foreign body	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Inflammation	18 (37%)	14 (28%)	10 (20%)	18 (36%)
Olfactory epithelium, metaplasia	2 (4%)	1 (2%)	3 (6%)	5 (10%)
Respiratory epithelium, hyperplasia	1 (2%)	3 (6%)	1 (2%)	4 (8%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Special Senses System				
Ear	(0)	(2)	(1)	(0)
Eye	(49)	(50)	(50)	(50)
Anterior chamber, inflammation				1 (2%)
Cornea, fibrosis	1 (2%)			
Cornea, inflammation			1 (2%)	
Iris, inflammation				2 (4%)
Iris, synechia	1 (2%)			
Lens, cataract	2 (4%)	1 (2%)	2 (4%)	
Retina, degeneration	6 (12%)	6 (12%)	10 (20%)	16 (32%)
Retina, retinal detachment	1 (2%)			
Harderian gland	(49)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Accumulation, hyaline droplet	1 (2%)		1 (2%)	2 (4%)
Hyperplasia, oncocytic				1 (2%)
Infarct	1 (2%)		1 (2%)	
Inflammation, acute			1 (2%)	
Mineralization	38 (78%)	43 (86%)	41 (82%)	34 (68%)
Nephropathy	46 (94%)	47 (94%)	48 (96%)	48 (96%)
Cortex, cyst	1 (2%)			1 (2%)
Papilla, necrosis	1 (2%)			
Pelvis, dilatation		1 (2%)		
Pelvis, inflammation	1 (2%)			1 (2%)
Pelvis, transitional epithelium, hyperplasia		1 (2%)	1 (2%)	15 (30%)
Ureter	(1)	(1)	(0)	(0)
Inflammation	1 (100%)			
Urethra	(1)	(0)	(0)	(0)
Inflammation	1 (100%)			
Urinary bladder	(49)	(50)	(50)	(50)
Inflammation	2 (4%)		1 (2%)	2 (4%)
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF KAVA KAVA EXTRACT

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1	1	2	2
Moribund	12	12	13	6
Natural deaths	3	2	11	8
Survivors				
Died last week of study		1		
Terminal sacrifice	34	34	24	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Periesophageal tissue, lipoma			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Mesentery	(10)	(9)	(7)	(9)
Oral mucosa	(0)	(1)	(2)	(0)
Squamous cell papilloma		1 (100%)	1 (50%)	
Pancreas	(50)	(50)	(50)	(50)
Adenoma, mixed cell			1 (2%)	
Acinus, adenoma			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Sarcoma, metastatic, stomach, glandular	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)			
Tooth	(5)	(3)	(4)	(2)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, lung	1 (2%)			
Schwannoma malignant, metastatic, lung				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Parathyroid gland	(44)	(47)	(44)	(47)
Adenoma				1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	29 (58%)	20 (40%)	24 (48%)	8 (16%)
Pars distalis, carcinoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma				1 (2%)
C-cell, adenoma	5 (10%)	6 (12%)	5 (10%)	4 (8%)
Follicular cell, adenoma	1 (2%)		1 (2%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(49)
Adenoma	2 (4%)	7 (14%)	3 (6%)	1 (2%)
Carcinoma	1 (2%)	1 (2%)		
Carcinoma, multiple	1 (2%)			
Ovary	(50)	(50)	(50)	(50)
Carcinoma, metastatic, lung				1 (2%)
Cystadenoma	1 (2%)			
Granulosa cell tumor benign			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Carcinosarcoma			1 (2%)	
Polyp stromal	6 (12%)	9 (18%)	8 (16%)	8 (16%)
Sarcoma stromal	1 (2%)		1 (2%)	
Bilateral, polyp stromal	1 (2%)			
Endometrium, adenoma				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(1)	(3)	(3)	(2)
Mediastinal, carcinoma, metastatic, lung				1 (50%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Carcinoma, metastatic, lung				1 (2%)
Thymus	(50)	(50)	(49)	(48)
Schwannoma malignant, metastatic, lung				1 (2%)
Thymoma benign		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)		
Carcinoma	1 (2%)	3 (6%)	1 (2%)	
Fibroadenoma	14 (28%)	17 (34%)	13 (26%)	4 (8%)
Fibroadenoma, multiple	10 (20%)	7 (14%)	2 (4%)	
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibroma	1 (2%)		1 (2%)	
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, osteosarcoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma			1 (2%)	
Osteosarcoma			1 (2%)	
Osteosarcoma, metastatic, skin			1 (2%)	
Skeletal muscle	(1)	(0)	(1)	(0)
Rhabdomyosarcoma			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign				1 (2%)
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma				1 (2%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma				1 (2%)
Carcinoma, metastatic, mammary gland		1 (2%)		
Osteosarcoma, metastatic, skin			1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Mediastinum, fibrosarcoma	1 (2%)			
Mediastinum, schwannoma malignant				1 (2%)
Nose	(50)	(50)	(50)	(50)
Pleura	(0)	(0)	(0)	(1)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(1)
Neural crest tumor	1 (100%)			1 (100%)
Eye	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(0)	(1)
Adenoma				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Adenolipoma	1 (2%)			
Leukemia mononuclear	5 (10%)	11 (22%)	6 (12%)	9 (18%)
Lymphoma malignant	1 (2%)			1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	45	44	30
Total primary neoplasms	91	89	78	46
Total animals with benign neoplasms	43	41	42	22
Total benign neoplasms	76	72	66	33
Total animals with malignant neoplasms	11	16	12	12
Total malignant neoplasms	13	17	12	12
Total animals with metastatic neoplasms	2	1	1	2
Total metastatic neoplasms	2	3	2	5
Total animals with uncertain neoplasms-benign or malignant	1			1
Total uncertain neoplasms	1			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 Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	3/50 (6%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate[b]	6.8%	2.3%	2.5%	2.4%
Terminal rate[c]	2/34 (6%)	1/34 (3%)	0/24 (0%)	1/34 (3%)
First incidence (days)	701	728 (T)	519	728 (T)
Poly-3 test[d]	P=0.371N	P=0.303N	P=0.333N	P=0.327N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.8%	4.5%	2.5%	2.4%
Terminal rate	2/34 (6%)	2/34 (6%)	0/24 (0%)	1/34 (3%)
First incidence (days)	701	728 (T)	519	728 (T)
Poly-3 test	P=0.293N	P=0.497N	P=0.333N	P=0.327N
Clitoral Gland: Adenoma				
Overall rate	2/50 (4%)	7/49 (14%)	3/50 (6%)	1/49 (2%)
Adjusted rate	4.6%	15.9%	7.4%	2.4%
Terminal rate	2/34 (6%)	6/34 (18%)	2/24 (8%)	0/34 (0%)
First incidence (days)	728 (T)	701	688	688
Poly-3 test	P=0.130N	P=0.077	P=0.460	P=0.520N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	8/49 (16%)	3/50 (6%)	1/49 (2%)
Adjusted rate	9.0%	18.2%	7.4%	2.4%
Terminal rate	2/34 (6%)	7/34 (21%)	2/24 (8%)	0/34 (0%)
First incidence (days)	615	701	688	688
Poly-3 test	P=0.050N	P=0.169	P=0.553N	P=0.200N
Mammary Gland: Fibroadenoma				
Overall rate	24/50 (48%)	24/50 (48%)	15/50 (30%)	4/50 (8%)
Adjusted rate	53.0%	52.6%	35.7%	9.6%
Terminal rate	20/34 (59%)	19/34 (56%)	8/24 (33%)	3/34 (9%)
First incidence (days)	450	615	615	688
Poly-3 test	P<0.001N	P=0.570N	P=0.075N	P<0.001N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	25/50 (50%)	24/50 (48%)	15/50 (30%)	4/50 (8%)
Adjusted rate	55.2%	52.6%	35.7%	9.6%
Terminal rate	21/34 (62%)	19/34 (56%)	8/24 (33%)	3/34 (9%)
First incidence (days)	450	615	615	688
Poly-3 test	P<0.001N	P=0.485N	P=0.048N	P<0.001N
Mammary Gland: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.3%	6.8%	2.5%	0.0%
Terminal rate	0/34 (0%)	3/34 (9%)	1/24 (4%)	0/34 (0%)
First incidence (days)	693	728 (T)	728 (T)	—[e]
Poly-3 test	P=0.188N	P=0.307	P=0.740	P=0.512N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.8%	6.8%	2.5%	0.0%
Terminal rate	2/34 (6%)	3/34 (9%)	1/24 (4%)	0/34 (0%)
First incidence (days)	693	728 (T)	728 (T)	—
Poly-3 test	P=0.075N	P=0.660N	P=0.339N	P=0.130N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	26/50 (52%)	25/50 (50%)	16/50 (32%)	4/50 (8%)
Adjusted rate	57.2%	54.8%	38.1%	9.6%
Terminal rate	21/34 (62%)	20/34 (59%)	9/24 (38%)	3/34 (9%)
First incidence (days)	450	615	615	688
Poly-3 test	P<0.001N	P=0.491N	P=0.052N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	29/50 (58%)	20/50 (40%)	24/50 (48%)	8/50 (16%)
Adjusted rate	63.0%	43.7%	54.5%	19.1%
Terminal rate	20/34 (59%)	15/34 (44%)	12/24 (50%)	6/34 (18%)
First incidence (days)	615	558	518	664
Poly-3 test	P<0.001N	P=0.046N	P=0.267N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	30/50 (60%)	20/50 (40%)	24/50 (48%)	8/50 (16%)
Adjusted rate	65.2%	43.7%	54.5%	19.1%
Terminal rate	21/34 (62%)	15/34 (44%)	12/24 (50%)	6/34 (18%)
First incidence (days)	615	558	518	664
Poly-3 test	P<0.001N	P=0.028N	P=0.200N	P<0.001N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	5/50 (10%)	6/50 (12%)	5/50 (10%)	5/50 (10%)
Adjusted rate	11.4%	13.5%	12.3%	12.0%
Terminal rate	5/34 (15%)	6/34 (18%)	3/24 (13%)	5/34 (15%)
First incidence (days)	728 (T)	728 (T)	647	728 (T)
Poly-3 test	P=0.567N	P=0.506	P=0.581	P=0.594
Uterus: Stromal Polyp				
Overall rate	7/50 (14%)	9/50 (18%)	8/50 (16%)	8/50 (16%)
Adjusted rate	15.6%	20.0%	19.4%	19.1%
Terminal rate	6/34 (18%)	7/34 (21%)	5/24 (21%)	6/34 (18%)
First incidence (days)	450	632	532	688
Poly-3 test	P=0.480	P=0.397	P=0.431	P=0.443
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	7/50 (14%)	9/50 (18%)	9/50 (18%)	8/50 (16%)
Adjusted rate	15.6%	20.0%	21.7%	19.1%
Terminal rate	6/34 (18%)	7/34 (21%)	5/24 (21%)	6/34 (18%)
First incidence (days)	450	632	532	688
Poly-3 test	P=0.485	P=0.397	P=0.329	P=0.443
All Organs: Mononuclear Cell Leukemia				
Overall rate	5/50 (10%)	11/50 (22%)	6/50 (12%)	9/50 (18%)
Adjusted rate	11.0%	24.0%	14.4%	20.7%
Terminal rate	1/34 (3%)	5/34 (15%)	0/24 (0%)	4/34 (12%)
First incidence (days)	506	605	518	380
Poly-3 test	P=0.322	P=0.085	P=0.440	P=0.166
All Organs: Osteoma or Osteosarcoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	7.3%	0.0%
Terminal rate	0/34 (0%)	0/34 (0%)	1/24 (4%)	0/34 (0%)
First incidence (days)	—	—	532	—
Poly-3 test	P=0.648N	—[f]	P=0.106	—

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	41/50 (82%)	42/50 (84%)	22/50 (44%)
Adjusted rate	91.7%	86.6%	89.8%	52.3%
Terminal rate	32/34 (94%)	30/34 (88%)	23/24 (96%)	19/34 (56%)
First incidence (days)	450	558	518	664
Poly-3 test	P<0.001N	P=0.313N	P=0.515N	P<0.001N
All Organs: Malignant Neoplasms				
Overall rate	11/50 (22%)	16/50 (32%)	12/50 (24%)	12/50 (24%)
Adjusted rate	23.2%	34.9%	27.6%	27.5%
Terminal rate	3/34 (9%)	10/34 (29%)	3/24 (13%)	5/34 (15%)
First incidence (days)	450	605	439	380
Poly-3 test	P=0.544N	P=0.153	P=0.404	P=0.409
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	45/50 (90%)	44/50 (88%)	30/50 (60%)
Adjusted rate	94.2%	93.8%	92.3%	68.8%
Terminal rate	32/34 (94%)	31/34 (91%)	23/24 (96%)	23/34 (68%)
First incidence (days)	450	558	439	380
Poly-3 test	P<0.001N	P=0.634N	P=0.519N	P<0.001N

(T) Terminal sacrifice

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

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

[c] Observed incidence at terminal kill

[d] 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**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE B3a
Historical Incidence of Adenoma of the Pituitary Gland (Pars Distalis) in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Isoeugenol (April 2002)	25/50
Kava Kava Extract (August 2004)	29/50
β-Myrcene (March 2002)	25/50
Pulegone (April 2003)	27/50
Total (%)	106/200 (53.0%)
Mean ± standard deviation	53.0% ± 3.8%
Range	50%-58%
Overall Historical Incidence: All Routes	
Total (%)	682/1,247 (54.7%)
Mean ± standard deviation	54.7% ± 11.3%
Range	32%-73%

[a] Data as of March 20, 2010

TABLE B3b
Historical Incidence of Fibroadenoma of the Mammary Gland in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Isoeugenol (April 2002)	26/50
Kava Kava Extract (August 2004)	24/50
β-Myrcene (March 2002)	17/50
Pulegone (April 2003)	21/50
Total (%)	88/200 (44.0%)
Mean ± standard deviation	44.0% ± 7.8%
Range	34%-52%
Overall Historical Incidence: All Routes	
Total (%)	655/1,250 (52.4%)
Mean ± standard deviation	52.4% ± 14.0%
Range	28%-86%

[a] Data as of March 20, 2010

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1	1	2	2
Moribund	12	12	13	6
Natural deaths	3	2	11	8
Survivors				
Died last week of study		1		
Terminal sacrifice	34	34	24	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Inflammation		1 (2%)	1 (2%)	
Perforation			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Necrosis			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	1 (2%)	8 (16%)	6 (12%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)		3 (6%)
Basophilic focus	44 (88%)	42 (84%)	41 (82%)	11 (22%)
Clear cell focus	12 (24%)	14 (28%)	11 (22%)	8 (16%)
Degeneration, cystic				1 (2%)
Eosinophilic focus	23 (46%)	31 (62%)	24 (48%)	23 (46%)
Fatty change, focal	1 (2%)			
Fatty change, diffuse	9 (18%)	10 (20%)	10 (20%)	11 (22%)
Hematopoietic cell proliferation	1 (2%)	3 (6%)		
Hepatodiaphragmatic nodule	5 (10%)	6 (12%)	1 (2%)	2 (4%)
Inflammation, chronic active	43 (86%)	40 (80%)	38 (76%)	35 (70%)
Mixed cell focus	15 (30%)	10 (20%)	9 (18%)	3 (6%)
Necrosis	2 (4%)	1 (2%)		
Pigmentation, hemosiderin	1 (2%)		1 (2%)	1 (2%)
Artery, necrosis	1 (2%)			
Bile duct, cyst		1 (2%)		
Bile duct, hyperplasia	12 (24%)	15 (30%)	10 (20%)	10 (20%)
Centrilobular, fatty change				2 (4%)
Centrilobular, necrosis		1 (2%)		1 (2%)
Hepatocyte, hypertrophy	5 (10%)	2 (4%)	3 (6%)	33 (66%)
Oval cell, hyperplasia		2 (4%)	2 (4%)	
Serosa, fibrosis	1 (2%)	1 (2%)		
Mesentery	(10)	(9)	(7)	(9)
Fat, necrosis	10 (100%)	9 (100%)	7 (100%)	9 (100%)
Oral mucosa	(0)	(1)	(2)	(0)
Hyperplasia, squamous			1 (50%)	
Pancreas	(50)	(50)	(50)	(50)
Atrophy	13 (26%)	10 (20%)	16 (32%)	17 (34%)
Basophilic focus	1 (2%)			
Inflammation				1 (2%)
Acinus, hyperplasia	1 (2%)	3 (6%)		
Acinus, metaplasia, hepatocyte		1 (2%)		4 (8%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy				1 (2%)

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion		1 (2%)	2 (4%)	
Hyperplasia, atypical	1 (2%)			
Inflammation	5 (10%)	7 (14%)	7 (14%)	13 (26%)
Ulcer	1 (2%)	1 (2%)	3 (6%)	7 (14%)
Epithelium, hyperplasia	5 (10%)	6 (12%)	8 (16%)	19 (38%)
Stomach, glandular	(50)	(50)	(50)	(50)
Amyloid deposition			1 (2%)	
Inflammation		2 (4%)		
Epithelium, hyperplasia			1 (2%)	
Epithelium, necrosis	1 (2%)	1 (2%)		
Tooth	(5)	(3)	(4)	(2)
Malformation	5 (100%)	3 (100%)	4 (100%)	2 (100%)
Peridontal tissue, inflammation				1 (50%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	42 (84%)	45 (90%)	45 (90%)	47 (94%)
Atrium, thrombosis		1 (2%)		1 (2%)
Valve, thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Amyloid deposition		1 (2%)		
Degeneration, cystic	3 (6%)	6 (12%)	7 (14%)	6 (12%)
Hemorrhage				1 (2%)
Hypertrophy	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Metaplasia, osseous			1 (2%)	
Necrosis	2 (4%)		1 (2%)	2 (4%)
Zona fasciculata, hyperplasia	6 (12%)	6 (12%)	4 (8%)	9 (18%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)		1 (2%)	1 (2%)
Necrosis		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)		3 (6%)	1 (2%)
Parathyroid gland	(44)	(47)	(44)	(47)
Pituitary gland	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Pars distalis, angiectasis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Pars distalis, hyperplasia	11 (22%)	20 (40%)	16 (32%)	23 (46%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	15 (30%)	15 (30%)	6 (12%)	4 (8%)
Follicle, cyst				2 (4%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)	4 (8%)	4 (8%)
General Body System				
None				

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Genital System				
Clitoral gland	(50)	(49)	(50)	(49)
Hyperplasia		3 (6%)	5 (10%)	1 (2%)
Inflammation	1 (2%)	2 (4%)		
Ovary	(50)	(50)	(50)	(50)
Cyst	7 (14%)	9 (18%)	6 (12%)	5 (10%)
Cyst, multiple	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Congestion		1 (2%)		
Hemorrhage		2 (4%)		1 (2%)
Necrosis		1 (2%)		
Endometrium, hyperplasia, adenomatous			1 (2%)	
Endometrium, hyperplasia, cystic	5 (10%)	5 (10%)	6 (12%)	10 (20%)
Stroma, hyperplasia			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Hyperplasia	26 (52%)	27 (54%)	19 (38%)	23 (46%)
Inflammation, histiocytic			1 (2%)	
Myelofibrosis	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Necrosis	1 (2%)			
Lymph node	(1)	(3)	(3)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Inflammation	2 (4%)			
Spleen	(50)	(50)	(50)	(50)
Accessory spleen			1 (2%)	
Fibrosis		3 (6%)		2 (4%)
Hematopoietic cell proliferation	19 (38%)	18 (36%)	17 (34%)	7 (14%)
Hyperplasia, histiocytic	1 (2%)		1 (2%)	
Pigmentation, hemosiderin	37 (74%)	37 (74%)	41 (82%)	42 (84%)
Lymphoid follicle, atrophy	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Lymphoid follicle, necrosis	1 (2%)			
Thymus	(50)	(50)	(49)	(48)
Atrophy	48 (96%)	43 (86%)	47 (96%)	45 (94%)
Infiltration cellular, plasma cell	1 (2%)			
Inflammation			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele			1 (2%)	
Hyperplasia			2 (4%)	
Skin	(50)	(50)	(50)	(50)
Epidermis, hyperkeratosis			1 (2%)	
Epidermis, hyperplasia, basal cell		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis	1 (2%)			
Skeletal muscle	(1)	(0)	(1)	(0)
Inflammation	1 (100%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage		2 (4%)	2 (4%)	
Infiltration cellular, mixed cell			1 (2%)	
Necrosis		3 (6%)	2 (4%)	
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Hemorrhage			1 (100%)	
Necrosis			1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Atelectasis	1 (2%)	1 (2%)		
Congestion	1 (2%)			1 (2%)
Hemorrhage				1 (2%)
Inflammation, chronic active	4 (8%)	5 (10%)	2 (4%)	7 (14%)
Alveolar epithelium, hyperplasia	11 (22%)	14 (28%)	6 (12%)	7 (14%)
Alveolar epithelium, hypertrophy	1 (2%)	1 (2%)		
Alveolar epithelium, metaplasia	1 (2%)			
Alveolar epithelium, metaplasia, squamous	1 (2%)	3 (6%)		
Alveolus, infiltration cellular, histiocyte	12 (24%)	17 (34%)	12 (24%)	16 (32%)
Mediastinum, foreign body	1 (2%)			
Mediastinum, inflammation, chronic active	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Foreign body	2 (4%)			
Inflammation	8 (16%)	2 (4%)	9 (18%)	8 (16%)
Glands, dilatation			1 (2%)	
Olfactory epithelium, metaplasia	1 (2%)		1 (2%)	
Respiratory epithelium, hyperplasia			1 (2%)	
Pleura	(0)	(0)	(0)	(1)
Mesothelium, hyperplasia				1 (100%)
Trachea	(50)	(50)	(50)	(50)
Peritracheal tissue, inflammation			1 (2%)	
Special Senses System				
Ear	(1)	(0)	(0)	(1)
Eye	(50)	(50)	(50)	(50)
Synechia			1 (2%)	
Cornea, fibrosis	1 (2%)			1 (2%)
Cornea, inflammation	1 (2%)		2 (4%)	2 (4%)
Lens, cataract	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Posterior chamber, inflammation				1 (2%)
Retina, degeneration	5 (10%)	5 (10%)	5 (10%)	12 (24%)
Retina, dysplasia			1 (2%)	
Retina, gliosis	1 (2%)		3 (6%)	1 (2%)
Retina, inflammation		1 (2%)		
Zymbal's gland	(0)	(0)	(0)	(1)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet		2 (4%)	1 (2%)	1 (2%)
Glomerulosclerosis		1 (2%)		
Infarct	2 (4%)	3 (6%)	4 (8%)	3 (6%)
Mineralization	43 (86%)	43 (86%)	45 (90%)	46 (92%)
Nephropathy	34 (68%)	35 (70%)	37 (74%)	43 (86%)
Cortex, cyst	2 (4%)			
Medulla, cyst	1 (2%)			
Papilla, necrosis			1 (2%)	1 (2%)
Pelvis, inflammation	1 (2%)	1 (2%)	5 (10%)	4 (8%)
Pelvis, transitional epithelium, hyperplasia			4 (8%)	6 (12%)
Pelvis, transitional epithelium, metaplasia			1 (2%)	
Renal tubule, dilatation			1 (2%)	
Renal tubule, necrosis			1 (2%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF KAVA KAVA EXTRACT

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	10	10	7
Natural deaths	13	7	5	7
Survivors				
Died last week of study	1		1	
Terminal sacrifice	33	33	34	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(49)	(50)	(49)	(47)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		1 (2%)
Carcinoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma	5 (10%)			1 (2%)
Hemangiosarcoma, metastatic, testes			1 (2%)	
Hepatoblastoma		4 (8%)	7 (14%)	9 (18%)
Hepatoblastoma, multiple			2 (4%)	3 (6%)
Hepatocellular adenoma	14 (28%)	13 (26%)	10 (20%)	12 (24%)
Hepatocellular adenoma, multiple	13 (26%)	19 (38%)	19 (38%)	23 (46%)
Hepatocellular carcinoma	16 (32%)	15 (30%)	19 (38%)	15 (30%)
Hepatocellular carcinoma, multiple	4 (8%)	3 (6%)	7 (14%)	5 (10%)
Hepatocholangiocarcinoma	4 (8%)			1 (2%)
Osteosarcoma, metastatic, skin	1 (2%)			
Mesentery	(3)	(6)	(2)	(3)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	8 (16%)	2 (4%)		2 (4%)
Squamous cell papilloma, multiple		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Tooth	(33)	(32)	(27)	(21)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)			
Osteosarcoma, metastatic, skin	1 (2%)			
Rhabdomyosarcoma			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign			2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Parathyroid gland	(47)	(44)	(46)	(44)
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, adenoma			1 (2%)	
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(49)	(50)
Follicular cell, adenoma				2 (4%)
General Body System				
Peritoneum	(0)	(0)	(0)	(2)
Genital System				
Coagulating gland	(0)	(1)	(1)	(0)
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(1)	(0)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Interstitial cell, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Hemangiosarcoma	2 (4%)			
Lymph node	(0)	(1)	(3)	(3)
Pancreatic, hepatoblastoma, metastatic, liver		1 (100%)		
Lymph node, mandibular	(50)	(50)	(50)	(49)
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Spleen	(50)	(50)	(49)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	2 (4%)	
Thymus	(48)	(47)	(47)	(49)
Hemangiosarcoma, metastatic, spleen		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, granular cell tumor benign	1 (2%)			
Subcutaneous tissue, hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, osteosarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Musculoskeletal System (continued)				
Skeletal muscle	(2)	(0)	(1)	(0)
Hemangiosarcoma	1 (50%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (50%)			
Rhabdomyosarcoma, metastatic, heart			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	9 (18%)	6 (12%)	7 (14%)	7 (14%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma	2 (4%)	4 (8%)	6 (12%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple		2 (4%)		
Carcinoma, metastatic, Harderian gland		1 (2%)		1 (2%)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Hemangiosarcoma, metastatic, testes			1 (2%)	
Hepatoblastoma, metastatic, liver		1 (2%)	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	5 (10%)	7 (14%)	7 (14%)	10 (20%)
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)			
Osteosarcoma, metastatic, skin	1 (2%)			
Rhabdomyosarcoma, metastatic, heart			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	5 (10%)	3 (6%)	6 (12%)
Carcinoma		1 (2%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Capsule, hemangioma	1 (2%)			
Renal tubule, adenoma	1 (2%)	3 (6%)		
Urethra	(2)	(4)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant	2 (4%)	3 (6%)	1 (2%)	6 (12%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	46	47	47
Total primary neoplasms	99	88	92	100
Total animals with benign neoplasms	34	38	35	37
Total benign neoplasms	57	53	43	56
Total animals with malignant neoplasms	32	27	36	32
Total malignant neoplasms	42	35	49	44
Total animals with metastatic neoplasms	9	10	9	13
Total metastatic neoplasms	17	12	12	14

[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 Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	5/50 (10%)	3/50 (6%)	6/50 (12%)
Adjusted rate[b]	11.4%	11.4%	6.7%	13.1%
Terminal rate[c]	4/34 (12%)	3/33 (9%)	3/34 (9%)	2/36 (6%)
First incidence (days)	668	437	730 (T)	547
Poly-3 test[d]	P=0.484	P=0.631	P=0.345N	P=0.535
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	4/50 (8%)	7/50 (14%)
Adjusted rate	11.4%	13.5%	9.0%	15.2%
Terminal rate	4/34 (12%)	3/33 (9%)	4/34 (12%)	3/36 (8%)
First incidence (days)	668	423	730 (T)	547
Poly-3 test	P=0.382	P=0.513	P=0.488N	P=0.414
Small Intestine (Duodenum or Jejunum): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.9%	2.4%	0.0%	2.3%
Terminal rate	3/34 (9%)	0/33 (0%)	0/34 (0%)	1/36 (3%)
First incidence (days)	730 (T)	695	—[e]	730 (T)
Poly-3 test	P=0.194N	P=0.313N	P=0.114N	P=0.299N
Kidney (Renal Tubule): Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.3%	7.1%	0.0%	0.0%
Terminal rate	1/34 (3%)	3/33 (9%)	0/34 (0%)	0/36 (0%)
First incidence (days)	730 (T)	730 (T)	—	—
Poly-3 test	P=0.149N	P=0.296	P=0.495N	P=0.497N
Liver: Hemangiosarcoma				
Overall rate	5/50 (10%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	11.2%	0.0%	0.0%	2.2%
Terminal rate	2/34 (6%)	0/33 (0%)	0/34 (0%)	0/36 (0%)
First incidence (days)	513	—	—	465
Poly-3 test	P=0.053N	P=0.035N	P=0.030N	P=0.098N
Liver: Hepatocholangiocarcinoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	9.0%	0.0%	0.0%	2.3%
Terminal rate	1/34 (3%)	0/33 (0%)	0/34 (0%)	1/36 (3%)
First incidence (days)	587	—	—	730 (T)
Poly-3 test	P=0.116N	P=0.066N	P=0.059N	P=0.179N
Liver: Hepatocellular Adenoma				
Overall rate	27/50 (54%)	32/50 (64%)	29/50 (58%)	35/50 (70%)
Adjusted rate	59.5%	68.8%	62.4%	75.0%
Terminal rate	22/34 (65%)	23/33 (70%)	22/34 (65%)	27/36 (75%)
First incidence (days)	570	431	580	574
Poly-3 test	P=0.092	P=0.231	P=0.471	P=0.077
Liver: Hepatocellular Carcinoma				
Overall rate	20/50 (40%)	18/50 (36%)	26/50 (52%)	20/50 (40%)
Adjusted rate	42.7%	40.3%	53.5%	43.8%
Terminal rate	11/34 (32%)	12/33 (36%)	15/34 (44%)	14/36 (39%)
First incidence (days)	455	431	455	574
Poly-3 test	P=0.409	P=0.490N	P=0.196	P=0.543

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	38/50 (76%)	39/50 (78%)	38/50 (76%)	40/50 (80%)
Adjusted rate	78.8%	82.7%	77.7%	85.7%
Terminal rate	26/34 (77%)	28/33 (85%)	25/34 (74%)	32/36 (89%)
First incidence (days)	455	431	455	574
Poly-3 test	P=0.266	P=0.410	P=0.548N	P=0.261
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	4/50 (8%)	9/50 (18%)	12/50 (24%)
Adjusted rate	0.0%	9.4%	20.1%	26.4%
Terminal rate	0/34 (0%)	2/33 (6%)	8/34 (24%)	8/36 (22%)
First incidence (days)	—	687	679	582
Poly-3 test	P<0.001	P=0.057	P=0.002	P<0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	20/50 (40%)	21/50 (42%)	30/50 (60%)	25/50 (50%)
Adjusted rate	42.7%	46.8%	61.7%	53.3%
Terminal rate	11/34 (32%)	14/33 (42%)	19/34 (56%)	16/36 (44%)
First incidence (days)	455	431	455	574
Poly-3 test	P=0.136	P=0.426	P=0.046	P=0.205
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	38/50 (76%)	39/50 (78%)	40/50 (80%)	42/50 (84%)
Adjusted rate	78.8%	82.7%	81.8%	88.7%
Terminal rate	26/34 (77%)	28/33 (85%)	27/34 (79%)	32/36 (89%)
First incidence (days)	455	431	455	574
Poly-3 test	P=0.125	P=0.410	P=0.452	P=0.142
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	9/50 (18%)	7/50 (14%)	8/50 (16%)	9/50 (18%)
Adjusted rate	20.3%	16.5%	17.5%	20.1%
Terminal rate	7/34 (21%)	7/33 (21%)	4/34 (12%)	8/36 (22%)
First incidence (days)	570	730 (T)	455	574
Poly-3 test	P=0.515	P=0.430N	P=0.469N	P=0.595N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	6/50 (12%)	6/50 (12%)	1/50 (2%)
Adjusted rate	4.6%	13.6%	13.5%	2.3%
Terminal rate	1/34 (3%)	3/33 (9%)	6/34 (18%)	1/36 (3%)
First incidence (days)	683	319	730 (T)	730 (T)
Poly-3 test	P=0.266N	P=0.134	P=0.139	P=0.496N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	11/50 (22%)	13/50 (26%)	13/50 (26%)	10/50 (20%)
Adjusted rate	24.7%	29.6%	28.4%	22.4%
Terminal rate	8/34 (24%)	10/33 (30%)	9/34 (27%)	9/36 (25%)
First incidence (days)	570	319	455	574
Poly-3 test	P=0.384N	P=0.393	P=0.439	P=0.494N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	8/50 (16%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	18.0%	7.1%	0.0%	4.5%
Terminal rate	4/34 (12%)	3/33 (9%)	0/34 (0%)	2/36 (6%)
First incidence (days)	648	730 (T)	—	730 (T)
Poly-3 test	P=0.015N	P=0.113N	P=0.003N	P=0.045N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	9/50 (18%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	20.2%	7.1%	0.0%	4.5%
Terminal rate	5/34 (15%)	3/33 (9%)	0/34 (0%)	2/36 (6%)
First incidence (days)	648	730 (T)	—	730 (T)
Poly-3 test	P=0.007N	P=0.070N	P=0.002N	P=0.025N
All Organs: Hemangiosarcoma				
Overall rate	6/50 (12%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	13.5%	4.6%	8.8%	2.2%
Terminal rate	3/34 (9%)	0/33 (0%)	1/34 (3%)	0/36 (0%)
First incidence (days)	513	546	623	465
Poly-3 test	P=0.060N	P=0.140N	P=0.356N	P=0.054N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	8/50 (16%)	3/50 (6%)	4/50 (8%)	1/50 (2%)
Adjusted rate	18.0%	6.9%	8.8%	2.2%
Terminal rate	5/34 (15%)	1/33 (3%)	1/34 (3%)	0/36 (0%)
First incidence (days)	513	546	623	465
Poly-3 test	P=0.014N	P=0.105N	P=0.165N	P=0.015N
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Adjusted rate	4.6%	7.1%	2.2%	13.4%
Terminal rate	2/34 (6%)	3/33 (9%)	1/34 (3%)	4/36 (11%)
First incidence (days)	730 (T)	730 (T)	730 (T)	596
Poly-3 test	P=0.085	P=0.488	P=0.491N	P=0.142
All Organs: Benign Neoplasms				
Overall rate	34/50 (68%)	38/50 (76%)	35/50 (70%)	37/50 (74%)
Adjusted rate	74.0%	81.7%	72.4%	78.3%
Terminal rate	27/34 (79%)	29/33 (88%)	24/34 (71%)	28/36 (78%)
First incidence (days)	570	431	455	547
Poly-3 test	P=0.471	P=0.251	P=0.522N	P=0.400
All Organs: Malignant Neoplasms				
Overall rate	32/50 (64%)	27/50 (54%)	36/50 (72%)	32/50 (64%)
Adjusted rate	65.8%	56.7%	73.7%	65.9%
Terminal rate	19/34 (56%)	16/33 (49%)	24/34 (71%)	20/36 (56%)
First incidence (days)	369	319	455	465
Poly-3 test	P=0.369	P=0.236N	P=0.264	P=0.583
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	46/50 (92%)	47/50 (94%)	47/50 (94%)
Adjusted rate	94.0%	93.2%	94.0%	95.6%
Terminal rate	31/34 (91%)	32/33 (97%)	31/34 (91%)	34/36 (94%)
First incidence (days)	369	319	455	465
Poly-3 test	P=0.410	P=0.600N	P=0.662	P=0.536

(T) Terminal sacrifice

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

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

[c] Observed incidence at terminal kill

[d] 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**.

[e] Not applicable; no neoplasms in animal group

TABLE C3a
Historical Incidence of Liver Neoplasms in Control Male B6C3F1 Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies					
Isoeugenol (May 2002)	24/50	8/50	3/50	11/50	30/50
Kava Kava Extract (August 2004)	27/50	20/50	0/50	20/50	38/50
β-Myrcene (April 2002)	26/50	14/50	4/50	16/50	34/50
Pulegone (April 2003)	22/50	13/50	1/50	13/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	22/50	17/50	2/50	19/50	34/50
Total (%)	121/250 (48.4%)	72/250 (28.8%)	10/250 (4.0%)	79/250 (31.6%)	165/250 (66.0%)
Mean ± standard deviation	48.4% ± 4.6%	28.8% ± 9.0%	4.0% ± 3.2%	31.6% ± 7.7%	66.0% ± 7.2%
Range	44%-54%	16%-40%	0%-8%	22%-40%	58%-76%
Overall Historical Incidence: All Routes					
Total (%)	684/1,248 (54.8%)	389/1,248 (31.2%)	50/1,248 (4.0%)	421/1,248 (33.7%)	889/1,248 (71.2%)
Mean ± standard deviation	54.8% ± 11.7%	31.2% ± 10.3%	4.0% ± 6.7%	33.7% ± 10.8%	71.2% ± 11.5%
Range	24%-72%	16%-56%	0%-34%	18%-58%	52%-92%

[a] Data as of March 22, 2010

TABLE C3b
Historical Incidence of Malignant Lymphoma in Control Male B6C3F1 Mice[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Isoeugenol (May 2002)	0/50
Kava Kava Extract (August 2004)	2/50
β-Myrcene (April 2002)	1/50
Pulegone (April 2003)	1/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	1/50
Total (%)	5/250 (2.0%)
Mean ± standard deviation	2.0% ± 1.4%
Range	0%-4%
Overall Historical Incidence: All Routes	
Total (%)	48/1,250 (3.8%)
Mean ± standard deviation	3.8% ± 3.0%
Range	0%-12%

[a] Data as of March 22, 2010

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	10	10	7
Natural deaths	13	7	5	7
Survivors				
Died last week of study	1		1	
Terminal sacrifice	33	33	34	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(49)	(50)	(49)	(47)
Infiltration cellular, mononuclear cell	1 (2%)		1 (2%)	1 (2%)
Inflammation, granulomatous			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Serosa, inflammation, granulomatous	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		1 (2%)	
Epithelium, hyperplasia	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Epithelium, hyperplasia		2 (4%)		
Peyer's patch, hyperplasia, lymphoid			1 (2%)	1 (2%)
Liver	(50)	(50)	(50)	(50)
Amyloid deposition			1 (2%)	1 (2%)
Angiectasis	3 (6%)	6 (12%)	7 (14%)	10 (20%)
Basophilic focus	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Clear cell focus	18 (36%)	18 (36%)	19 (38%)	21 (42%)
Degeneration, cystic	1 (2%)		1 (2%)	1 (2%)
Eosinophilic focus	28 (56%)	32 (64%)	42 (84%)	43 (86%)
Fatty change	32 (64%)	34 (68%)	27 (54%)	24 (48%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	1 (2%)	2 (4%)		1 (2%)
Hepatodiaphragmatic nodule			1 (2%)	
Infarct		1 (2%)	2 (4%)	
Infiltration cellular, mononuclear cell	49 (98%)	47 (94%)	47 (94%)	48 (96%)
Inflammation, suppurative				1 (2%)
Mineralization	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Mixed cell focus	15 (30%)	28 (56%)	15 (30%)	12 (24%)
Necrosis	3 (6%)	10 (20%)	7 (14%)	13 (26%)
Tension lipidosis	5 (10%)	2 (4%)	3 (6%)	2 (4%)
Vacuolization cytoplasmic		1 (2%)		1 (2%)
Bile duct, cyst				1 (2%)
Centrilobular, fatty change		1 (2%)		1 (2%)
Centrilobular, hypertrophy		34 (68%)	30 (60%)	39 (78%)
Mesentery	(3)	(6)	(2)	(3)
Inflammation, chronic	1 (33%)			
Fat, necrosis	2 (67%)	6 (100%)	2 (100%)	3 (100%)

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Inflammation, granulomatous			1 (2%)	
Inflammation, chronic			1 (2%)	
Acinus, atrophy	1 (2%)		1 (2%)	
Acinus, hypertrophy	2 (4%)			
Duct, cyst				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	43 (86%)	36 (72%)	40 (80%)	36 (72%)
Parotid gland, atrophy	1 (2%)			
Parotid gland, hyperplasia			1 (2%)	
Parotid gland, mineralization		1 (2%)		
Submandibular gland, atrophy		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	19 (38%)	22 (44%)	24 (48%)	24 (48%)
Mineralization			1 (2%)	
Necrosis	8 (16%)	11 (22%)	12 (24%)	10 (20%)
Epithelium, hyperplasia	18 (36%)	22 (44%)	25 (50%)	22 (44%)
Stomach, glandular	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	2 (4%)		2 (4%)	1 (2%)
Mineralization		1 (2%)		
Ulcer	1 (2%)			
Epithelium, hyperplasia	2 (4%)			
Glands, ectasia	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Tooth	(33)	(32)	(27)	(21)
Dysplasia	33 (100%)	32 (100%)	27 (100%)	21 (100%)
Inflammation, suppurative		1 (3%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	14 (28%)	16 (32%)	11 (22%)	14 (28%)
Inflammation, suppurative		1 (2%)		1 (2%)
Inflammation, chronic		1 (2%)		
Artery, infiltration cellular, mononuclear cell	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Myocardium, mineralization	1 (2%)	4 (8%)		1 (2%)
Valve, inflammation				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hypertrophy	1 (2%)	2 (4%)		2 (4%)
Infiltration cellular, mononuclear cell	1 (2%)			
Vacuolization cytoplasmic	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Subcapsular, hyperplasia	44 (88%)	45 (90%)	48 (96%)	49 (98%)
Zona reticularis, hyperplasia	5 (10%)	3 (6%)	2 (4%)	4 (8%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	37 (74%)	35 (70%)	36 (72%)	33 (66%)
Parathyroid gland	(47)	(44)	(46)	(44)
Cyst	1 (2%)	1 (2%)		1 (2%)
Infiltration cellular, mononuclear cell	1 (2%)			
Pituitary gland	(49)	(50)	(50)	(50)
Cyst	1 (2%)		1 (2%)	2 (4%)
Pars distalis, hyperplasia	1 (2%)		1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(49)	(50)
Atrophy	1 (2%)			
Cyst	4 (8%)		1 (2%)	
Follicle, hyperplasia	1 (2%)		1 (2%)	
General Body System				
Peritoneum	(0)	(0)	(0)	(2)
Inflammation, chronic				2 (100%)
Genital System				
Coagulating gland	(0)	(1)	(1)	(0)
Hyperplasia		1 (100%)		
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	2 (4%)		3 (6%)	1 (2%)
Infiltration cellular, mononuclear cell	29 (58%)	22 (44%)	33 (66%)	28 (56%)
Inflammation, chronic		1 (2%)		
Penis	(0)	(1)	(0)	(0)
Infiltration cellular, polymorphonuclear		1 (100%)		
Preputial gland	(50)	(50)	(50)	(50)
Cyst	4 (8%)	5 (10%)	5 (10%)	3 (6%)
Inflammation	10 (20%)	4 (8%)	9 (18%)	13 (26%)
Prostate	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Fibrosis			1 (2%)	
Hyperplasia	1 (2%)			1 (2%)
Infiltration cellular, mononuclear cell	29 (58%)	31 (62%)	38 (76%)	29 (58%)
Inflammation	4 (8%)	9 (18%)	4 (8%)	2 (4%)
Mineralization		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Fibrosis	2 (4%)			
Hyperplasia				1 (2%)
Infiltration cellular, mononuclear cell	4 (8%)	4 (8%)	6 (12%)	
Inflammation	6 (12%)	5 (10%)		2 (4%)
Testes	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Mineralization	1 (2%)			
Germinal epithelium, atrophy	2 (4%)	4 (8%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(0)	(1)	(3)	(3)
Renal, hyperplasia, lymphoid			1 (33%)	
Lymph node, mandibular	(50)	(50)	(50)	(49)
Hyperplasia, lymphoid		3 (6%)	2 (4%)	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Atrophy	1 (2%)			
Hyperplasia, lymphoid		1 (2%)	2 (4%)	
Spleen	(50)	(50)	(49)	(50)
Angiectasis			1 (2%)	
Hematopoietic cell proliferation	16 (32%)	19 (38%)	21 (43%)	15 (30%)
Hyperplasia, lymphoid	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Lymphoid follicle, atrophy	6 (12%)	5 (10%)	3 (6%)	6 (12%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Hematopoietic System (continued)				
Thymus	(48)	(47)	(47)	(49)
Atrophy	41 (85%)	41 (87%)	46 (98%)	43 (88%)
Ectopic thyroid		1 (2%)		
Hyperplasia, histiocytic	1 (2%)	1 (2%)		
Infiltration cellular, histiocyte			2 (4%)	
Inflammation, chronic active				1 (2%)
Epithelial cell, hyperplasia		1 (2%)		1 (2%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			2 (4%)	
Hemorrhage				1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)		1 (2%)
Ulcer	1 (2%)	1 (2%)	9 (18%)	4 (8%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, hyperostosis	1 (2%)			
Skeletal muscle	(2)	(0)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Hypothalamus, compression			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation		2 (4%)	3 (6%)	2 (4%)
Metaplasia, osseous			1 (2%)	
Mineralization			2 (4%)	
Alveolar epithelium, hyperplasia	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	6 (12%)	5 (10%)	1 (2%)
Bronchus, hyperplasia	2 (4%)	2 (4%)		1 (2%)
Bronchus, infiltration cellular, histiocyte			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Inflammation, chronic	9 (18%)	8 (16%)	7 (14%)	5 (10%)
Polyp, inflammatory		2 (4%)		1 (2%)
Nasolacrimal duct, inflammation, suppurative	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Respiratory epithelium, hyperplasia	1 (2%)		1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)	1 (2%)	
Epithelium, cytoplasmic alteration			1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Degeneration		1 (2%)		
Anterior chamber, inflammation, suppurative				1 (2%)
Cornea, inflammation, chronic		1 (2%)	1 (2%)	2 (4%)
Retrobulbar, inflammation, chronic				1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hyperplasia	4 (8%)	3 (6%)	1 (2%)	8 (16%)
Infiltration cellular, mononuclear cell	36 (72%)	30 (60%)	34 (68%)	39 (78%)
Inflammation, granulomatous			1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition			1 (2%)	
Hydronephrosis	6 (12%)	1 (2%)		1 (2%)
Inflammation	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Metaplasia, osseous		2 (4%)		2 (4%)
Mineralization	3 (6%)	1 (2%)		
Nephropathy	32 (64%)	40 (80%)	37 (74%)	38 (76%)
Thrombosis		1 (2%)		
Cortex, medulla, necrosis	6 (12%)			
Papilla, necrosis	3 (6%)			3 (6%)
Renal tubule, cyst			3 (6%)	
Renal tubule, dilatation		1 (2%)		
Renal tubule, hyperplasia	6 (12%)	20 (40%)	13 (26%)	10 (20%)
Renal tubule, mineralization	31 (62%)	42 (84%)	38 (76%)	31 (62%)
Renal tubule, pigmentation, lipofuscin	1 (2%)		1 (2%)	2 (4%)
Urethra	(2)	(4)	(0)	(1)
Cyst		1 (25%)		
Inflammation	2 (100%)	4 (100%)		1 (100%)
Necrosis				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	20 (40%)	25 (50%)	26 (52%)	20 (40%)
Inflammation	4 (8%)	2 (4%)		
Transitional epithelium, cytoplasmic alteration				1 (2%)
Transitional epithelium, hyperplasia		1 (2%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF KAVA KAVA EXTRACT

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1			1
Moribund	4	4	1	7
Natural deaths	7	12	4	5
Survivors				
Terminal sacrifice	38	34	45	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(48)	(49)	(50)
Liposarcoma, metastatic, skin		1 (2%)		
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Liposarcoma, metastatic, skin		1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Hemangioma				1 (2%)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Hepatoblastoma			1 (2%)	
Hepatocellular adenoma	8 (16%)	7 (14%)	8 (16%)	4 (8%)
Hepatocellular adenoma, multiple		4 (8%)	6 (12%)	1 (2%)
Hepatocellular carcinoma	2 (4%)	12 (24%)	7 (14%)	8 (16%)
Hepatocellular carcinoma, multiple	1 (2%)	1 (2%)	1 (2%)	
Liposarcoma, metastatic, skin		1 (2%)		
Sarcoma	1 (2%)			
Mesentery	(8)	(10)	(5)	(6)
Pancreas	(49)	(50)	(50)	(50)
Liposarcoma, metastatic, skin		1 (2%)		
Salivary glands	(49)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma			2 (4%)	
Squamous cell papilloma	3 (6%)	3 (6%)	2 (4%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Tooth	(1)	(1)	(2)	(2)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Fibrous histiocytoma, metastatic, skin				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)		1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Carcinoma			1 (2%)	
Parathyroid gland	(48)	(44)	(48)	(48)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	2 (4%)	1 (2%)	1 (2%)	
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Genital System				
Clitoral gland	(49)	(48)	(50)	(50)
Ovary	(49)	(50)	(50)	(50)
Cystadenoma	5 (10%)	9 (18%)	9 (18%)	4 (8%)
Granulosa cell tumor benign			1 (2%)	
Hemangioma		3 (6%)	1 (2%)	
Hemangiosarcoma				1 (2%)
Luteoma	1 (2%)			
Teratoma benign		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Leiomyosarcoma				1 (2%)
Polyp stromal	2 (4%)			2 (4%)
Vagina	(0)	(0)	(0)	(1)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		2 (4%)		
Lymph node	(3)	(3)	(0)	(0)
Lymph node, mandibular	(48)	(49)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(49)	(48)	(50)	(49)
Hemangiosarcoma			1 (2%)	
Liposarcoma, metastatic, skin		1 (2%)		
Mast cell tumor malignant	1 (2%)			
Thymus	(50)	(50)	(49)	(47)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)			
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Fibrosarcoma		1 (2%)		
Fibrous histiocytoma				1 (2%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, liposarcoma		1 (2%)		
Subcutaneous tissue, schwannoma malignant		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		2 (4%)		1 (2%)
Skeletal muscle	(3)	(1)	(0)	(0)
Liposarcoma, metastatic, skin		1 (100%)		
Rhabdomyosarcoma	3 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(1)	(0)	(0)	(1)
Spinal cord	(1)	(0)	(0)	(1)
Sarcoma, metastatic, liver	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	4 (8%)	5 (10%)	2 (4%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)			
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Iris, melanoma benign			1 (2%)	
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	5 (10%)	5 (10%)	2 (4%)	5 (10%)
Bilateral, adenoma				1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Hemangioma			1 (2%)	
Urinary bladder	(50)	(48)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	3 (6%)			2 (4%)
Lymphoma malignant	6 (12%)	7 (14%)	1 (2%)	6 (12%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	39	38	35	30
Total primary neoplasms	53	70	58	44
Total animals with benign neoplasms	26	26	27	19
Total benign neoplasms	33	41	39	22
Total animals with malignant neoplasms	19	24	17	18
Total malignant neoplasms	20	29	19	22
Total animals with metastatic neoplasms	4	5	3	3
Total metastatic neoplasms	6	10	3	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 Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	5/50 (10%)	2/50 (4%)	6/50 (12%)
Adjusted rate[b]	10.9%	10.9%	4.2%	13.1%
Terminal rate[c]	3/38 (8%)	4/34 (12%)	2/45 (4%)	5/37 (14%)
First incidence (days)	421	723	729 (T)	548
Poly-3 test[d]	P=0.473	P=0.626N	P=0.198N	P=0.503
Liver: Hepatocellular Adenoma				
Overall rate	8/50 (16%)	11/50 (22%)	14/50 (28%)	5/50 (10%)
Adjusted rate	17.6%	23.7%	29.2%	11.0%
Terminal rate	7/38 (18%)	9/34 (27%)	14/45 (31%)	5/37 (14%)
First incidence (days)	560	669	729 (T)	729 (T)
Poly-3 test	P=0.212N	P=0.322	P=0.141	P=0.275N
Liver: Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	13/50 (26%)	8/50 (16%)	8/50 (16%)
Adjusted rate	6.7%	28.1%	16.5%	17.2%
Terminal rate	3/38 (8%)	9/34 (27%)	6/45 (13%)	3/37 (8%)
First incidence (days)	729 (T)	701	534	604
Poly-3 test	P=0.337	P=0.007	P=0.126	P=0.109
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	10/50 (20%)	21/50 (42%)	20/50 (40%)	13/50 (26%)
Adjusted rate	22.1%	45.1%	41.2%	28.0%
Terminal rate	9/38 (24%)	16/34 (47%)	18/45 (40%)	8/37 (22%)
First incidence (days)	560	669	534	604
Poly-3 test	P=0.542	P=0.015	P=0.036	P=0.338
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	3/50 (6%)	13/50 (26%)	9/50 (18%)	8/50 (16%)
Adjusted rate	6.7%	28.1%	18.5%	17.2%
Terminal rate	3/38 (8%)	9/34 (27%)	7/45 (16%)	3/37 (8%)
First incidence (days)	729 (T)	701	534	604
Poly-3 test	P=0.328	P=0.007	P=0.080	P=0.109
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	10/50 (20%)	21/50 (42%)	20/50 (40%)	13/50 (26%)
Adjusted rate	22.1%	45.1%	41.2%	28.0%
Terminal rate	9/38 (24%)	16/34 (47%)	18/45 (40%)	8/37 (22%)
First incidence (days)	560	669	534	604
Poly-3 test	P=0.542	P=0.015	P=0.036	P=0.338
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	5/50 (10%)	2/50 (4%)
Adjusted rate	4.5%	8.7%	10.3%	4.4%
Terminal rate	1/38 (3%)	1/34 (3%)	4/45 (9%)	2/37 (5%)
First incidence (days)	684	708	554	729 (T)
Poly-3 test	P=0.514N	P=0.350	P=0.248	P=0.691N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.4%	2.2%	6.3%	4.4%
Terminal rate	1/38 (3%)	1/34 (3%)	3/45 (7%)	1/37 (3%)
First incidence (days)	581	729 (T)	729 (T)	678
Poly-3 test	P=0.500	P=0.494N	P=0.525	P=0.692N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	8/50 (16%)	4/50 (8%)
Adjusted rate	8.8%	10.8%	16.5%	8.8%
Terminal rate	2/38 (5%)	2/34 (6%)	7/45 (16%)	3/37 (8%)
First incidence (days)	581	708	554	678
Poly-3 test	P=0.546	P=0.510	P=0.210	P=0.642N
Ovary: Hemangioma				
Overall rate	0/49 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.5%	2.1%	0.0%
Terminal rate	0/37 (0%)	3/34 (9%)	1/45 (2%)	0/37 (0%)
First incidence (days)	—[e]	729 (T)	729 (T)	—
Poly-3 test	P=0.350N	P=0.128	P=0.518	—[f]
Ovary: Cystadenoma				
Overall rate	5/49 (10%)	9/50 (18%)	9/50 (18%)	4/50 (8%)
Adjusted rate	11.4%	19.5%	18.6%	8.8%
Terminal rate	4/37 (11%)	8/34 (24%)	8/45 (18%)	4/37 (11%)
First incidence (days)	684	697	534	729 (T)
Poly-3 test	P=0.302N	P=0.220	P=0.252	P=0.481N
Skeletal Muscle: Rhabdomyosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.7%	0.0%	0.0%	0.0%
Terminal rate	2/38 (5%)	0/34 (0%)	0/45 (0%)	0/37 (0%)
First incidence (days)	639	—	—	—
Poly-3 test	P=0.045N	P=0.115N	P=0.109N	P=0.118N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.7%	6.5%	4.2%	0.0%
Terminal rate	3/38 (8%)	3/34 (9%)	2/45 (4%)	0/37 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.070N	P=0.650N	P=0.470N	P=0.116N
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	0/50 (0%)
Adjusted rate	6.7%	6.5%	8.4%	0.0%
Terminal rate	3/38 (8%)	3/34 (9%)	4/45 (9%)	0/37 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.111N	P=0.650N	P=0.536	P=0.116N
All Organs: Hemangioma				
Overall rate	0/50 (0%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	6.5%	6.3%	4.4%
Terminal rate	0/38 (0%)	3/34 (9%)	3/45 (7%)	1/37 (3%)
First incidence (days)	—	729 (T)	729 (T)	669
Poly-3 test	P=0.322	P=0.124	P=0.131	P=0.241
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.2%	10.9%	8.4%	6.6%
Terminal rate	1/38 (3%)	4/34 (12%)	4/45 (9%)	2/37 (5%)
First incidence (days)	729 (T)	724	729 (T)	669
Poly-3 test	P=0.417	P=0.108	P=0.200	P=0.311

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
All Organs: Histiocytic Sarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate	6.5%	0.0%	0.0%	4.4%
Terminal rate	0/38 (0%)	0/34 (0%)	0/45 (0%)	1/37 (3%)
First incidence (days)	544	—	—	725
Poly-3 test	P=0.527N	P=0.118N	P=0.112N	P=0.506N
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	7/50 (14%)	1/50 (2%)	6/50 (12%)
Adjusted rate	13.4%	15.1%	2.1%	13.2%
Terminal rate	6/38 (16%)	5/34 (15%)	1/45 (2%)	5/37 (14%)
First incidence (days)	729 (T)	669	729 (T)	678
Poly-3 test	P=0.424N	P=0.526	P=0.046N	P=0.609N
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	26/50 (52%)	27/50 (54%)	19/50 (38%)
Adjusted rate	56.0%	55.8%	55.1%	41.2%
Terminal rate	22/38 (58%)	20/34 (59%)	25/45 (56%)	17/37 (46%)
First incidence (days)	421	669	534	548
Poly-3 test	P=0.075N	P=0.575N	P=0.545N	P=0.108N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	24/50 (48%)	17/50 (34%)	18/50 (36%)
Adjusted rate	40.5%	50.3%	35.0%	38.3%
Terminal rate	13/38 (34%)	14/34 (41%)	15/45 (33%)	11/37 (30%)
First incidence (days)	544	521	534	548
Poly-3 test	P=0.288N	P=0.228	P=0.366N	P=0.496N
All Organs: Benign or Malignant Neoplasms				
Overall rate	39/50 (78%)	38/50 (76%)	35/50 (70%)	30/50 (60%)
Adjusted rate	80.4%	79.3%	71.3%	63.1%
Terminal rate	29/38 (76%)	26/34 (77%)	32/45 (71%)	22/37 (60%)
First incidence (days)	421	521	534	548
Poly-3 test	P=0.019N	P=0.551N	P=0.206N	P=0.045N

(T) Terminal sacrifice

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

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

[c] Observed incidence at terminal kill

[d] 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**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE D3
Historical Incidence of Liver Neoplasms in Control Female B6C3F1 Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma	Hepatocellular Carcinoma or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies				
Isoeugenol (May 2002)	11/49	3/49	13/49	3/49
Kava Kava Extract (August 2004)	8/50	3/50	10/50	3/50
β-Myrcene (April 2002)	6/50	1/50	7/50	1/50
Pulegone (April 2003)	13/49	5/49	17/49	5/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	3/49	2/49	4/49	2/49
Total (%)	41/247 (16.6%)	14/247 (5.7%)	51/247 (20.7%)	14/247 (5.7%)
Mean ± standard deviation	16.6% ± 8.1%	5.7% ± 3.0%	20.7% ± 10.4%	5.7% ± 3.0%
Range	6%-27%	2%-10%	8%-35%	2%-10%
Overall Historical Incidence: All Routes				
Total (%)	353/1,296 (27.2%)	136/1,296 (10.5%)	428/1,296 (33.0%)	138/1,296 (10.7%)
Mean ± standard deviation	27.2% ± 16.5%	10.5% ± 9.7%	33.0% ± 18.8%	10.6% ± 9.7%
Range	2%-62%	0%-46%	6%-76%	0%-46%

[a] Data as of March 22, 2010

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1			1
Moribund	4	4	1	7
Natural deaths	7	12	4	5
Survivors				
Terminal sacrifice	38	34	45	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		
Gallbladder	(50)	(48)	(49)	(50)
Cyst	1 (2%)	1 (2%)		1 (2%)
Epithelium, cytoplasmic alteration				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Metaplasia, squamous				1 (2%)
Epithelium, hyperplasia			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Ulcer			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Epithelium, hyperplasia				1 (2%)
Peyer's patch, hyperplasia			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)		2 (4%)	
Basophilic focus		1 (2%)	4 (8%)	1 (2%)
Clear cell focus	3 (6%)		1 (2%)	5 (10%)
Cyst			1 (2%)	
Eosinophilic focus	9 (18%)	7 (14%)	16 (32%)	26 (52%)
Fatty change	39 (78%)	33 (66%)	42 (84%)	23 (46%)
Hematopoietic cell proliferation	1 (2%)	4 (8%)	4 (8%)	1 (2%)
Infiltration cellular, mononuclear cell	45 (90%)	47 (94%)	49 (98%)	44 (88%)
Mixed cell focus	12 (24%)	13 (26%)	14 (28%)	7 (14%)
Necrosis	5 (10%)	4 (8%)	5 (10%)	1 (2%)
Tension lipidosis	3 (6%)	8 (16%)	7 (14%)	4 (8%)
Vacuolization cytoplasmic				1 (2%)
Centrilobular, hypertrophy		20 (40%)	48 (96%)	49 (98%)
Mesentery	(8)	(10)	(5)	(6)
Fat, necrosis	8 (100%)	10 (100%)	5 (100%)	6 (100%)
Pancreas	(49)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell				1 (2%)
Inflammation, chronic	2 (4%)			
Acinus, atrophy			1 (2%)	
Acinus, hypertrophy				1 (2%)
Acinus, vacuolization cytoplasmic			1 (2%)	
Duct, cyst		1 (2%)	1 (2%)	1 (2%)
Salivary glands	(49)	(49)	(50)	(50)
Atrophy		1 (2%)		
Infiltration cellular, mononuclear cell	34 (69%)	33 (67%)	31 (62%)	26 (52%)
Duct, submandibular gland, hyperplasia			1 (2%)	
Parotid gland, hyperplasia				1 (2%)

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion		1 (2%)	14 (28%)	11 (22%)
Inflammation, chronic	3 (6%)	6 (12%)	21 (42%)	22 (44%)
Ulcer		2 (4%)	3 (6%)	6 (12%)
Epithelium, hyperplasia	3 (6%)	6 (12%)	23 (46%)	24 (48%)
Stomach, glandular	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	2 (4%)	1 (2%)		1 (2%)
Mineralization	1 (2%)			
Ulcer				2 (4%)
Epithelium, hyperplasia			1 (2%)	2 (4%)
Glands, ectasia	1 (2%)	1 (2%)	2 (4%)	
Tooth	(1)	(1)	(2)	(2)
Dysplasia	1 (100%)	1 (100%)	2 (100%)	2 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Mineralization		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Thrombosis		1 (2%)		
Artery, infiltration cellular, mononuclear cell	1 (2%)	4 (8%)	3 (6%)	
Myocardium, mineralization		2 (4%)		1 (2%)
Valve, inflammation		2 (4%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Mineralization	1 (2%)			
Vacuolization cytoplasmic	1 (2%)		4 (8%)	
Subcapsular, hyperplasia	49 (98%)	50 (100%)	50 (100%)	50 (100%)
Zona reticularis, hyperplasia				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)		2 (4%)	2 (4%)
Vacuolization cytoplasmic				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	4 (8%)	1 (2%)	
Parathyroid gland	(48)	(44)	(48)	(48)
Cyst		1 (2%)		
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis			1 (2%)	
Cyst	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Pars distalis, hyperplasia	4 (8%)	4 (8%)	7 (14%)	5 (10%)
Thyroid gland	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Ectopic thymus	1 (2%)			
Infiltration cellular, mononuclear cell	1 (2%)			
Follicle, hyperplasia	1 (2%)			
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Inflammation, chronic				1 (100%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Genital System				
Clitoral gland	(49)	(48)	(50)	(50)
Inflammation	1 (2%)			
Ovary	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Cyst	4 (8%)	10 (20%)	6 (12%)	4 (8%)
Hemorrhage	1 (2%)			
Thrombosis		1 (2%)	2 (4%)	
Uterus	(50)	(50)	(50)	(50)
Inflammation, suppurative	3 (6%)	1 (2%)		
Metaplasia, squamous	1 (2%)	2 (4%)		
Endometrium, hyperplasia, cystic	42 (84%)	44 (88%)	42 (84%)	36 (72%)
Vagina	(0)	(0)	(0)	(1)
Vacuolization cytoplasmic				1 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis		2 (4%)	1 (2%)	1 (2%)
Necrosis				1 (2%)
Lymph node	(3)	(3)	(0)	(0)
Mediastinal, hyperplasia, lymphoid	1 (33%)			
Mediastinal, hyperplasia, plasma cell		1 (33%)		
Lymph node, mandibular	(48)	(49)	(50)	(50)
Hyperplasia, lymphoid	4 (8%)	4 (8%)	4 (8%)	2 (4%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, plasma cell		1 (2%)		
Inflammation, granulomatous				1 (2%)
Spleen	(49)	(48)	(50)	(49)
Hematopoietic cell proliferation	26 (53%)	24 (50%)	20 (40%)	10 (20%)
Hyperplasia, lymphoid	7 (14%)	6 (13%)	6 (12%)	5 (10%)
Lymphoid follicle, atrophy	4 (8%)	2 (4%)	4 (8%)	2 (4%)
Thymus	(50)	(50)	(49)	(47)
Atrophy	39 (78%)	44 (88%)	46 (94%)	40 (85%)
Hyperplasia, histiocytic	1 (2%)		2 (4%)	1 (2%)
Infiltration cellular, histiocyte	5 (10%)			2 (4%)
Epithelial cell, hyperplasia	1 (2%)			
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)			
Inflammation, chronic active	1 (2%)			
Metaplasia, squamous	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	
Inflammation, chronic	1 (2%)		1 (2%)	
Ulcer	1 (2%)	1 (2%)		
Subcutaneous tissue, fibrosis		1 (2%)		
Subcutaneous tissue, necrosis			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrosis	15 (30%)	8 (16%)	7 (14%)	9 (18%)
Joint, inflammation, chronic		1 (2%)		
Skeletal muscle	(3)	(1)	(0)	(0)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Choroid plexus, infiltration cellular, mononuclear cell	1 (2%)			
Hypothalamus, compression	2 (4%)			
Peripheral nerve	(1)	(0)	(0)	(1)
Infiltration cellular, mononuclear cell	1 (100%)			
Axon, degeneration	1 (100%)			1 (100%)
Spinal cord	(1)	(0)	(0)	(1)
Axon, degeneration	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Inflammation	1 (2%)		2 (4%)	
Inflammation, acute		1 (2%)		
Metaplasia, osseous		1 (2%)		
Mineralization		1 (2%)		
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)		1 (2%)
Alveolus, infiltration cellular, histiocyte		1 (2%)		1 (2%)
Serosa, fibrosis		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative				1 (2%)
Inflammation, chronic	4 (8%)	2 (4%)		
Glands, dilatation			1 (2%)	
Nasolacrimal duct, inflammation, suppurative			1 (2%)	
Olfactory epithelium, degeneration	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Anterior chamber, inflammation, suppurative	1 (2%)			
Harderian gland	(50)	(49)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	
Hyperplasia	3 (6%)	4 (8%)	5 (10%)	5 (10%)
Infiltration cellular, mononuclear cell	38 (76%)	42 (86%)	42 (84%)	38 (76%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Hydronephrosis		1 (2%)		
Inflammation	1 (2%)	1 (2%)		1 (2%)
Metaplasia, osseous	3 (6%)	1 (2%)	1 (2%)	5 (10%)
Mineralization	12 (24%)	20 (40%)	7 (14%)	13 (26%)
Nephropathy	21 (42%)	25 (50%)	26 (52%)	20 (40%)
Papilla, necrosis	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Pelvis, cyst			1 (2%)	
Renal tubule, hyperplasia	3 (6%)	1 (2%)	2 (4%)	
Urinary bladder	(50)	(48)	(50)	(50)
Infiltration cellular, mononuclear cell	34 (68%)	34 (71%)	40 (80%)	34 (68%)
Inflammation		1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Testing procedures used in the first study, conducted at BioReliance Corporation (Rockville, MD) followed protocols reported by Zeiger *et al.* (1992); in the test conducted at SITEK Research Laboratories (Rockville, MD), a slightly modified procedure was used, and that is described below. Kava kava extract was tested at both laboratories as a coded sample. The study conducted at SITEK Research Laboratories used the same lot of kava kava extract that was used for the 2-week, 3-month, and 2-year studies (lot no. 9077SDK). In the tests conducted at BioReliance Corporation, kava kava extract was incubated with the *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) for 20 minutes at 37° C. Two different concentrations of S9 were tested, 10% and 30%. 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 2 days incubation at 37° C.

The modified protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation and employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. Incubation of bacterial strains with kava kava and subsequent plating were carried out as described above for the traditional protocol.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of kava kava extract. At both laboratories, the assay limit dose of 10,000 µg/plate was selected as the high dose. All trials were repeated and those that were conducted with S9 activation enzymes were repeated using the same or higher concentrations of S9.

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 toxicity study, peripheral blood samples were obtained from five male and five 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 micronucleated cells in 2,000 normochromatic erythrocytes (NCEs; mature erythrocytes) per animal. In addition, the percentage of polychromatic erythrocytes (PCEs; reticulocytes) in a population of 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). 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

Kava kava extract was tested for bacterial mutagenicity in two independent assays using several strains of bacteria (*S. typhimurium* tester strains TA97, TA98, TA100, and TA1535 and *E. coli* strain WP2 *uvrA*/pKM101), with and without exogenous metabolic activation supplied by induced rat or hamster liver S9. Concentrations of kava kava extract ranged from 33 to 10,000 μg/plate. No increase in mutant colonies was seen in any of the tester strains, under any activation condition (Tables E1 and E2).

Peripheral blood samples from the mice in the 3-month study were evaluated for frequency of micronucleated erythrocytes; kava kava extract was administered via gavage, and doses ranged from 0.125 to 2.0 g/kg per day. No increases in the frequencies of micronucleated erythrocytes were observed in either male or female mice, and no significant changes in the percentage of reticulocytes in peripheral blood were observed (Table E3). These results indicate that treatment with kava kava extract did not produce chromosomal damage or bone marrow toxicity in male or female B6C3F1 mice.

TABLE E1
Mutagenicity Testing of Kava Kava Extract 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	84 ± 10	96 ± 4	108 ± 5	97 ± 4	124 ± 11	81 ± 15
	33	108 ± 2	85 ± 3				
	100	110 ± 13	92 ± 6	109 ± 11	85 ± 15	125 ± 12	64 ± 6
	333	105 ± 12	95 ± 3	112 ± 3	100 ± 10	124 ± 11	69 ± 4
	1,000	75 ± 8	73 ± 3[b]	107 ± 10	85 ± 3	121 ± 22	73 ± 8
	3,333	10 ± 8[c]	7 ± 2[c]	96 ± 2[d]	13 ± 11[d]	116 ± 15[d]	22 ± 8[d]
	10,000			6 ± 3[c]	0 ± 0[c]	8 ± 2[c]	5 ± 5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[e]		353 ± 12	270 ± 10	371 ± 30	1,007 ± 74	449 ± 8	462 ± 29
TA1535	0	16 ± 2	15 ± 2	14 ± 1	11 ± 1	13 ± 2	16 ± 2
	33	16 ± 2	14 ± 2			11 ± 1	
	100	10 ± 3	14 ± 1	10 ± 2	13 ± 2	12 ± 1	18 ± 2
	333	18 ± 4	14 ± 1	12 ± 2	13 ± 2	12 ± 1	17 ± 1
	1,000	16 ± 0[d]	12 ± 2	14 ± 2	11 ± 1	11 ± 1[d]	12 ± 1
	3,333	1 ± 1[c]	3 ± 1[c]	10 ± 4[d]	10 ± 1[d]	2 ± 1[c]	15 ± 3[d]
	10,000			3 ± 2[c]	1 ± 1[c]		7 ± 1[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		274 ± 21	236 ± 7	53 ± 2	162 ± 2	253 ± 21	127 ± 17
TA97	0	122 ± 8	100 ± 3	116 ± 3	164 ± 9		
	33	139 ± 13	95 ± 5				
	100	138 ± 12	102 ± 6	125 ± 5	182 ± 20		
	333	128 ± 10	76 ± 9	115 ± 1	151 ± 11		
	1,000	110 ± 18[d]	79 ± 2	123 ± 3	168 ± 19		
	3,333	5 ± 2[c]	29 ± 7[c]	103 ± 9[d]	153 ± 12[d]		
	10,000			31 ± 8[c]	94 ± 14[c]		
Trial summary		Negative	Negative	Negative	Negative		
Positive control		443 ± 88	397 ± 39	538 ± 10	986 ± 222		
		With 10% rat S9	**With 10% rat S9**	**With 30% rat S9**			
TA97 (continued)	0	125 ± 3	101 ± 17	180 ± 13			
	33						
	100	144 ± 1	80 ± 8	173 ± 8			
	333	133 ± 3	90 ± 2	211 ± 9			
	1,000	163 ± 9	87 ± 5	199 ± 9			
	2,000		84 ± 17				
	3,333	128 ± 13[d]	75 ± 15[c]	222 ± 17[d]			
	10,000	15 ± 2[c]		88 ± 8[c]			
Trial summary		Equivocal	Negative	Negative			
Positive control		1,272 ± 26	661 ± 38	411 ± 6			

TABLE E1
Mutagenicity Testing of Kava Kava Extract 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	11 ± 1	21 ± 1	19 ± 0	10 ± 1	17 ± 1	13 ± 3
	33	12 ± 1	17 ± 2				
	100	10 ± 1	17 ± 2	21 ± 4	10 ± 2	24 ± 4	12 ± 1
	333	8 ± 3[f]	13 ± 1	19 ± 5	8 ± 2	21 ± 2	11 ± 2
	1,000	8 ± 3	16 ± 2	17 ± 4	9 ± 1	20 ± 2	8 ± 2
	3,333	3 ± 2[c]	1 ± 1[c]	15 ± 1[d]	3 ± 1[d]	15 ± 2[d]	7 ± 4[d]
	10,000			1 ± 1[c]	1 ± 1[c]	4 ± 1[c]	3 ± 2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		321 ± 13	342 ± 14	330 ± 18	832 ± 99	301 ± 17	347 ± 26

[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] Slight toxicity
[c] Slight toxicity and precipitate
[d] Precipitate
[e] 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.
[f] Contamination

TABLE E2
Mutagenicity Testing of Kava Kava Extract (lot number 9077SDK) in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	57 ± 4	67 ± 7	69 ± 2	78 ± 2
	500	59 ± 9			88 ± 9
	1,500	54 ± 12	53 ± 4	69 ± 8	78 ± 6
	2,500	55 ± 9	49 ± 1	76 ± 8	67 ± 1
	3,500	57 ± 5	42 ± 1	41 ± 4	51 ± 2
	5,000	51 ± 3	27 ± 1	24 ± 2	39 ± 5
	7,500	30 ± 4	23 ± 1	21 ± 2	39 ± 3
	10,000	20 ± 4			Toxic
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		421 ± 20	769 ± 63	827 ± 18	861 ± 11
TA98	0	20 ± 2	21 ± 0	28 ± 2	33 ± 4
	500	17 ± 2		29 ± 4	
	1,500	14 ± 2	22 ± 2	27 ± 3	26 ± 1
	2,500	11 ± 1	15 ± 1	25 ± 6	29 ± 3
	3,500	11 ± 2	11 ± 2	31 ± 1	30 ± 2
	5,000	14 ± 2	9 ± 1	23 ± 1	28 ± 3
	7,500	5 ± 2	5 ± 1	9 ± 2	20 ± 2
	10,000	5 ± 1		6 ± 1	
Trial summary		Negative	Negative	Negative	Negative
Positive control		629 ± 16	649 ± 13	1,079 ± 95	951 ± 41
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	125 ± 3	149 ± 7	199 ± 17	207 ± 7
	2,500	117 ± 12	143 ± 14	242 ± 10	218 ± 8
	3,500	108 ± 14	132 ± 8	217 ± 9	213 ± 12
	5,000	111 ± 5	125 ± 4	193 ± 13	218 ± 30
	7,500	95 ± 2	74 ± 4	179 ± 10	152 ± 4
	10,000	93 ± 1	76 ± 6	166 ± 6	136 ± 15
Trial summary		Negative	Negative	Negative	Negative
Positive control		2,161 ± 37	859 ± 52	1,250 ± 39	1,015 ± 12

[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.

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Kava Kava Extract by Gavage for 3 Months[a]

Compound	Dose (g/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn Oil[d]	0	5	2.30 ± 0.41		3.480 ± 0.16
Kava kava extract	0.125	5	1.90 ± 0.37	0.7317	3.600 ± 0.18
	0.25	5	2.40 ± 0.24	0.4419	3.880 ± 0.34
	0.5	5	2.90 ± 0.68	0.2024	3.560 ± 0.31
	1.0	5	1.70 ± 0.41	0.8289	3.600 ± 0.28
	2.0	5	3.40 ± 0.58	0.0723	3.340 ± 0.11
			P=0.050[e]		
Female					
Corn Oil	0	5	1.50 ± 0.35		3.980 ± 0.27
Kava kava extract	0.125	5	2.20 ± 0.25	0.1247	4.740 ± 0.32
	0.25	5	1.80 ± 0.64	0.3006	3.500 ± 0.34
	0.5	5	1.80 ± 0.25	0.3006	3.740 ± 0.53
	1.0	5	1.40 ± 0.29	0.5737	3.120 ± 0.37
	2.0	5	1.60 ± 0.33	0.4287	3.340 ± 0.42
			P=0.730		

[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≤0.005
[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 Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
n						
Day 4	10	10	10	10	10	8
Day 23	10	10	10	10	10	8
Week 14	10	10	9	10	10	7
Hematology						
Hematocrit (auto) (%)						
Day 4	45.6 ± 0.6	45.0 ± 0.4	45.2 ± 0.7	45.0 ± 0.6	45.8 ± 0.5	47.1 ± 0.6
Day 23	51.8 ± 0.7	50.0 ± 0.8	50.1 ± 0.6	49.5 ± 0.5	50.1 ± 0.6	49.3 ± 0.4
Week 14	48.9 ± 0.4	49.1 ± 0.5	48.0 ± 0.3	48.5 ± 0.3	49.9 ± 0.6	49.8 ± 1.4
Hemoglobin (g/dL)						
Day 4	13.5 ± 0.2	13.5 ± 0.1	13.7 ± 0.2	13.5 ± 0.2	13.7 ± 0.2	14.2 ± 0.2*
Day 23	15.4 ± 0.2	15.0 ± 0.2	15.0 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	14.7 ± 0.1**
Week 14	15.4 ± 0.1	15.4 ± 0.2	15.0 ± 0.1	15.1 ± 0.1	15.4 ± 0.2	15.2 ± 0.4
Erythrocytes (10^6/µL)						
Day 4	7.34 ± 0.12	7.27 ± 0.07	7.38 ± 0.12	7.28 ± 0.09	7.47 ± 0.08	7.79 ± 0.11
Day 23	8.52 ± 0.12	8.35 ± 0.11	8.28 ± 0.10	8.18 ± 0.09	8.34 ± 0.07	8.20 ± 0.07
Week 14	8.83 ± 0.08	8.84 ± 0.09	8.70 ± 0.07	8.73 ± 0.07	8.95 ± 0.11	9.14 ± 0.22
Reticulocytes (10^3/µL)						
Day 4	446.9 ± 32.2	503.5 ± 22.4	451.4 ± 26.6	483.7 ± 24.2	426.1 ± 18.3	287.9 ± 23.3**
Day 23	277.0 ± 9.9	284.0 ± 8.9	298.6 ± 9.2	321.7 ± 9.1**	315.0 ± 8.3**	433.7 ± 43.6**
Week 14	202.5 ± 2.2	207.5 ± 7.4	195.9 ± 7.5	217.8 ± 4.7*	266.0 ± 31.3**	260.9 ± 11.1**
Mean cell volume (fL)						
Day 4	62.2 ± 0.4	61.9 ± 0.3	61.3 ± 0.4	61.8 ± 0.2	61.2 ± 0.2	60.5 ± 0.2**
Day 23	60.8 ± 0.2	59.8 ± 0.2*	60.5 ± 0.1	60.5 ± 0.2	60.0 ± 0.3	60.1 ± 0.2
Week 14	55.4 ± 0.3	55.6 ± 0.2	55.2 ± 0.2	55.6 ± 0.2	55.8 ± 0.2	54.5 ± 0.4
Mean cell hemoglobin (pg)						
Day 4	18.4 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.3 ± 0.1	18.3 ± 0.1
Day 23	18.1 ± 0.1	18.0 ± 0.1	18.2 ± 0.1	18.2 ± 0.1	17.9 ± 0.0	17.9 ± 0.2
Week 14	17.5 ± 0.1	17.4 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	17.2 ± 0.1*	16.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	29.6 ± 0.1	30.0 ± 0.2	30.3 ± 0.2*	29.9 ± 0.2	29.9 ± 0.1	30.2 ± 0.1
Day 23	29.7 ± 0.1	30.1 ± 0.2	30.0 ± 0.2	30.1 ± 0.2	29.8 ± 0.2	29.7 ± 0.3
Week 14	31.5 ± 0.2	31.4 ± 0.2	31.3 ± 0.1	31.2 ± 0.1	30.9 ± 0.1**	30.5 ± 0.1**
Platelets (10^3/µL)						
Day 4	1,045.0 ± 35.0	1,150.0 ± 35.0	1,068.0 ± 43.0	1,062.0 ± 38.0	1,116.0 ± 45.0	1,224.0 ± 26.0**
Day 23	985.3 ± 34.9	993.8 ± 19.7	955.9 ± 36.4	1,032.5 ± 33.0	984.4 ± 39.4	1,035.1 ± 50.8
Week 14	729.6 ± 14.8	695.4 ± 22.6	735.0 ± 14.1	750.9 ± 10.8	769.9 ± 18.1	798.7 ± 17.6*
Leukocytes (10^3/µL)						
Day 4	9.48 ± 0.45	9.81 ± 0.32	10.37 ± 0.44	9.66 ± 0.52	9.70 ± 0.45	8.28 ± 0.83
Day 23	12.08 ± 0.67	12.99 ± 0.44	12.11 ± 0.52	11.90 ± 0.29	11.53 ± 0.68	11.24 ± 0.58
Week 14	9.06 ± 0.69	7.56 ± 0.77	8.63 ± 0.44	7.20 ± 0.67	7.26 ± 0.59	7.21 ± 0.62
Segmented neutrophils (10^3/µL)						
Day 4	0.93 ± 0.05	1.01 ± 0.03	1.01 ± 0.06	1.15 ± 0.05**	1.19 ± 0.07**	1.67 ± 0.18**
Day 23	1.01 ± 0.06	1.06 ± 0.05	1.05 ± 0.03	1.05 ± 0.03	1.03 ± 0.05	1.11 ± 0.08
Week 14	1.29 ± 0.11	1.26 ± 0.11	1.25 ± 0.07	1.04 ± 0.07	1.35 ± 0.11	1.30 ± 0.05
Lymphocytes (10^3/µL)						
Day 4	8.24 ± 0.42	8.45 ± 0.30	9.00 ± 0.40	8.19 ± 0.48	8.16 ± 0.39	6.36 ± 0.88
Day 23	10.70 ± 0.59	11.51 ± 0.38	10.69 ± 0.50	10.46 ± 0.28	10.14 ± 0.61	9.72 ± 0.55
Week 14	7.52 ± 0.55	6.07 ± 0.63	7.14 ± 0.37	5.98 ± 0.59	5.73 ± 0.49	5.73 ± 0.57
Monocytes (10^3/µL)						
Day 4	0.23 ± 0.03	0.24 ± 0.02	0.26 ± 0.02	0.23 ± 0.01	0.24 ± 0.02	0.19 ± 0.02
Day 23	0.24 ± 0.02	0.29 ± 0.03	0.26 ± 0.02	0.25 ± 0.01	0.26 ± 0.03	0.31 ± 0.02
Week 14	0.13 ± 0.02	0.14 ± 0.03	0.14 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.02

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male (continued)						
n						
Day 4	10	10	10	10	10	8
Day 23	10	10	10	10	10	8
Week 14	10	10	9	10	10	7
Hematology (continued)						
Basophils (10^3/μL)						
Day 4	0.046 ± 0.008	0.061 ± 0.003	0.061 ± 0.009	0.055 ± 0.006	0.053 ± 0.005	0.034 ± 0.004
Day 23	0.077 ± 0.013	0.082 ± 0.007	0.069 ± 0.006	0.083 ± 0.010	0.066 ± 0.005	0.065 ± 0.003
Week 14	0.038 ± 0.006	0.033 ± 0.006	0.032 ± 0.004	0.028 ± 0.006	0.036 ± 0.007	0.024 ± 0.006
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.06 ± 0.01	0.03 ± 0.01
Day 23	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
Week 14	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01*	0.05 ± 0.01*	0.04 ± 0.00**
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	11.0 ± 0.5	10.9 ± 0.7	11.9 ± 0.4	11.4 ± 0.5	11.4 ± 0.5	13.8 ± 1.3
Day 23	13.1 ± 0.4	12.5 ± 0.3	12.4 ± 0.4	12.6 ± 0.5	12.8 ± 0.4	12.6 ± 0.3
Week 14	12.5 ± 0.5	12.1 ± 0.7	13.2 ± 0.5	12.9 ± 0.6	12.6 ± 0.9	15.7 ± 0.6*
Creatinine (mg/dL)						
Day 4	0.46 ± 0.02	0.44 ± 0.02	0.46 ± 0.02	0.45 ± 0.02	0.47 ± 0.02	0.45 ± 0.03
Day 23	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.50 ± 0.02	0.50 ± 0.00
Week 14	0.54 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.58 ± 0.01	0.59 ± 0.02	0.60 ± 0.03
Glucose (mg/dL)						
Day 4	161 ± 3	157 ± 3	164 ± 2	160 ± 2	149 ± 3*	143 ± 3**
Day 23	151 ± 1	155 ± 3	152 ± 2	159 ± 3	160 ± 4	145 ± 2
Week 14	165 ± 3	175 ± 4	166 ± 4	169 ± 4	156 ± 5	146 ± 3**
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	5.9 ± 0.1
Day 23	6.9 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	6.9 ± 0.1
Week 14	6.8 ± 0.1	6.7 ± 0.0	6.8 ± 0.1	7.1 ± 0.1**	7.4 ± 0.1**	7.9 ± 0.3**
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.4 ± 0.1	4.3 ± 0.1	4.2 ± 0.1
Day 23	4.8 ± 0.1	4.7 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.9 ± 0.1	4.8 ± 0.0
Week 14	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.0**	5.0 ± 0.1**	5.3 ± 0.1**
Cholesterol (mg/dL)						
Day 4	95 ± 1	100 ± 1**	99 ± 2*	114 ± 2**	124 ± 3**	132 ± 6**
Day 23	87 ± 1	87 ± 2	91 ± 2	97 ± 2**	97 ± 3**	94 ± 3**
Week 14	77 ± 1	75 ± 1	79 ± 1	83 ± 2*	86 ± 2**	92 ± 4**
Triglycerides (mg/dL)						
Day 4	66 ± 4	74 ± 7	72 ± 4	83 ± 7	88 ± 8	89 ± 10
Day 23	173 ± 16	141 ± 16	150 ± 10	146 ± 14	111 ± 9**	114 ± 8**
Week 14	113 ± 11	104 ± 9	118 ± 9	115 ± 11	88 ± 8	94 ± 13
Alanine aminotransferase (IU/L)						
Day 4	54 ± 2	57 ± 1	53 ± 2	53 ± 1	55 ± 2	73 ± 5*
Day 23	49 ± 2	47 ± 1	48 ± 1	47 ± 2	44 ± 1	54 ± 2
Week 14	71 ± 5	53 ± 2**	47 ± 1**	40 ± 1**	45 ± 2**	63 ± 4**
Alkaline phosphatase (IU/L)						
Day 4	656 ± 20	677 ± 10	659 ± 13	652 ± 12	662 ± 15	685 ± 30
Day 23	492 ± 9	472 ± 6	481 ± 6	457 ± 12*	424 ± 10**	442 ± 13**
Week 14	225 ± 6	221 ± 5	215 ± 6	209 ± 5	202 ± 5**	209 ± 9*

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male (continued)						
n						
Day 4	10	10	10	10	10	8
Day 23	10	10	10	10	10	8
Week 14	10	10	9	10	10	7
Clinical Chemistry (continued)						
Creatine kinase (IU/L)						
Day 4	241 ± 11	237 ± 20	247 ± 27	212 ± 13	228 ± 27	257 ± 34
Day 23	219 ± 21	169 ± 20	218 ± 26	232 ± 37	156 ± 9	221 ± 40
Week 14	87 ± 8	94 ± 8	101 ± 13	108 ± 12	93 ± 11	134 ± 28
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	15 ± 0	16 ± 1	16 ± 1	16 ± 1	20 ± 2
Day 23	21 ± 1	19 ± 1	20 ± 1	20 ± 1	18 ± 1*	20 ± 1
Week 14	18 ± 1	16 ± 1	13 ± 1**	13 ± 1**	14 ± 1**	16 ± 3*
γ-Glutamyltransferase (IU/L)						
Day 4	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.3	0.3 ± 0.2	0.1 ± 0.1	2.8 ± 0.6**
Day 23	0.0 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.9 ± 0.2**
Week 14	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.1**
Bile salts (μmol/L)						
Day 4	11.2 ± 2.0	10.1 ± 1.7	7.2 ± 1.0	6.5 ± 0.9	7.9 ± 1.5	13.3 ± 2.7
Day 23	10.6 ± 2.2	12.9 ± 2.1	8.7 ± 2.3	7.9 ± 1.6	7.7 ± 1.5	8.4 ± 2.2
Week 14	8.6 ± 1.8	6.0 ± 1.1	5.9 ± 1.2	7.2 ± 1.3	6.1 ± 1.1	9.1 ± 1.1
Female						
Hematology						
n						
Day 23	7	10	10	9	10	10
Week 14	9	10	10	10	9	6
Hematocrit (auto) (%)						
Day 23	48.3 ± 0.5	48.6 ± 0.8	48.3 ± 0.4	49.0 ± 0.3	49.0 ± 0.7	50.2 ± 0.6
Week 14	47.4 ± 0.2	47.7 ± 0.3	47.5 ± 0.4	47.7 ± 0.4	47.4 ± 0.5	48.7 ± 1.1
Hemoglobin (g/dL)						
Day 23	14.9 ± 0.1	15.0 ± 0.2	14.9 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	15.0 ± 0.2
Week 14	15.6 ± 0.1	15.6 ± 0.1	15.6 ± 0.1	15.5 ± 0.2	15.2 ± 0.2	15.5 ± 0.3
Erythrocytes (10^6/μL)						
Day 23	8.14 ± 0.07	8.21 ± 0.13	8.13 ± 0.05	8.30 ± 0.06	8.22 ± 0.10	8.43 ± 0.08
Week 14	8.32 ± 0.04	8.37 ± 0.06	8.30 ± 0.06	8.37 ± 0.06	8.38 ± 0.08	8.70 ± 0.19
Reticulocytes (10^3/μL)						
Day 23	158.4 ± 4.4	152.9 ± 3.0	161.8 ± 4.5	155.3 ± 4.2	167.1 ± 4.3	212.1 ± 13.4**
Week 14	176.1 ± 5.8	180.4 ± 5.0	165.5 ± 4.4	174.7 ± 5.8	171.1 ± 7.9	165.7 ± 9.5
Mean cell volume (fL)						
Day 23	59.3 ± 0.3	59.2 ± 0.2	59.4 ± 0.4	59.1 ± 0.3	59.6 ± 0.3	59.5 ± 0.3
Week 14	57.0 ± 0.1	56.9 ± 0.1	57.2 ± 0.1	56.9 ± 0.2	56.6 ± 0.2	55.9 ± 0.1**
Mean cell hemoglobin (pg)						
Day 23	18.3 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	17.8 ± 0.1
Week 14	18.7 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	18.5 ± 0.1	18.1 ± 0.1**	17.8 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 23	30.9 ± 0.2	30.9 ± 0.3	30.9 ± 0.2	31.0 ± 0.2	30.8 ± 0.2	30.0 ± 0.1**
Week 14	32.8 ± 0.2	32.7 ± 0.1	32.8 ± 0.2	32.5 ± 0.2	32.1 ± 0.2*	31.8 ± 0.3*
Platelets (10^3/μL)						
Day 23	860.7 ± 13.8	846.1 ± 25.1	877.4 ± 23.4	844.8 ± 29.0	830.0 ± 15.8	839.7 ± 32.2
Week 14	730.3 ± 31.7	673.5 ± 22.1	693.2 ± 18.7	706.4 ± 23.5	649.9 ± 22.1	630.7 ± 10.7

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Female (continued)						
Hematology (continued)						
n						
Day 23	7	10	10	9	10	10
Week 14	9	10	10	10	9	6
Leukocytes (10^3/μL)						
Day 23	10.41 ± 0.63	9.43 ± 0.57	9.23 ± 0.64	9.47 ± 0.72	10.63 ± 0.40	9.39 ± 0.43
Week 14	8.48 ± 0.61	7.46 ± 0.38	8.12 ± 0.28	8.22 ± 0.29	8.60 ± 0.39	7.48 ± 0.42
Segmented neutrophils (10^3/μL)						
Day 23	0.83 ± 0.08	0.74 ± 0.07	0.75 ± 0.08	0.90 ± 0.10	1.02 ± 0.05	0.96 ± 0.06
Week 14	1.09 ± 0.06	1.14 ± 0.07	1.28 ± 0.10	1.23 ± 0.06	1.24 ± 0.09	1.35 ± 0.06
Lymphocytes (10^3/μL)						
Day 23	9.31 ± 0.56	8.41 ± 0.51	8.23 ± 0.55	8.31 ± 0.64	9.29 ± 0.37	8.11 ± 0.39
Week 14	7.14 ± 0.54	6.08 ± 0.39	6.59 ± 0.23	6.74 ± 0.23	7.11 ± 0.36	5.91 ± 0.43
Monocytes (10^3/μL)						
Day 23	0.17 ± 0.02	0.17 ± 0.03	0.17 ± 0.02	0.16 ± 0.02	0.23 ± 0.02*	0.24 ± 0.02*
Week 14	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.15 ± 0.02	0.14 ± 0.01
Basophils (10^3/μL)						
Day 23	0.040 ± 0.004	0.039 ± 0.006	0.028 ± 0.004	0.032 ± 0.006	0.039 ± 0.003	0.031 ± 0.003
Week 14	0.032 ± 0.004	0.033 ± 0.003	0.036 ± 0.004	0.044 ± 0.003	0.033 ± 0.003	0.033 ± 0.008
Eosinophils (10^3/μL)						
Day 23	0.07 ± 0.01	0.08 ± 0.02	0.05 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Week 14	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00*	0.06 ± 0.01**	0.04 ± 0.00**
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	10	10	9	6
Urea nitrogen (mg/dL)						
Day 4	13.2 ± 0.9	12.2 ± 0.7	12.2 ± 0.3	13.4 ± 0.7	12.3 ± 0.5	12.8 ± 0.6
Day 23	13.1 ± 0.5	13.0 ± 0.5	13.4 ± 0.5	13.3 ± 0.5	12.3 ± 0.6	11.1 ± 0.3**
Week 14	12.3 ± 0.5	14.3 ± 0.5*	13.1 ± 0.6	13.0 ± 0.4	14.1 ± 0.6	15.3 ± 0.8**
Creatinine (mg/dL)						
Day 4	0.41 ± 0.01	0.41 ± 0.01	0.43 ± 0.02	0.40 ± 0.00	0.40 ± 0.00	0.42 ± 0.02
Day 23	0.45 ± 0.02	0.48 ± 0.01	0.47 ± 0.02	0.47 ± 0.02	0.48 ± 0.01	0.44 ± 0.02
Week 14	0.51 ± 0.01	0.52 ± 0.01	0.50 ± 0.00	0.50 ± 0.00	0.47 ± 0.02	0.48 ± 0.02
Glucose (mg/dL)						
Day 4	138 ± 3	133 ± 3	137 ± 2	134 ± 3	130 ± 2*	125 ± 4**
Day 23	152 ± 3	151 ± 5	149 ± 4	149 ± 4	140 ± 3**	127 ± 4**
Week 14	142 ± 5	155 ± 5	142 ± 4	139 ± 4	140 ± 7	129 ± 2
Total protein (g/dL)						
Day 4	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.0	6.2 ± 0.0*	6.1 ± 0.1	5.9 ± 0.1
Day 23	5.8 ± 0.1	6.0 ± 0.1	6.1 ± 0.1*	6.2 ± 0.0**	6.2 ± 0.1**	6.3 ± 0.1**
Week 14	6.4 ± 0.1	6.7 ± 0.1**	6.5 ± 0.1*	6.8 ± 0.1**	6.8 ± 0.1**	7.2 ± 0.1**
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	4.3 ± 0.0
Day 23	4.4 ± 0.0	4.5 ± 0.1	4.5 ± 0.0*	4.6 ± 0.0**	4.6 ± 0.0**	4.6 ± 0.1**
Week 14	4.6 ± 0.0	4.8 ± 0.0**	4.8 ± 0.0*	4.9 ± 0.1**	4.9 ± 0.0**	5.1 ± 0.0**
Cholesterol (mg/dL)						
Day 4	85 ± 3	92 ± 3	89 ± 2	109 ± 2**	112 ± 2**	128 ± 5**
Day 23	73 ± 1	78 ± 2	83 ± 1**	90 ± 1**	100 ± 2**	107 ± 3**
Week 14	69 ± 2	83 ± 2**	75 ± 2*	92 ± 2**	99 ± 2**	105 ± 4**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Kava Kava Extract

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	10	10	9	6
Triglycerides (mg/dL)						
Day 4	76 ± 6	78 ± 6	66 ± 2	78 ± 4	68 ± 6	74 ± 4
Day 23	47 ± 6	51 ± 4	51 ± 5	53 ± 4	55 ± 5	64 ± 6
Week 14	56 ± 4	62 ± 7	52 ± 5	53 ± 4	57 ± 5	55 ± 8
Alanine aminotransferase (IU/L)						
Day 4	43 ± 1	43 ± 2	40 ± 1	42 ± 1	43 ± 2	57 ± 2**
Day 23	37 ± 1	37 ± 1	35 ± 1	35 ± 1	37 ± 1	55 ± 4*
Week 14	45 ± 2	50 ± 4	43 ± 2	38 ± 1	43 ± 2	62 ± 4
Alkaline phosphatase (IU/L)						
Day 4	531 ± 10	542 ± 9	515 ± 7	515 ± 13	508 ± 10	558 ± 18
Day 23	320 ± 5	333 ± 10	305 ± 10	303 ± 6	276 ± 6**	331 ± 11
Week 14	197 ± 7	176 ± 4	178 ± 4	163 ± 4**	169 ± 5*	214 ± 15
Creatine kinase (IU/L)						
Day 4	253 ± 49	177 ± 13	227 ± 38	409 ± 115	315 ± 46	242 ± 54
Day 23	248 ± 35[b]	218 ± 42	229 ± 37	243 ± 47	183 ± 30	157 ± 30
Week 14	222 ± 27	163 ± 31	158 ± 29	139 ± 24	231 ± 76	154 ± 21
Sorbitol dehydrogenase (IU/L)						
Day 4	14 ± 1	14 ± 1	15 ± 0	14 ± 1	14 ± 1	14 ± 1
Day 23	13 ± 1	14 ± 1	14 ± 1	15 ± 1	15 ± 1	14 ± 0
Week 14	13 ± 1	15 ± 1	12 ± 1	12 ± 1	12 ± 1	12 ± 1
γ-Glutamyltransferase (IU/L)						
Day 4	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.9 ± 0.2	4.3 ± 0.7**
Day 23	0.5 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	1.0 ± 0.1	3.9 ± 0.4**
Week 14	1.3 ± 0.3	1.5 ± 0.3	1.4 ± 0.2	1.7 ± 0.3	3.1 ± 0.3**	15.3 ± 1.0**
Bile salts (μmol/L)						
Day 4	7.3 ± 1.2	9.2 ± 1.9	8.8 ± 1.3	6.3 ± 1.1	7.3 ± 1.6	11.2 ± 1.4
Day 23	12.2 ± 2.4	10.4 ± 1.8	10.0 ± 1.4	5.7 ± 0.8	8.6 ± 1.3	10.8 ± 1.6
Week 14	9.6 ± 2.3	13.5 ± 2.6	11.3 ± 1.5	9.0 ± 1.4	9.8 ± 1.2	12.2 ± 1.9

* 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

TABLE F2
Clinical Chemistry Data for Rats in the 2-Year Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Male				
n				
Month 6	10	10	10	10
Month 12	10	10	10	10
Month 18	10	9	10	10
Urea nitrogen (mg/dL)				
Month 6	13.7 ± 0.4	13.9 ± 0.5	13.8 ± 0.3	15.4 ± 0.4*
Month 12	11.1 ± 0.3	11.7 ± 0.4	10.5 ± 0.3	11.7 ± 0.3
Month 18	10.2 ± 0.3	11.7 ± 0.4	10.6 ± 0.5	12.3 ± 0.4**
Creatinine (mg/dL)				
Month 6	0.67 ± 0.02	0.69 ± 0.02	0.70 ± 0.00	0.71 ± 0.01
Month 12	0.66 ± 0.02	0.67 ± 0.02	0.72 ± 0.01*	0.70 ± 0.00*
Month 18	0.60 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.69 ± 0.02**
Glucose (mg/dL)				
Month 6	148 ± 4	150 ± 6	143 ± 6	138 ± 4
Month 12	118 ± 5	129 ± 11	126 ± 9	110 ± 3
Month 18	113 ± 3	119 ± 7	110 ± 2	119 ± 8
Total protein (g/dL)				
Month 6	7.2 ± 0.1	7.3 ± 0.1	7.4 ± 0.1*	7.8 ± 0.1**
Month 12	7.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1	8.0 ± 0.1**
Month 18	7.2 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.6 ± 0.1**
Albumin (g/dL)				
Month 6	4.8 ± 0.0	4.9 ± 0.0	5.0 ± 0.1*	5.3 ± 0.1**
Month 12	5.1 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	5.4 ± 0.0**
Month 18	4.7 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.1**
Cholesterol (mg/dL)				
Month 6	94 ± 2	95 ± 2	102 ± 2*	108 ± 2**
Month 12	122 ± 3	118 ± 3	117 ± 4	128 ± 5
Month 18	121 ± 4	121 ± 5	125 ± 7	137 ± 7
Triglycerides (mg/dL)				
Month 6	265 ± 20	232 ± 15	252 ± 22	311 ± 28
Month 12	331 ± 49	410 ± 29	311 ± 26	453 ± 40
Month 18	380 ± 23	333 ± 32	373 ± 46	332 ± 21
Alanine aminotransferase (IU/L)				
Month 6	102 ± 7	81 ± 3*	67 ± 6**	55 ± 1**
Month 12	113 ± 8	88 ± 3*	79 ± 4**	72 ± 4**
Month 18	108 ± 8	103 ± 9	66 ± 4**	60 ± 2**
Alkaline phosphatase (IU/L)				
Month 6	234 ± 7	225 ± 5	212 ± 3*	203 ± 5**
Month 12	198 ± 7	204 ± 4	178 ± 4	187 ± 6
Month 18	194 ± 6	195 ± 5	174 ± 4*	145 ± 5**
Creatine kinase (IU/L)				
Month 6	266 ± 43	237 ± 45	235 ± 48	165 ± 32
Month 12	319 ± 52[b]	489 ± 82	728 ± 139*	514 ± 89
Month 18	379 ± 102	482 ± 138	272 ± 44	337 ± 53
Sorbitol dehydrogenase (IU/L)				
Month 6	25 ± 1	24 ± 1	20 ± 2**	15 ± 1**
Month 12	66 ± 5	60 ± 5	66 ± 9	50 ± 4
Month 18	35 ± 2	29 ± 3	26 ± 2**	26 ± 1**
γ-Glutamyltransferase (IU/L)				
Month 6	0.5 ± 0.2	0.0 ± 0.0*	0.1 ± 0.1	0.6 ± 0.2
Month 12	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.2
Month 18	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	1.1 ± 0.2**
Bile salts (μmol/L)				
Month 6	6.2 ± 1.2	7.4 ± 1.5	5.6 ± 1.2	5.6 ± 0.7
Month 12	3.8 ± 0.5	8.0 ± 1.6	4.5 ± 0.7	9.9 ± 1.6**
Month 18	9.4 ± 1.9	10.1 ± 2.4	9.5 ± 1.5	16.4 ± 3.5

TABLE F2
Clinical Chemistry Data for Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Female				
n				
Month 6	10	10	10	10
Month 12	10	10	10	10
Month 18	10	10	10	8
Urea nitrogen (mg/dL)				
Month 6	14.6 ± 0.5	15.2 ± 0.4	13.2 ± 0.6	14.7 ± 0.5
Month 12	13.4 ± 0.3	12.7 ± 0.2	13.8 ± 0.4	12.7 ± 0.5
Month 18	13.1 ± 0.3	12.8 ± 0.7	12.8 ± 0.4	12.3 ± 0.5
Creatinine (mg/dL)				
Month 6	0.67 ± 0.02	0.63 ± 0.02	0.66 ± 0.02	0.65 ± 0.02
Month 12	0.62 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.61 ± 0.01
Month 18	0.67 ± 0.02	0.64 ± 0.02	0.68 ± 0.01	0.64 ± 0.02
Glucose (mg/dL)				
Month 6	148 ± 5	143 ± 3	145 ± 5	145 ± 4
Month 12	135 ± 9	147 ± 11	126 ± 4	128 ± 3
Month 18	116 ± 4	108 ± 5	116 ± 4	115 ± 3
Total protein (g/dL)				
Month 6	6.8 ± 0.1	7.1 ± 0.1	7.3 ± 0.1**	7.3 ± 0.1**
Month 12	7.3 ± 0.1	7.4 ± 0.1	7.7 ± 0.1*	7.5 ± 0.1
Month 18	7.6 ± 0.1	7.7 ± 0.3	7.8 ± 0.1	8.0 ± 0.1
Albumin (g/dL)				
Month 6	4.9 ± 0.1	5.1 ± 0.1	5.3 ± 0.1**	5.3 ± 0.1**
Month 12	5.1 ± 0.1	5.2 ± 0.1	5.5 ± 0.1*	5.4 ± 0.0
Month 18	5.2 ± 0.1	5.3 ± 0.2	5.5 ± 0.1	5.7 ± 0.1**
Cholesterol (mg/dL)				
Month 6	90 ± 3	99 ± 3*	106 ± 3**	128 ± 3**
Month 12	105 ± 2	112 ± 3	128 ± 5**	149 ± 3**
Month 18	111 ± 4	121 ± 5	120 ± 3	166 ± 6**
Triglycerides (mg/dL)				
Month 6	148 ± 20	187 ± 18	169 ± 25	122 ± 13
Month 12	305 ± 27	337 ± 38	365 ± 32	151 ± 26*
Month 18	445 ± 48	565 ± 72	415 ± 44	268 ± 25*
Alanine aminotransferase (IU/L)				
Month 6	66 ± 5	61 ± 5	48 ± 2**	45 ± 2**
Month 12	65 ± 2	58 ± 3*	54 ± 2**	50 ± 2**
Month 18	67 ± 2	66 ± 6	59 ± 2*	55 ± 2**
Alkaline phosphatase (IU/L)				
Month 6	231 ± 9	215 ± 8	197 ± 7*	184 ± 7**
Month 12	209 ± 5	186 ± 7	196 ± 6	136 ± 7**
Month 18	230 ± 7	232 ± 25	220 ± 8	149 ± 7**
Creatine kinase (IU/L)				
Month 6	195 ± 33	313 ± 100	146 ± 25	152 ± 27
Month 12	410 ± 129	211 ± 43	244 ± 53	228 ± 59
Month 18	232 ± 20	255 ± 30	469 ± 88*	417 ± 160
Sorbitol dehydrogenase (IU/L)				
Month 6	15 ± 1	15 ± 1	13 ± 1	14 ± 1
Month 12	32 ± 2	25 ± 2*	28 ± 2	24 ± 2*
Month 18	23 ± 1	21 ± 1	21 ± 2	20 ± 1
γ-Glutamyltransferase (IU/L)				
Month 6	1.7 ± 0.2	2.2 ± 0.3	1.6 ± 0.3	4.3 ± 0.3**
Month 12	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.2	3.5 ± 0.6**
Month 18	0.5 ± 0.2	0.4 ± 0.3	0.5 ± 0.2	4.4 ± 0.7**

TABLE F2
Clinical Chemistry Data for Rats in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.1 g/kg	0.3 g/kg	1.0 g/kg
Female (continued)				
n				
Month 6	10	10	10	10
Month 12	10	10	10	10
Month 18	10	10	10	8
Bile salts (μmol/L)				
Month 6	7.1 ± 1.3	5.2 ± 0.9	4.1 ± 0.4	11.3 ± 1.8
Month 12	4.9 ± 0.5	6.6 ± 0.9	7.8 ± 0.7*	7.3 ± 0.9
Month 18	5.5 ± 0.8	10.9 ± 2.5*	11.6 ± 1.3**	15.4 ± 2.0**

* 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

TABLE F3
Hematology Data for Mice in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
n	10	10	10	10	10	6
Male						
Hematocrit (auto) (%)	49.1 ± 0.5	48.6 ± 0.5	47.9 ± 0.6	49.5 ± 0.5	49.4 ± 0.3	48.2 ± 0.6
Hemoglobin (g/dL)	15.1 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.2 ± 0.1
Erythrocytes (10^6/µL)	9.96 ± 0.12	9.96 ± 0.10	9.84 ± 0.13	10.07 ± 0.12	10.09 ± 0.10	9.82 ± 0.16
Reticulocytes (10^3/µL)	247.0 ± 6.2	247.5 ± 6.8	249.7 ± 5.1	247.8 ± 7.1	255.8 ± 6.1	258.8 ± 8.4
Mean cell volume (fL)	49.3 ± 0.3	48.8 ± 0.2	48.7 ± 0.4	49.2 ± 0.2	48.9 ± 0.3	49.1 ± 0.3
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.4 ± 0.3
Mean cell hemoglobin concentration (g/dL)	30.7 ± 0.1	31.1 ± 0.2	31.5 ± 0.2*	31.0 ± 0.2	31.1 ± 0.2	31.4 ± 0.4
Platelets (10^3/µL)	1,153.9 ± 23.7	1,190.2 ± 70.6	1,197.1 ± 31.1	1,148.6 ± 28.6	1,286.5 ± 43.3*	1,361.7 ± 51.4**
Leukocytes (10^3/µL)	4.81 ± 0.46	4.91 ± 0.35	4.80 ± 0.41	4.13 ± 0.42	4.34 ± 0.26	4.05 ± 0.52
Segmented neutrophils (10^3/µL)	0.66 ± 0.05	0.61 ± 0.05	0.62 ± 0.07	0.61 ± 0.06	0.57 ± 0.04	0.62 ± 0.07
Lymphocytes (10^3/µL)	3.99 ± 0.46	4.15 ± 0.32	3.99 ± 0.36	3.35 ± 0.34	3.64 ± 0.22	3.29 ± 0.46
Monocytes (10^3/µL)	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Basophils (10^3/µL)	0.013 ± 0.002	0.013 ± 0.002	0.011 ± 0.002	0.012 ± 0.002	0.014 ± 0.002	0.012 ± 0.002
Eosinophils (10^3/µL)	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
Female						
Hematocrit (auto) (%)	50.4 ± 1.2	50.6 ± 1.0	48.2 ± 0.5	49.8 ± 0.7	50.0 ± 0.8	48.7 ± 1.0
Hemoglobin (g/dL)	16.4 ± 0.3	16.4 ± 0.3	15.8 ± 0.1	16.2 ± 0.2	16.3 ± 0.2	15.8 ± 0.3
Erythrocytes (10^6/µL)	10.50 ± 0.20	10.52 ± 0.18	10.17 ± 0.10	10.41 ± 0.14	10.54 ± 0.17	10.16 ± 0.20
Reticulocytes (10^3/µL)	295.3 ± 16.2	318.5 ± 14.6	276.2 ± 13.7	270.9 ± 20.7	262.5 ± 12.4	261.1 ± 41.6
Mean cell volume (fL)	47.9 ± 0.3	48.1 ± 0.3	47.4 ± 0.2	47.8 ± 0.3	47.5 ± 0.2	48.0 ± 0.2
Mean cell hemoglobin (pg)	15.6 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.5 ± 0.0
Mean cell hemoglobin concentration (g/dL)	32.6 ± 0.3	32.3 ± 0.2	32.8 ± 0.2	32.6 ± 0.1	32.5 ± 0.1	32.4 ± 0.2
Platelets (10^3/µL)	854.7 ± 71.9	876.8 ± 75.7	1,009.5 ± 59.0	932.3 ± 59.9	1,027.5 ± 59.6	994.2 ± 49.6
Leukocytes (10^3/µL)	3.62 ± 0.26	3.55 ± 0.31	4.25 ± 0.35	4.04 ± 0.31	3.66 ± 0.16	4.33 ± 0.79
Segmented neutrophils (10^3/µL)	0.35 ± 0.04	0.47 ± 0.06	0.44 ± 0.03	0.51 ± 0.11	0.37 ± 0.04	0.62 ± 0.13
Lymphocytes (10^3/µL)	3.10 ± 0.20	2.94 ± 0.26	3.61 ± 0.32	3.31 ± 0.25	3.12 ± 0.12	3.48 ± 0.61
Monocytes (10^3/µL)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.10 ± 0.03	0.09 ± 0.02	0.09 ± 0.01
Basophils (10^3/µL)	0.015 ± 0.003	0.016 ± 0.005	0.014 ± 0.004	0.015 ± 0.004	0.009 ± 0.004	0.023 ± 0.008
Eosinophils (10^3/µL)	0.10 ± 0.03	0.07 ± 0.01	0.13 ± 0.03	0.11 ± 0.03	0.08 ± 0.01	0.12 ± 0.03

* 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.

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 Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	187 ± 5	187 ± 7	185 ± 6	184 ± 5	186 ± 6	176 ± 4
Heart						
Absolute	0.68 ± 0.02	0.68 ± 0.02	0.67 ± 0.02	0.68 ± 0.01	0.69 ± 0.03	0.67 ± 0.01
Relative	3.664 ± 0.034	3.654 ± 0.066	3.624 ± 0.037	3.681 ± 0.046	3.680 ± 0.087	3.795 ± 0.065
R. Kidney						
Absolute	0.83 ± 0.03	0.84 ± 0.04	0.86 ± 0.03	0.84 ± 0.03	0.88 ± 0.04	0.87 ± 0.02
Relative	4.443 ± 0.081	4.483 ± 0.083	4.651 ± 0.070	4.562 ± 0.102	4.735 ± 0.117*	4.963 ± 0.043**
Liver						
Absolute	10.15 ± 0.37	10.12 ± 0.35	10.14 ± 0.22	10.73 ± 0.54	11.51 ± 0.49*	12.43 ± 0.33**
Relative	54.309 ± 0.978	54.233 ± 0.823	54.735 ± 0.766	58.338 ± 1.576*	61.768 ± 1.206**	70.721 ± 1.371**
Lung						
Absolute	1.44 ± 0.12	1.35 ± 0.09	1.29 ± 0.11	1.65 ± 0.21	1.29 ± 0.11	1.23 ± 0.07
Relative	7.670 ± 0.556	7.255 ± 0.493	6.937 ± 0.568	9.060 ± 1.310	6.901 ± 0.457	6.999 ± 0.316
R. Testis						
Absolute	1.026 ± 0.030	1.046 ± 0.025	1.051 ± 0.016	1.030 ± 0.020	1.052 ± 0.032	1.030 ± 0.029
Relative	5.497 ± 0.086	5.612 ± 0.127	5.681 ± 0.125	5.617 ± 0.081	5.656 ± 0.087	5.857 ± 0.101
Thymus						
Absolute	0.456 ± 0.026	0.432 ± 0.022	0.444 ± 0.015	0.447 ± 0.029	0.472 ± 0.036	0.374 ± 0.024
Relative	2.450 ± 0.160	2.318 ± 0.129	2.399 ± 0.058	2.433 ± 0.139	2.523 ± 0.133	2.125 ± 0.132
Female						
n	5	5	5	5	5	4
Necropsy body wt	134 ± 1	137 ± 2	137 ± 2	135 ± 3	137 ± 2	132 ± 3
Heart						
Absolute	0.52 ± 0.01	0.54 ± 0.02	0.54 ± 0.01	0.52 ± 0.02	0.54 ± 0.01	0.53 ± 0.01
Relative	3.880 ± 0.067	3.954 ± 0.109	3.945 ± 0.054	3.815 ± 0.051	3.907 ± 0.111	3.980 ± 0.057
R. Kidney						
Absolute	0.67 ± 0.02	0.64 ± 0.02	0.67 ± 0.01	0.66 ± 0.02	0.70 ± 0.01	0.70 ± 0.02
Relative	5.019 ± 0.154	4.664 ± 0.056	4.918 ± 0.092	4.896 ± 0.085	5.102 ± 0.138	5.312 ± 0.070
Liver						
Absolute	6.35 ± 0.18	6.66 ± 0.19	6.90 ± 0.22	7.47 ± 0.29**	8.15 ± 0.13**	9.15 ± 0.29**
Relative	47.293 ± 1.079	48.505 ± 0.773	50.316 ± 0.870*	55.048 ± 0.930**	59.371 ± 0.481**	69.335 ± 1.704**
Lung						
Absolute	0.97 ± 0.03	1.24 ± 0.12	1.11 ± 0.09	1.17 ± 0.17	1.08 ± 0.10	0.97 ± 0.02
Relative	7.226 ± 0.185	9.003 ± 0.718	8.087 ± 0.581	8.592 ± 1.136	7.839 ± 0.652	7.356 ± 0.208
Thymus						
Absolute	0.360 ± 0.006	0.376 ± 0.019	0.385 ± 0.025	0.376 ± 0.014	0.324 ± 0.011	0.332 ± 0.026
Relative	2.679 ± 0.026	2.735 ± 0.109	2.815 ± 0.191	2.777 ± 0.074	2.357 ± 0.060	2.510 ± 0.182

* Significantly different (P≤0.05) from the vehicle control group by Williams' 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 G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
n	10	10	9	10	10	7
Necropsy body wt	346 ± 6	334 ± 6	334 ± 5	339 ± 4	316 ± 7**	286 ± 6**
Heart						
Absolute	0.940 ± 0.025	0.911 ± 0.028	0.915 ± 0.021	0.939 ± 0.026	0.918 ± 0.023	0.855 ± 0.014
Relative	2.717 ± 0.051	2.723 ± 0.054	2.741 ± 0.044	2.766 ± 0.065	2.909 ± 0.051*	2.991 ± 0.059**
R. Kidney						
Absolute	0.933 ± 0.015	0.929 ± 0.027	0.936 ± 0.016	0.985 ± 0.012*	1.005 ± 0.014**	1.039 ± 0.011**
Relative	2.699 ± 0.034	2.777 ± 0.043	2.801 ± 0.022	2.904 ± 0.028**	3.189 ± 0.047**	3.633 ± 0.046**
Liver						
Absolute	11.05 ± 0.47[b]	11.05 ± 0.29[b]	12.09 ± 0.35*[b]	12.42 ± 0.20**[b]	13.20 ± 0.33**[b]	15.06 ± 0.21**[c]
Relative	32.088 ± 0.589[b]	33.356 ± 0.256[b]	35.982 ± 0.816**[b]	37.441 ± 0.643**[b]	41.560 ± 0.862**[b]	51.923 ± 1.125**[c]
Lung						
Absolute	1.676 ± 0.057	1.581 ± 0.056	1.601 ± 0.043	1.596 ± 0.079	1.500 ± 0.040*	1.482 ± 0.089*
Relative	4.842 ± 0.119	4.735 ± 0.159	4.803 ± 0.147	4.706 ± 0.228	4.745 ± 0.068	5.189 ± 0.328
R. Testis						
Absolute	1.473 ± 0.035	1.444 ± 0.040	1.477 ± 0.031	1.455 ± 0.018	1.486 ± 0.018	1.460 ± 0.026
Relative	4.258 ± 0.069	4.321 ± 0.082	4.422 ± 0.059	4.293 ± 0.062	4.715 ± 0.072**	5.104 ± 0.084**
Thymus						
Absolute	0.292 ± 0.013	0.284 ± 0.018	0.288 ± 0.008	0.285 ± 0.010	0.255 ± 0.009*	0.214 ± 0.010**
Relative	0.843 ± 0.032	0.847 ± 0.043	0.860 ± 0.017	0.841 ± 0.029	0.809 ± 0.028	0.749 ± 0.037
Female						
n	9	10	10	10	9	6
Necropsy body wt	197 ± 2	196 ± 2	193 ± 2	196 ± 4	190 ± 4	178 ± 4**
Heart						
Absolute	0.634 ± 0.016	0.620 ± 0.011	0.630 ± 0.011	0.629 ± 0.014	0.638 ± 0.016	0.623 ± 0.012
Relative	3.214 ± 0.065	3.159 ± 0.059	3.263 ± 0.062	3.216 ± 0.072	3.362 ± 0.045	3.512 ± 0.084**
R. Kidney						
Absolute	0.673 ± 0.016	0.696 ± 0.020	0.701 ± 0.009	0.731 ± 0.024*	0.746 ± 0.018**	0.776 ± 0.015**
Relative	3.410 ± 0.064	3.542 ± 0.080	3.630 ± 0.037*	3.727 ± 0.068**	3.932 ± 0.046**	4.369 ± 0.098**
Liver						
Absolute	6.418 ± 0.106	6.747 ± 0.160	6.817 ± 0.173	8.062 ± 0.253**	8.973 ± 0.269**	10.290 ± 0.464**
Relative	32.519 ± 0.343	34.336 ± 0.641	35.262 ± 0.626*	41.108 ± 0.769**	47.224 ± 0.810**	57.838 ± 2.062**
Lung						
Absolute	1.186 ± 0.038	1.158 ± 0.057	1.159 ± 0.056	1.228 ± 0.048	1.133 ± 0.057	1.144 ± 0.075
Relative	6.015 ± 0.201	5.882 ± 0.249	5.990 ± 0.264	6.273 ± 0.222	5.966 ± 0.263	6.461 ± 0.477
Thymus						
Absolute	0.287 ± 0.040	0.231 ± 0.011	0.229 ± 0.009	0.240 ± 0.013	0.219 ± 0.014*	0.179 ± 0.020**
Relative	1.449 ± 0.192	1.176 ± 0.056	1.183 ± 0.039	1.225 ± 0.062	1.154 ± 0.067	1.007 ± 0.110*

* 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] n=4
[c] n=3

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male						
n	5	5	5	5	5	3
Necropsy body wt	23.1 ± 0.6	22.4 ± 0.8	23.1 ± 0.6	22.2 ± 0.6	22.9 ± 0.4	22.4 ± 0.4
Heart						
Absolute	0.15 ± 0.02	0.15 ± 0.01	0.13 ± 0.00	0.12 ± 0.01	0.13 ± 0.00	0.16 ± 0.00
Relative	6.383 ± 0.576	6.692 ± 0.786	5.477 ± 0.268	5.420 ± 0.337	5.601 ± 0.168	7.074 ± 0.052
R. Kidney						
Absolute	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.22 ± 0.01
Relative	9.965 ± 0.135	10.082 ± 0.248	10.117 ± 0.294	9.997 ± 0.249	10.476 ± 0.232	9.759 ± 0.131
Liver						
Absolute	1.31 ± 0.05	1.26 ± 0.03	1.26 ± 0.02	1.29 ± 0.04	1.39 ± 0.03	1.64 ± 0.05**
Relative	56.321 ± 1.162	56.434 ± 1.304	54.660 ± 1.392	58.245 ± 0.808	60.694 ± 0.608*	72.956 ± 1.602**
Lung						
Absolute	0.24 ± 0.03	0.26 ± 0.03	0.20 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.31 ± 0.03
Relative	10.374 ± 1.274	11.579 ± 1.564	8.467 ± 0.484	8.026 ± 0.296	8.726 ± 0.462	13.907 ± 1.592
R. Testis						
Absolute	0.101 ± 0.004	0.100 ± 0.003	0.107 ± 0.003	0.106 ± 0.006	0.102 ± 0.003	0.097 ± 0.003
Relative	4.357 ± 0.124	4.480 ± 0.259	4.657 ± 0.151	4.786 ± 0.228	4.465 ± 0.112	4.346 ± 0.212
Thymus						
Absolute	0.049 ± 0.003	0.051 ± 0.003	0.049 ± 0.002	0.046 ± 0.003	0.045 ± 0.002	0.050 ± 0.004
Relative	2.101 ± 0.109	2.269 ± 0.178	2.136 ± 0.145	2.069 ± 0.124	1.967 ± 0.107	2.229 ± 0.178
Female						
n	5	5	5	5	5	5
Necropsy body wt	20.3 ± 0.3	19.5 ± 0.7	19.2 ± 0.8	19.5 ± 0.6	20.0 ± 0.4	19.6 ± 0.5
Heart						
Absolute	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.01	0.11 ± 0.00	0.11 ± 0.01	0.11 ± 0.00
Relative	5.391 ± 0.159	5.446 ± 0.216	5.655 ± 0.199	5.422 ± 0.140	5.537 ± 0.230	5.464 ± 0.121
R. Kidney						
Absolute	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.00	0.18 ± 0.01
Relative	8.732 ± 0.224	8.517 ± 0.232	9.013 ± 0.329	8.088 ± 0.171	8.064 ± 0.122	9.160 ± 0.280
Liver						
Absolute	1.04 ± 0.02	1.01 ± 0.03	1.04 ± 0.05	1.06 ± 0.04	1.14 ± 0.03	1.23 ± 0.05**
Relative	51.182 ± 0.422	51.931 ± 0.665	54.051 ± 0.735*	54.040 ± 0.867*	56.708 ± 0.222**	62.877 ± 1.537**
Lung						
Absolute	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.00	0.17 ± 0.01
Relative	8.612 ± 0.330	8.864 ± 0.328	9.007 ± 0.548	8.771 ± 0.176	8.840 ± 0.246	8.696 ± 0.328
Thymus						
Absolute	0.072 ± 0.002	0.069 ± 0.001	0.069 ± 0.003	0.071 ± 0.002	0.069 ± 0.002	0.074 ± 0.002
Relative	3.554 ± 0.137	3.548 ± 0.184	3.618 ± 0.158	3.618 ± 0.094	3.467 ± 0.173	3.774 ± 0.121

* Significantly different (P≤0.05) from the vehicle control group by Williams' 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 G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.125 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
n	10	10	10	10	10	6
Male						
Necropsy body wt	34.4 ± 0.9	35.5 ± 1.0	35.4 ± 0.9	36.0 ± 1.0	35.3 ± 0.9	32.4 ± 0.4
Heart						
Absolute	0.159 ± 0.004	0.161 ± 0.006	0.152 ± 0.004	0.157 ± 0.005	0.156 ± 0.004	0.154 ± 0.005
Relative	4.615 ± 0.098	4.535 ± 0.121	4.319 ± 0.113	4.375 ± 0.112	4.412 ± 0.094	4.755 ± 0.183
R. Kidney						
Absolute	0.266 ± 0.008	0.272 ± 0.008	0.270 ± 0.006	0.274 ± 0.007	0.278 ± 0.007	0.263 ± 0.010
Relative	7.758 ± 0.223	7.680 ± 0.155	7.675 ± 0.269	7.605 ± 0.144	7.880 ± 0.184	8.095 ± 0.229
Liver						
Absolute	1.414 ± 0.052[b]	1.406 ± 0.096[b]	1.455 ± 0.085[b]	1.606 ± 0.060[b]	1.600 ± 0.052[b]	1.904 ± 0.048**[c]
Relative	42.102 ± 0.501[b]	39.241 ± 1.794[b]	41.841 ± 2.165[b]	44.618 ± 1.530[b]	47.513 ± 0.631*[b]	60.831 ± 1.534**[c]
Lung						
Absolute	0.185 ± 0.004	0.199 ± 0.011	0.178 ± 0.006	0.184 ± 0.006	0.184 ± 0.005	0.183 ± 0.004
Relative	5.422 ± 0.237	5.630 ± 0.264	5.065 ± 0.224	5.100 ± 0.128	5.245 ± 0.239	5.636 ± 0.109
R. Testis						
Absolute	0.117 ± 0.004	0.119 ± 0.003	0.117 ± 0.003	0.115 ± 0.003	0.119 ± 0.002	0.118 ± 0.003
Relative	3.404 ± 0.118	3.366 ± 0.066	3.304 ± 0.085	3.200 ± 0.090	3.368 ± 0.067	3.631 ± 0.054
Thymus						
Absolute	0.037 ± 0.002	0.038 ± 0.003	0.041 ± 0.003	0.043 ± 0.002	0.040 ± 0.002	0.037 ± 0.002
Relative	1.077 ± 0.053	1.068 ± 0.063	1.169 ± 0.072	1.203 ± 0.049	1.119 ± 0.045	1.147 ± 0.065
Female						
Necropsy body wt	28.1 ± 0.9	28.5 ± 0.4	27.7 ± 0.8	29.1 ± 0.3	27.1 ± 0.8	27.0 ± 0.5
Heart						
Absolute	0.146 ± 0.007	0.153 ± 0.006	0.139 ± 0.004	0.149 ± 0.007	0.142 ± 0.008	0.161 ± 0.012
Relative	5.226 ± 0.295	5.369 ± 0.237	5.051 ± 0.214	5.096 ± 0.200	5.262 ± 0.318	5.970 ± 0.461
R. Kidney						
Absolute	0.159 ± 0.004	0.162 ± 0.003	0.157 ± 0.003	0.166 ± 0.003	0.156 ± 0.004	0.175 ± 0.003*
Relative	5.698 ± 0.161	5.702 ± 0.108	5.701 ± 0.141	5.698 ± 0.110	5.777 ± 0.107	6.505 ± 0.116**
Liver						
Absolute	1.123 ± 0.029	1.199 ± 0.016	1.156 ± 0.030	1.223 ± 0.031*	1.248 ± 0.050**	1.648 ± 0.032**
Relative	40.191 ± 1.237	42.211 ± 0.930	41.866 ± 0.937	41.947 ± 0.829	45.964 ± 0.718**	61.212 ± 1.306**
Lung						
Absolute	0.268 ± 0.017	0.286 ± 0.023	0.228 ± 0.013	0.261 ± 0.014	0.258 ± 0.017	0.273 ± 0.024
Relative	9.564 ± 0.517	10.064 ± 0.862	8.283 ± 0.501	8.954 ± 0.518	9.610 ± 0.712	10.161 ± 0.920
Thymus						
Absolute	0.052 ± 0.002	0.050 ± 0.002	0.045 ± 0.002	0.052 ± 0.004	0.047 ± 0.002	0.043 ± 0.003
Relative	1.850 ± 0.053	1.763 ± 0.082	1.639 ± 0.059	1.786 ± 0.145	1.761 ± 0.096	1.581 ± 0.079

* 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] n=4
[c] n=2

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 Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
n	10	9	10	10
Weights (g)				
Necropsy body wt	346 ± 6	334 ± 5	339 ± 4	316 ± 7**
L. Cauda epididymis	0.1412 ± 0.0037	0.1257 ± 0.0090	0.1274 ± 0.0081	0.1408 ± 0.0045
L. Epididymis	0.4489 ± 0.0069	0.4152 ± 0.0126	0.4167 ± 0.0115	0.4403 ± 0.0071
L. Testis	1.5738 ± 0.0321	1.5664 ± 0.0364	1.5464 ± 0.0270	1.5855 ± 0.0284
Spermatid measurements				
Spermatid heads (10^3/mg testis)	122.5 ± 4.9	130.5 ± 3.8	126.6 ± 3.5	131.8 ± 3.7
Spermatid heads (10^6/testis)	172.50 ± 5.50	176.81 ± 7.27	174.13 ± 4.73	182.75 ± 3.78
Epididymal spermatozoal measurements				
Sperm motility (%)	80.6 ± 0.8	79.3 ± 1.1	78.7 ± 0.7	81.4 ± 1.3
Sperm (10^3/mg cauda epididymis)	640 ± 44	757 ± 74	640 ± 37	583 ± 48
Sperm (10^6/cauda epididymis)	90.63 ± 7.14	90.14 ± 4.03	79.25 ± 3.12	80.63 ± 4.52

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (cauda epididymis, epididymis, and testis weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Number weighed at necropsy	9	10	10	9
Necropsy body wt (g)	197 ± 2	193 ± 2	196 ± 4	190 ± 4
Proportion of regular cycling females[b]	9/9	10/10	10/10	9/9
Estrous cycle length (days)	5.1 ± 0.11	5.0 ± 0.00	5.0 ± 0.00	5.2 ± 0.15
Estrous stages (% of cycle)				
Diestrus	50.9	52.5	55.0	53.7
Proestrus	21.3	21.7	19.2	13.9
Estrus	20.4	20.8	21.7	19.4
Metestrus	5.6	4.2	1.7	9.3
Uncertain diagnoses	1.9	0.8	2.5	3.7

[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 dose groups and between the vehicle control group and each dosed group indicated the dosed females did not have extended estrus or diestrus.

[b] Number of females with a regular cycle/number of females cycling

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.4 ± 0.9	35.4 ± 0.9	36.0 ± 1.0	35.3 ± 0.9
L. Cauda epididymis	0.0131 ± 0.0006	0.0159 ± 0.0012	0.0145 ± 0.0006	0.0152 ± 0.0011
L. Epididymis	0.0427 ± 0.0015	0.0445 ± 0.0025	0.0433 ± 0.0016	0.0452 ± 0.0016
L. Testis	0.1140 ± 0.0033	0.1119 ± 0.0022	0.1144 ± 0.0029	0.1133 ± 0.0013
Spermatid measurements				
Spermatid heads (10^3/mg testis)	196.2 ± 6.4	205.9 ± 6.8	197.1 ± 4.2	197.0 ± 6.5
Spermatid heads (10^6/testis)	21.54 ± 0.80	21.85 ± 0.69	21.49 ± 0.88	21.18 ± 0.74
Epididymal spermatozoal measurements				
Sperm motility (%)	81.2 ± 1.1	80.4 ± 1.1	82.5 ± 1.3	81.3 ± 0.7
Sperm (10^3/mg cauda epididymis)	580.3 ± 118.0	573.0 ± 105.9	456.6 ± 86.0	700.8 ± 82.8
Sperm (10^6/cauda epididymis)	7.53 ± 1.48	8.59 ± 1.40	6.85 ± 1.46	10.25 ± 1.01

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body, cauda epididymis, epididymis, and testis weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Kava Kava Extract[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	28.1 ± 0.9	27.7 ± 0.8	29.1 ± 0.3	27.1 ± 0.8
Proportion of regular cycling females[b]	9/10	9/9	8/10	8/9
Estrous cycle length (days)	4.3 ± 0.13	4.1 ± 0.10[c]	4.4 ± 0.16	4.5 ± 0.20[c]
Estrous stages (% of cycle)				
Diestrus	47.5	40.0	34.2	35.0
Proestrus	0.0	0.0	0.0	0.0
Estrus	30.8	36.7	42.5	39.2
Metestrus	21.7	23.3	23.3	23.3
Uncertain diagnoses	0.0	0.0	0.0	2.5

[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 dose groups and between the vehicle control group and each dosed group indicated the dosed females did not have extended estrus or 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.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Kava Kava Extract

Kava kava extract was obtained from Cosmopolitan Trading Co. (Seattle, WA) in one lot (9077SDK), which was received in several batches. Lot 9077SDK, Batch 02 was used in the 2-week studies. Prior to the start of the 3-month studies, the study laboratory (Battelle Columbus Operations, Columbus, OH) combined lot 9077SDK, Batches 02, 03, and 04 into a single batch (and assigned a lot number of 082203) that was used during the 3-month studies. Lot 9077SDK, Batch 05 was used during the 2-year studies. Identity, purity, stability, and weight loss on drying analyses were conducted by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO). Reports on analyses performed in support of the kava kava extract studies are on file at the National Institute of Environmental Health Sciences.

The identity and purity of lot 9077SDK were determined using compendial methods to obtain the component profile. The bulk density of Batch 02 was determined to be 0.46396 g/mL, and that of Batch 05 was 0.4678 g/mL. Estimations of the distributions of particle sizes for Batches 02 through 05 were determined using a sieve method (Table I1). For these determinations, each sieve was weighed empty, including the top cover and bottom pan. The series of sieves was stacked in descending order (μm) between the top cover and bottom pan. A weighed kava kava extract sample (~50 gm) was placed on the top sieve and covered. The assembled stack of sieves was shaken on a mechanical shaker after which each of the sieves was reweighed. The sieves were reassembled in descending order, shaken for 5 minutes, and then reweighed. This shaking and weighing pattern was continued until an endpoint was reached when the weight of the extract on each sieve did not change more than 5% between two consecutive cycles for sieves containing more than 5% of the total sample weight or 20% between two consecutive cycles for sieves containing less than 5% of the total sample weight.

To evaluate organic constituents of the test article, water, methanol, and methylene chloride extracts were prepared according to a proposed USP monograph (USP, 2000, 2002). A combination of chromatographic and spectrometric techniques was used to characterize the test article; an authentic standard of kavain was used for quantitation. Thin-layer chromatography (TLC) was performed on Batches 02 and 05 of lot 9077SDK. High-performance liquid chromatography (HPLC) was used to determine the composition of all four batches of the bulk chemical using a Waters liquid chromatograph and ultraviolet (UV) light detector (Waters Corporation, Milford, MA), and a YMC Basic S 5 μm column (250 mm × 4.6 mm; YMC Company, Ltd., through Waters Corporation, Milford, MA) maintained at 35° C. For these assays, the isocratic solvent program utilized a mobile phase of 0.1% phosphoric acid:acetonitrile:isopropyl alcohol (64:20:16, v/v/v) at a flow rate of 0.6 mL/minutes, and absorbance was recorded at 220 nm. Several components of Batches 02 and 05 of the test article were tentatively identified using various mass spectrometry techniques including liquid chromatography/mass spectrometry (LC/MS), gas chromatography/mass spectrometry (GC/MS), and matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS; Batch 05 only).

As determined by weight loss on drying, moisture content for lot 9077SDK was 5.42%, 5.04%, 5.35%, and 5.57% for Batches 02 through 05, respectively. TLC plates were visualized with visible and UV (254 nm) light after spraying with a *p*-anisaldehyde reagent; kavain was observed in all three extracts of Batches 02 and 05 of the test article, and methysticin was observed in the three extracts of Batch 02. HPLC/UV profiles of methanolic (Batches 02 and 05) and aqueous (Batch 02) extracts of the test article indicated the presence of 10 components, eight of which were identified using specifications provided by the Institute for Nutraceutical Advancement (INA, 2001). In Batch 05, these 10 components were shown to account for all but 1.96% of the total peak area. LC/MS analyses of methanolic and aqueous extracts of Batches 02 and 05 confirmed the presence of six kavalactones quantitated in the HPLC/UV analyses of these extracts and tentatively identified seven other components. The identified kavalactones included methysticin, dihydromethysticin, kavain, dihydrokavain, yangonin, and desmethoxyyangonin. GC/MS analyses of Batches 02 and 05 performed on hexane, methylene chloride, acetone, and methanol (Batch 02 only) extracts of the bulk material confirmed the presence and identity of kavalactones in organic extracts of the test article. Additional confirmation of the presence and identity of the kavalactones was

obtained from MALDI-TOF/MS analyses of aliquots of Batch 05 diluted with methanol:water (70:30, v/v). Using the validated HPLC/UV method previously described, six major kavalactones were quantitated in methanolic extracts of all four batches of lot 9077SDK (Table I2), and the results were very consistent across all the batches. A representative HPLC profile of the kavalactones identified in Lot 9077SDK is presented in Figure I1. Aliquots of Batches 02 and 05 of lot 9077SDK were sent to Covance Laboratories, Inc. (Madison, WI) for nutritional and contaminant testing using standard methods. Organochlorine and organophosphorus pesticide levels were below the detection limits for the analytical assay. Lead, mercury, and cadmium levels were less than the limits of detection, which were respectively 50, 25, and 2,000 ppb; selenium was present at 24 ppb. *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine were present at 1.4 and 31.2 ppb, respectively.

Taken together, these data indicate that all four batches of the test article were kava kava extract and their composition was consistent with the expected composition of typical kava kava extract. Results of the nutritional and contaminant tests were deemed acceptable for use in these studies.

The bulk material was stored at room temperature, protected from light, in white, 5 gallon plastic drums and its stability was monitored using HPLC/UV analysis. No degradation was observed over the course of the studies.

Corn Oil

USP-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 2-week, 3-month, and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing kava kava extract with corn oil to give the required concentrations (Table I3). The dose formulations were stored at room temperature in clear glass bottles sealed with Teflon®-lined lids in amber plastic bags for up to 37 days (2-week studies) or 42 days (3-month and 2-year studies).

Homogeneity studies of ~200 and ~400 mg/mL dose formulations were performed by the analytical chemistry laboratory while studies of 12.5, 20, 25, 100, 200, and 400 mg/mL dose formulations were performed by the study laboratory; all of these studies used the HPLC/UV system previously described. Stability studies of a 12.5 mg/mL dose formulation were also performed by the analytical chemistry laboratory using HPLC/UV analysis by the system previously described. Homogeneity was confirmed, and stability was confirmed for at least 42 days for dose formulations stored in amber glass vials sealed with Teflon®-lined septa and crimped caps at room temperature and approximately 5° C, and for up to 3 hours under simulated animal room conditions.

The study laboratory determined that a 400 mg/mL dose formulation of kava kava extract in corn oil was gavagable using a 20-gauge gavage needle.

Periodic analyses of the dose formulations of kava kava extract were conducted by the study laboratory using an HPLC/UV system similar to the one previously described. During the 2-week studies, the dose formulations were analyzed once; all six dose formulations for rats and mice were within 10% of the target concentrations (Table I4). Animal room samples of these dose formulations were also analyzed; four of five for rats and all five for mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I5). Of the dose formulations analyzed during the studies, all 18 for rats and mice were within 10% of the target concentrations; 14 of 15 animal room samples for rats and 13 of 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 11 weeks; animal room samples were also analyzed (Table I6). Of the dose formulations analyzed during the studies, 29 of 30 for rats and all 30 for mice were within 10% of the target concentrations; all 12 animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

TABLE I1
Particle Size Distribution in Lot 9077SDK in the Gavage Studies of Kava Kava Extract

U.S. Sieve Number	Sieve Size (µm)	Batch 02 Percent Through	Batch 03 Percent Through	Batch 04 Percent Through	Batch 05 Percent Through
40	425	99.0	98.0	97.0	98.2
60	250	92.3	93.6	91.6	94.8
70	212	89.1	91.2	89.4	93.6
80	180	84.9	85.4	75.1	91.8
100	150	55.7	71.5	47.3	88.2
120	125	49.9	66.9	16.5	83.6
140	106	36.6	51.4	0.6	77.0
200	75	15.4	25.4	0.0	53.3
270	53	1.9	3.1	0.0	26.1

TABLE I2
Major Kavalactones in Lot 9077SDK in the Gavage Studies of Kava Kava Extract[a]

Batch Numbers	Methysticin	Dihydro-methysticin	Kavain	Dihydrokavain	Yangonin	Desmethoxy-yangonin	Total
02	3.11	3.05	6.55	6.90	3.62	2.15	25.38
03	2.97	3.17	6.25	7.04	4.19	2.12	25.74
04	2.99	3.21	6.30	7.05	4.19	2.13	25.87
05	3.20	3.39	6.44	7.53	4.14	2.29	26.99

[a] Values are presented as percentages calculated as mg extracted kavalactone/mg kava kava extract analyzed × 100

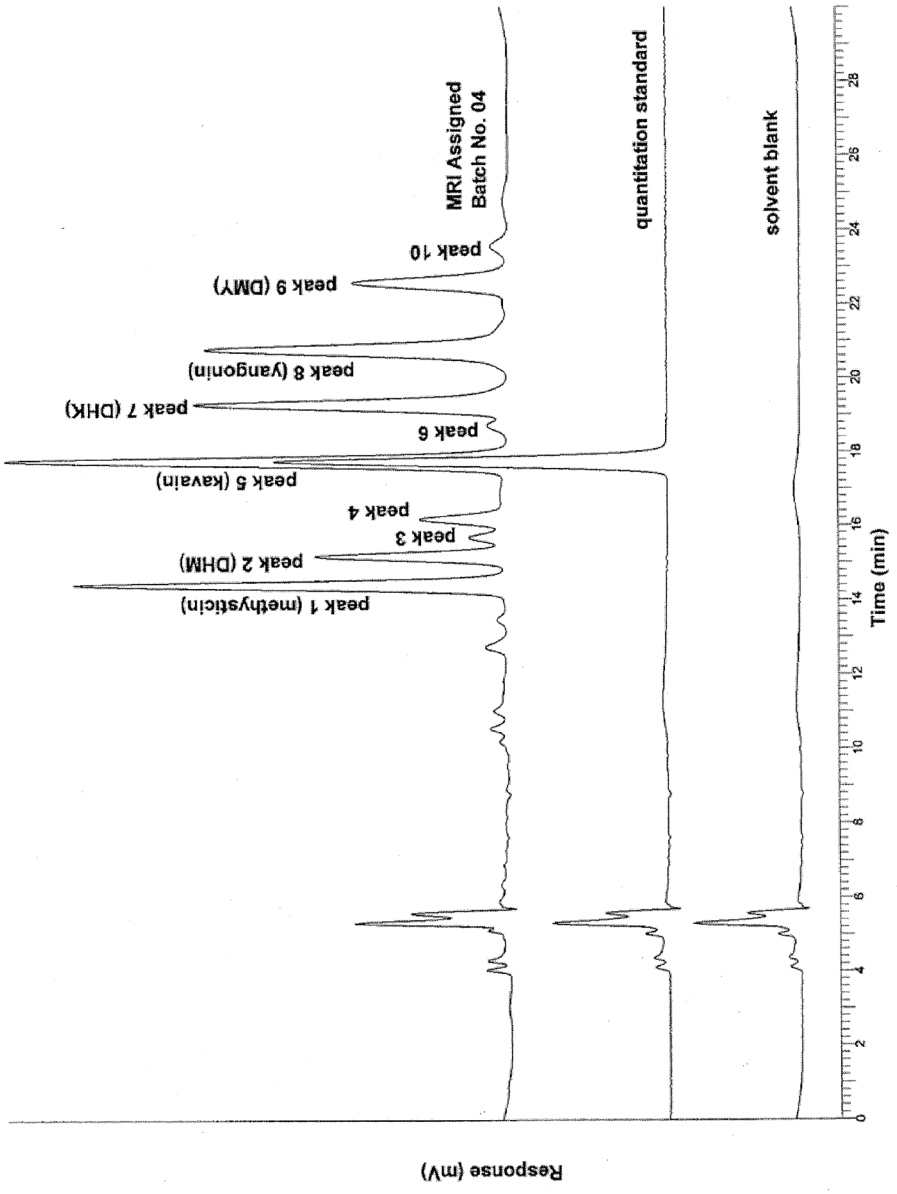

FIGURE I1
High-Performance Liquid Chromatography Quantitation of Kavalactones in Kava Kava Extract

TABLE I3
Preparation and Storage of Dose Formulations in the Gavage Studies of Kava Kava Extract

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
The test article was passed through a 40-mesh sieve. For the 12.5, 25, 50, and 100 mg/mL formulations, the appropriate amount of sieved test article was weighed into a Nalgene® carboy equipped with a spigot. Enough corn oil was added to wet the test article, and a smooth slurry was formed by stirring with a spatula. The spatula and sides of the carboy were rinsed with corn oil. The contents of the carboy were diluted to approximately half of the total final volume with corn oil, and the contents were stirred at a vigorous vortex for approximately 15 minutes with an overhead stirrer. While the formulation was being stirred, approximately 200 mL of it were dispensed into a beaker and poured back into the carboy. The beaker was rinsed into the carboy with corn oil, and the contents of the carboy were diluted to final volume with corn oil. The contents of the carboy were stirred at a vigorous vortex for approximately 5 minutes with an overhead stirrer. For the 200 and 400 mg/mL formulations, the appropriate amount of sieved test article was weighed into a beaker. A small amount of the test article was transferred from the beaker to a Nalgene® carboy, and enough corn oil was added to wet the test article, and a smooth slurry was formed by stirring with a spatula. Increments of test article and corn oil were added to the carboy until all test article and corn oil were incorporated and a smooth paste was formed. The spatula and beaker were rinsed with corn oil into the carboy, and the contents were diluted to final volume. The contents of the carboy were stirred at a vigorous vortex for approximately 15 minutes with an overhead stirrer. While the formulation was being stirred, approximately 200 mL of it were dispensed into a beaker and poured back into the carboy. The contents of the carboy were stirred at a vigorous vortex for approximately 5 minutes using an overhead stirrer. The dose formulations were prepared once during the studies.	Same as 2-week studies except that the appearance of the formulations was compared with photographs taken during the prestart work to confirm that the test article was dissolved. The dose formulations were prepared five times during the studies.	Same as 3-month studies (the 20 and 60 mg/mL dose formulations were prepared in the same manner as the 100 mg/mL or less dose formulations) except that for the 200 mg/mL formulations prepared from July 18, 2005, until the end of the studies, the appropriate amount of sieved test article was weighed directly into a Nalgene® carboy equipped with a spigot. The dose formulations were prepared approximately every 4 weeks.

TABLE I3
Preparation and Storage of Dose Formulations in the Gavage Studies of Kava Kava Extract

2-Week Studies	3-Month Studies	2-Year Studies
Chemical Lot Number		
9077SDK, Batch 02	9077SDK, Batches 02, 03, 04 (combined and assigned the lot number 082203)	9077SDK, Batch 05
Maximum Storage Time		
37 days	42 days	42 days
Storage Conditions		
Stored in clear glass bottles enclosed in amber plastic bags at room temperature	Stored in clear glass bottles enclosed in amber plastic bags at room temperature	Stored in clear glass bottles enclosed in amber plastic bags at room temperature
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of Kava Kava Extract

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
January 29, 2003	February 3-4, 2003	12.5	11.56	–8
		25	24.76	–1
		50	47.55	–5
		100	100.7	+1
		200	206.4	+3
		400	427.4	+7
	March 10-12, 2003[c]	25	22.99	–8
		50	43.25[d]	–14
		100	98.33	–2
		200	184.8	–8
		400	372.7	–7
	March 10-12, 2003[e]	12.5	11.37	–9
		25	23.31	–7
		50	45.59[f]	–9
		100	96.87	–3
		200	203.6	+2

[a] The 12.5 and 400 mg/mL dose formulations were used for mice or rats only, respectively.
[b] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 25 mg/mL=0.125 g/kg, 50 mg/mL=0.25 g/kg, 100 mg/mL=0.5 g/kg, 200 mg/mL=1.0 g/kg, 400 mg/mL=2.0 g/kg. For mice, dosing volume=10 mL/kg; 12.5 mg/mL=0.125 g/kg, 25 mg/mL=0.25 g/kg, 50 mg/mL=0.5 g/kg, 100 mg/mL=1.0 g/kg, 200 mg/mL=2.0 g/kg.
[c] Animal room samples for rats
[d] Results of triplicate analyses
[e] Animal room samples for mice
[f] Results of quadruplicate analyses

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Kava Kava Extract

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
September 3, 2003	September 4-5, 2003	12.5	12.65	+1
		25	25.91	+4
		50	49.71	–1
		100	99.62	0
		200	201.9	+1
		400	410.7	+3
	October 16-17, 2003[c]	25	25.65	+3
		50	49.45	–1
		100	97.91	–2
		200	196.7	–2
		400	403.1	+1
	October 16-17, 2003[d]	12.5	10.64	–15
		25	24.68	–1
		50	48.65	–3
		100	97.21	–3
		200	196.6	–2
September 25, 2003	September 30-October 1, 2003[e]	12.5	12.26	–2
		25	24.52	–2
		50	52.11	+4
		100	101.0	+1
		200	196.1	–2
		400	392.9	–2
	November 7-8, 2003[c]	25	24.84	–1
		50	51.04	+2
		100	102.5	+3
		200	200.5	0
		400	401.4	0
	November 7-8, 2003[d]	12.5	12.33	–1
		25	24.33	–3
		50	50.37	+1
		100	101.8	+2
		200	199.5	0

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Kava Kava Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
November 13, 2003	November 14-15, 2003	12.5	12.79	+2
		25	25.40	+2
		50	50.33	+1
		100	102.4	+2
		200	200.3	0
		400	394.5	–1
	January 2-5, 2004[c]	25	25.00	0
		50	43.98	–12
		100	97.61	–2
		200	197.4	–1
		400	386.6	–3
	January 2-5, 2004[d]	12.5	12.52	0
		25	25.08	0
		50	44.54[f]	–11
		100	92.89	–7
		200	184.7	–8

[a] The 12.5 and 400 mg/mL dose formulations were used for mice or rats only, respectively.
[b] Results of triplicate analyses. For rats, dosing volume=5 mL/kg; 25 mg/mL=0.125 g/kg, 50 mg/mL=0.25 g/kg, 100 mg/mL=0.5 g/kg, 200 mg/mL=1.0 g/kg, 400 mg/mL=2.0 g/kg. For mice, dosing volume=10 mL/kg; 12.5 mg/mL=0.125 g/kg, 25 mg/mL=0.25 g/kg, 50 mg/mL=0.5 g/kg, 100 mg/mL=1.0 g/kg, 200 mg/mL=2.0 g/kg.
[c] Animal room samples for rats
[d] Animal room samples for mice
[e] All samples analyzed on these dates were analyzed in duplicate.
[f] Insufficient sample to analyze in triplicate

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Kava Kava Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
August 4, 2004	August 5-8, 2004	20	20.32	+2
	September 8-9, 2004[b]	20	19.75	–1
August 10, 2004	August 10-11, 2004	60	58.84	–2
		200	199.7	0
	September 8-9, 2004[b]	60	54.87	–9
		200	191.0	–5
October 20, 2004	October 21-22, 2004	60	58.27	–3
		200	178.9[c]	–11
	October 28-29 and November 1, 2004	20	18.71	–6
January 10, 2005	January 11-13, 2005	20	19.35	–3
		60	57.16	–5
		200	194.2	–3
March 30, 2005	March 31-April 1, 2005	20	19.83	–1
		60	59.74	0
		200	196.0	–2
	May 9-10, 2005[b]	20	19.42	–3
		60	59.28	–1
		200	202.5	+1
June 20, 2005	June 22-23, 2005	20	20.23	+1
		60	58.88	–2
		200	193.4	–3
September 7, 2005	September 8-9, 2005	20	19.20	–4
		60	56.79	–5
		200	201.7	+1
November 29, 2005	December 1-3, 2005	20	20.85	+4
		60	62.19	+4
		200	203.5	+2
	January 6-7, 2006[b]	20	19.91	0
		60	59.23	–1
		200	200.3	0
February 15, 2006	February 17-18, 2006	20	20.86	+4
		60	59.66	–1
		200	206.6	+3
May 8, 2006	May 10-11, 2006	20	19.24	–4
		60	60.64	+1
		200	184.3	–8

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Kava Kava Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
July 26, 2006	July 29, 2006	20	21.15	+6
		60	63.02	+5
		200	201.7	+1
	September 13-14, 2006[b]	20	20.65	+3
		60	61.08	+2
		200	197.5	–1
Mice				
August 10, 2004	August 10-11, 2004	25	23.98	–4
		50	50.41	+1
		100	96.49	–4
	September 8-9, 2004[b]	25	23.45	–6
		50	47.15	–6
		100	92.68	–7
October 20, 2004	October 21-22, 2004	25	24.56	–2
		50	48.14	–4
		100	96.69	–3
January 10, 2005	January 11-13, 2005	25	23.36	–7
		50	46.94	–6
		100	97.65	–2
March 30, 2005	March 31-April 1, 2005	25	24.58	–2
		50	47.81	–4
		100	95.25	–5
	May 9-10, 2005[b]	25	23.81	–5
		50	47.07	–6
		100	95.66	–4
June 20, 2005	June 22-23, 2005	25	24.78	–1
		50	50.16	0
		100	101.4	+1
September 7, 2005	September 8-9, 2005	25	24.03	–4
		50	47.50	–5
		100	96.16	–4
November 29, 2005	December 1-3, 2005	25	24.70	–1
		50	51.09	+2
		100	100.8	+1
	January 6-7, 2006[b]	25	24.91	0
		50	50.02	0
		100	98.09	–2
February 15, 2006	February 17-18, 2006	25	24.71	–1
		50	50.88	+2
		100	101.5	+2

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Kava Kava Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
May 8, 2006	May 10-11, 2006	25	23.67	–5
		50	48.87	–2
		100	94.68	–5
July 26, 2006	July 29, 2006	25	24.94	0
		50	50.43	+1
		100	101.7	+2
	August 29, 2006[b]	25	23.46	–6
		50	47.36	–5
		100	97.01	–3

[a] Results of triplicate analyses. For rats, dosing volume=5 mL/kg; 20 mg/mL=0.1 g/kg, 60 mg/mL=0.3 g/kg, 200 mg/mL=1.0 g/kg. For mice, dosing volume=10 mL/kg; 25 mg/mL=0.25 g/kg, 50 mg/mL=0.5 g/kg, 100 mg/mL=1.0 g/kg.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration, but used at NTP's direction.

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)	14.6 ± 0.69	13.5 – 16.3	25
Crude fat (% by weight)	8.3 ± 0.37	7.6 – 9.3	25
Crude fiber (% by weight)	9.3 ± 0.45	8.4 – 10.0	25
Ash (% by weight)	5.0 ± 0.24	4.6 – 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)	4,048 ± 884	2,340 – 6,160	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.8 ± 19.39	52.0 – 124.0	20
Thiamine (ppm)[b]	7.7 ± 1.15	6.3 – 10.5	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 (%)	0.977 ± 0.049	0.895 – 1.080	25
Phosphorus (%)	0.568 ± 0.030	0.515 – 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)	0.23 ± 0.061	0.16 – 0.39	25
Cadmium (ppm)	0.05 ± 0.010	0.036 – 0.086	25
Lead (ppm)	0.09 ± 0.017	0.07 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.28 ± 0.100	0.18 – 0.49	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	13.1 ± 5.59	4.76 – 23.7	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]	4.9 ± 1.66	2.2 – 9.9	25
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.22	1.0 – 6.3	25
N-Nitrosopyrrolidine (ppb)[e]	2.2 ± 0.80	1.1 – 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	0.132 ± 0.130	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.254 ± 0.250	0.020 – 0.997	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.

In the 3-month studies, serum samples were collected from five male and five female sentinel rats and mice at 1 month and at the end of the studies. For the 2-year studies, serum samples were collected from five male and four or five female sentinel rats and mice at 1, 6, 12, and 18 (mice only) months; five male and five female special study vehicle control rats at 18 months; and from five male and five female 1.0 g/kg rats and mice at study termination. Fecal samples were taken from four male and five female mice at 18 months in the 2-year study for *Helicobacter spp.* by polymerase chain reaction testing. Blood from each animal was collected and allowed to clot, and the serum was separated. 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)	1 month, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	1 month, study termination
Immunofluorescence Assay	
Parvovirus	1 month, study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	1, 6, 12, 18 months, study termination
RCV/SDA	1, 6, 12, 18 months, study termination
Sendai	1, 6, 12, 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, 18 months, study termination
RCV/SDA	1 month
Sendai	1 month

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

RESULTS

All test results were negative.

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