NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF RIDDELLIINE

(CAS NO. 23246-96-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

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

May 2003

NTP TR 508

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

FOREWORD

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

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

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Technical Reports printed since 1982 appears on the inside back cover.

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SUMMARY

Background

Riddelliine is a naturally occurring alkaloid found in certain plants in the western United States rangeland. Traces of riddelline have been found in the meat of animals grazing on these plants, and cattle, horses, and sheep have died from such exposure.

Methods

We deposited solutions of riddelliine through a tube directly into the stomachs of male and female rats and mice five days per week for two years. Male and female rats received concentrations of 1 milligram of riddelliine per kilogram of body weight, and male and female mice received concentrations of 3 milligrams of riddelliine per kilogram of body weight. Other groups of female rats and male mice also received 1/3, 1/10, or 1/30 as much as the highest concentration.

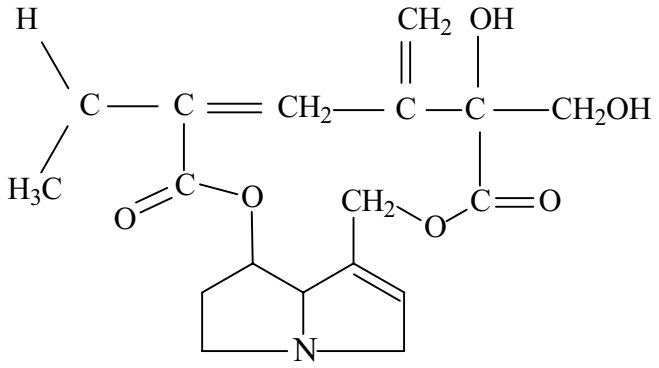
Results

Of 100 rats receiving 1 mg/kg, all but three died before the end of the study. Liver tumors were the cause of death for most of these animals; the incidences of mononuclear cell leukemia were also increased in exposed rats. Over half the male and female mice receiving 3 mg/kg also died before the end of the study. Male mice had more liver tumors and female mice more lung tumors than did animals not exposed to the chemical.

Conclusions

We conclude that riddelliine caused liver neoplasms in male and female rats and male mice and lung neoplasms in female mice, and also leukemia in male and female rats.

ABSTRACT



RIDDELLIINE

CAS No. 23246-96-0

Chemical Formula: $C_{18}H_{23}NO_6$ Molecular Weight: 349.4

Synonyms: 13,19-Didehydro-12,18-dihydroxy senecionan-11,16-dione; 3-ethylidine-3,4,5,6,9,11,13,14,14a,14b-decahydro-6-hydroxy-6-(hydroxymethyl)-5-methylene(1,6)di-oxacyclododecino(2,3,4-*gh*)-pyrrolizidine-2,7-dione; *trans*-15-ethylidine-12b-hydroxy-12a-hydroxymethyl-13-methylenesenec-1-enine

Riddelliine belongs to a class of toxic pyrrolizidine alkaloids and is isolated from plants of the genera *Crotalaria*, *Amsinckia*, and *Senecio* that grow in the western United States. Cattle, horses, and sheep that ingest these plants succumb to their toxic effects. Riddelliine residues have been found in meat, milk, and honey, and the plants may contaminate human food sources. Riddelliine was nominated for study by the Food and Drug Administration because of its potential for human exposure and its economic impact on the livestock industry and because the toxicity of other pyrrolizidine alkaloids suggests riddelliine may be carcinogenic. Male and female F344/N rats and B6C3F$_1$ mice received riddelliine (approximately 92% pure) by gavage. Female rats and male and female mice were dosed for 2 years; due to high mortality, the study in male rats was terminated at week 72. *In vitro* genetic toxicology studies were conducted in *Salmonella typhimurium* and in cultured Chinese hamster ovary (CHO) cells. In addition, riddelliine was evaluated *in vivo* for induction of micronuclei in mouse bone marrow and peripheral blood erythrocytes and for induction of S-phase DNA synthesis and unscheduled DNA synthesis in the liver of rats and mice. Riddelliine-induced DNA adduct levels were determined in liver tissue obtained from female rats admininstered riddelliine for 3 or 6 months.

2-Year Study in Rats

Groups of 50 male and 50 female rats were administered 0 or 1 mg riddelliine/kg body weight in sodium phosphate buffer by gavage 5 days per week; additional groups of 50 female rats received 0.01, 0.033, 0.1, or 0.33 mg/kg. A wide dose range was used in female rats to better characterize the dose-response curve. Females were dosed for 105 weeks; due to high mortality, male rats were terminated at week 72.

All but three 1 mg/kg males died before week 70, and all 1 mg/kg females died before week 97. Mean body weights of 1 mg/kg males and females were less than those of the vehicle controls throughout most of the study. The only clinical finding related to riddelliine administration was a general debilitation of the animals prior to death.

Hemangiosarcomas were present in the liver of 86% of males and 76% of females in the 1 mg/kg groups, and this neoplasm was considered the cause of the large

number of early deaths in these groups. The incidences of hepatocellular adenoma and mononuclear cell leukemia in 1 mg/kg males and females were significantly increased. Nonneoplastic lesions related to riddelliine treatment occurred in the liver and kidney of males and females.

Analyses of liver tissue from female rats treated with riddelliine for 3 or 6 months yielded eight DNA adducts; these were the same as DNA adducts formed *in vitro* by the metabolism of riddelliine by human liver microsomes in the presence of calf thymus DNA.

2-Year Study in Mice

Groups of 50 male and 50 female mice were administered riddelliine in sodium phosphate buffer by gavage at doses of 0 or 3 mg/kg, 5 days per week, for 105 weeks; additional groups of 50 male mice received 0.1, 0.3, or 1 mg/kg for 105 weeks. A wide dose range was used in male mice to better characterize the dose-response curve.

Survival of males and females administered 3 mg/kg was significantly less than that of the vehicle controls. Mean body weights of 3 mg/kg mice were less than those of the vehicle controls throughout most of the study.

Hemangiosarcomas of the liver were present in 62% of males in the 3 mg/kg group. The incidences of hepatocellular neoplasms occurred with negative trends in male mice and were significantly decreased in 3 mg/kg females. The incidences of alveolar/bronchiolar neoplasms in 3 mg/kg females were significantly increased. Nonneoplastic lesions related to riddelliine administration occurred in the liver and kidney of males and females and in the lung and arteries (multiple tissues) of females.

Genetic Toxicology

Riddelliine was mutagenic in *S. typhimurium* strain TA100 with, but not without, S9 activation; no significant mutagenic activity was detected in strain TA98 or TA1535, with or without S9. A small, dose-related increase in mutant colonies seen in strain TA97 with S9 was judged to be equivocal. Riddelliine induced sister chromatid exchanges in cultured CHO cells with and without S9. Chromosomal aberrations were induced in CHO cells only in the presence of S9. Following 4 or 13 weeks of daily gavage treatment with riddelliine, no increases in the frequency of micronucleated erythrocytes were noted in the peripheral blood of male or female $B6C3F_1$ mice. Use of a single intraperitoneal injection protocol, however, produced a small but significant increase in the frequency of micronucleated erythrocytes in peripheral blood of male Swiss mice 48 hours after injection; bone marrow analysis 24 hours after injection demonstrated a small but insignificant increase in the frequency of micronuclei. Unscheduled DNA synthesis was detected in cultured hepatocytes from male and female rats and mice following 5 or 30 days of riddelliine treatment by gavage. In addition, an S-phase DNA synthesis was observed in cultured hepatocytes of male and female rats treated for either time period.

Conclusions

Under the conditions of these studies, there was *clear evidence of carcinogenic activity** of riddelliine in male and female F344/N rats based on increased incidences of hemangiosarcoma in the liver. The increased incidences of hepatocellular adenoma and mononuclear cell leukemia in male and female rats were also considered to be treatment related. There was *clear evidence of carcinogenic activity* of riddelliine in male $B6C3F_1$ mice based on increased incidences of hemangiosarcoma in the liver. There was *clear evidence of carcinogenic activity* in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.

Administration of riddelliine by gavage resulted in nonneoplastic lesions in the liver and kidney of male and female rats; the liver and kidney of male and female mice; and the lung and arteries (multiple tissues) of female mice.

Decreased incidences of hepatocellular neoplasms in male and female mice were related to riddelliine administration.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Riddelliine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses in sodium phosphate buffer by gavage	Vehicle control or 1 mg/kg	Vehicle control, 0.01, 0.033, 0.1, 0.33, or 1 mg/kg	Vehicle control, 0.1, 0.3, 1, or 3 mg/kg	Vehicle control or 3 mg/kg
Body weights	1 mg/kg group less than the vehicle control group	1 mg/kg group less than the vehicle control group	3 mg/kg group less than the vehicle control group	3 mg/kg group less than the vehicle control group
Survival rates	49/50, 3/50	33/50, 22/50, 28/50, 22/50, 29/50, 0/50	39/50, 41/50, 40/50, 38/50, 20/50	34/50, 17/50
Nonneoplastic effects	Liver: regenerative hepatocyte hyperplasia (0/50, 49/50); hepatocyte cytomegaly (0/50, 32/50); focal necrosis (0/50, 23/50); eosinophilic focus (3/50, 15/50); mixed cell focus (3/50, 7/50); hemorrhage (0/50, 4/50) Kidney: renal tubule necrosis (0/50, 6/50)	Liver: regenerative hepatocyte hyperplasia (0/50, 0/50, 0/50, 0/50, 8/50, 50/50); hepatocyte cytomegaly (0/50, 0/50, 7/50, 23/50, 32/50, 29/50); focal necrosis (4/50, 2/50, 3/50, 4/50, 4/50, 15/50); eosinophilic focus (1/50, 2/50, 6/50, 4/50, 12/50, 13/50); mixed cell focus (8/50, 10/50, 10/50, 11/50, 23/50, 5/50); clear cell focus (9/50, 8/50, 9/50, 13/50, 22/50, 2/50); bile duct hyperplasia (2/50, 1/50, 4/50, 4/50, 3/50, 10/50); hemorrhage (0/50, 0/50, 2/50, 0/50, 1/50, 7/50) Kidney: renal tubule necrosis (0/50, 0/50, 0/50, 1/50, 1/50, 6/50); transitional epithelium hyperplasia (1/50, 1/50, 1/50, 1/50, 0/50, 5/50)	Liver: hepatocyte cytomegaly (4/50, 4/50, 16/50, 33/50, 43/50); hepatocyte karyomegaly (4/50, 4/50, 15/50, 33/50, 43/50); centrilobular hepatocyte necrosis (0/50, 1/50, 3/50, 4/50, 10/50) hemorrhage focal (0/50, 2/50, 1/50, 6/50, 21/50); severity of focal hepatocyte necrosis (1.3, 1.3, 1.8, 2.2, 2.6) Kidney: severity of nephropathy (1.3, 1.5, 1.8, 2.1, 2.8); glomerulosclerosis (0/49, 1/49, 0/50, 42/50, 41/50); renal tubule karyomegaly (0/49, 0/49, 0/50, 0/50, 12/50); renal tubule dilatation (16/49, 17/49, 24/50, 29/50, 22/50)	Liver: hepatocyte cytomegaly (0/49, 49/50); hepatocyte karyomegaly (0/49, 49/50); bile duct hyperplasia (0/49, 28/50); mixed cellular infiltration (29/49, 41/50) Kidney: nephropathy (18/49, 47/50); severity of nephropathy (1.3, 3.4); glomerulosclerosis (0/49, 40/50); renal tubule hyaline droplet accumulation (2/49, 14/50); renal tubule pigmentation (2/49, 27/50) Lung: alveolar epithelial hyperplasia (1/50, 6/50) Arteries, chronic arterial inflammation (includes focal): small intestine (duodenum) (0/47, 13/46); large intestine (cecum) (0/48, 18/47); kidney (1/49, 16/50); mesentery (1/23, 19/29); ovary (0/49, 26/48); spleen (0/49, 6/50); uterus (0/49, 21/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Riddelliine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Neoplastic effects	Liver: hemangiosarcoma (0/50, 43/50); hepatocellular adenoma (0/50, 4/50) Mononuclear cell leukemia (all organs): (2/50, 9/50)	Liver: hemangiosarcoma (0/50, 0/50, 0/50, 0/50, 3/50, 38/50); hepatocellular adenoma (1/50, 0/50, 0/50, 0/50, 1/50, 7/50) Mononuclear cell leukemia (all organs): (12/50, 8/50, 13/50, 18/50, 18/50, 14/50)	Liver: hemangiosarcoma (2/50, 1/50, 0/50, 2/50, 31/50)	Lung: alveolar/bronchiolar adenoma (1/50, 9/50); alveolar/bronchiolar carcinoma (1/50, 4/50); alveolar/bronchiolar adenoma or carcinoma (2/50, 13/50)
Decreased incidences	None	None	Liver: hepatocellular adenoma (16/50, 18/50, 14/50, 5/50, 0/50); hepatocellular carcinoma (23/50, 21/50, 19/50, 20/50, 3/50); hepatocellular adenoma or carcinoma (36/50, 39/50, 33/50, 23/50, 3/50)	Liver: hepatocellular adenoma (9/49, 0/50); hepatocellular carcinoma (8/49, 0/50); hepatocellular adenoma or carcinoma (16/49, 0/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:		Positive in strain TA100 with rat and hamster S9, negative without S9; negative in strains TA98 and TA1535 with and without S9; negative in strain TA97 without S9, equivocal with S9		
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro:*		Positive with and without S9		
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro:*		Positive with S9, negative without S9		
Micronucleus test in mice:				
Single injection study, bone marrow		Negative		
Single injection study, peripheral blood		Positive, but not confirmed with a repeat test		
4-week gavage study, peripheral blood		Negative		
13-week gavage study, peripheral blood		Negative		
Unscheduled DNA synthesis				
5-day gavage study		Positive in male and female rats and mice		
30-day gavage study		Positive in male rats and male and female mice; negative in female rats		
S-Phase DNA synthesis				
5-day gavage study		Positive in male and female rats; negative in male and female mice		
30-day gavage study		Positive in male and female rats and male mice; negative in female mice		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in 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 in 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 BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on riddelliine on October 18, 2001, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee 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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* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On October 18, 2001, the draft Technical Report on the toxicology and carcinogenesis studies of riddelliine received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of riddelliine by describing the structure and occurrence of the chemical, the results of the previously published 13-week studies, and the design of the present 2-year studies. He discussed the survival and body weight effects and the chemical-related neoplasms observed in the exposed animals. The proposed conclusions were *clear evidence of carcinogenic activity* in male and female F344/N rats and $B6C3F_1$ mice.

Dr. M.W. Chou, NCTR, described companion studies to characterize the metabolism of riddelliine by rat and human liver microsomes and the DNA adducts formed in both systems.

Dr. Drinkwater, a principal reviewer, expressed reservations about the unbalanced study design, with male rats and female mice each having just one dosed group. He also questioned whether the hepatocellular neoplasms and leukemia in the male and female rats could definitely be attributed to chemical exposure.

Dr. Piegorsch, the second principal reviewer, also questioned the unbalanced study design but did not feel that any neoplastic effects went undetected in these studies. He noted the potential benefits for risk assessment from the multiple-dose studies in female rats and male mice.

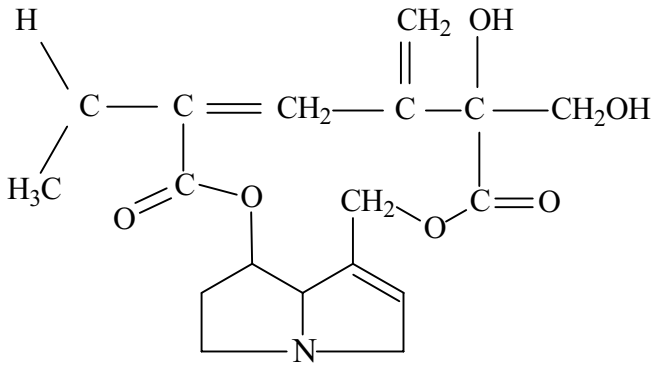
Dr. Medinsky, the third principal reviewer, agreed with the proposed conclusions and noted the importance of the DNA adduct studies in characterizing the genotoxic mechanism for this chemical.

Dr. J.R. Bucher, NIEHS, explained the rationale for use of the unbalanced dosing scheme. In preliminary 13-week studies, riddelliine caused liver neoplasms in female mice. Because the amount of test material was limited and the carcinogenicity of the chemical was already established, the NTP decided to use several lower dose concentrations in one sex for each rodent species to try to determine dose-response relationships or no-observed-effect levels.

Dr. J.K. Haseman, NIEHS, explained that the hepatocellular adenomas and leukemia in female rats were not part of the "*clear evidence*" conclusion but were still considered attributable to chemical administration and by themselves would have constituted "*some evidence*" of carcinogenic activity. The increase in leukemia incidence was deemed more significant after survival-adjusted analyses, reflecting the early deaths of many animals. Dr. J.R. Hailey, NIEHS, added that mononuclear cell leukemia normally occurs after 18 months in Fischer rats, while most of the female rats in the top dose group had already died by that time.

Dr. Medinsky moved that the conclusions be accepted as written, and Dr. Drinkwater seconded the motion, which was accepted unanimously with five votes.

INTRODUCTION



RIDDELLIINE

CAS No. 23246-96-0

Chemical Formula: $C_{18}H_{23}NO_6$ Molecular Weight: 349.4

Synonyms: 13,19-Didehydro-12,18-dihydroxy senecionan-11,16-dione; 3-ethylidine-3,4,5,6,9,11,13,14,14a,14b-decahydro-6-hydroxy-6-(hydroxymethyl)-5-methylene(1,6)di-oxacyclododecino(2,3,4-*gh*)-pyrrolizidine-2,7-dione; *trans*-15-ethylidine-12b-hydroxy-12a-hydroxymethyl-13-methylenesenec-1-enine

Chemical and Physical Properties

Riddelliine, a crystalline solid, is a macrocyclic diester of retronecine (an unsaturated alcohol) and riddelliic acid (an oxygenated, branched, dicarboxylic acid). Riddelliine has a melting point of 198° C, is soluble in water as the hydrochloride and in chloroform, is slightly soluble in acetone and in ethanol, and is sparingly soluble in water. The solid is stable at room temperature in diffuse light for at least 1 year (Cheeke, 1979). Alcoholic and aqueous solutions of riddelliine are stable at room temperature when protected from light.

Production, Use, and Human Exposure

Riddelliine is produced commercially only as a reference standard and as a research material for laboratories and clinics. Accurate production data for riddelliine are not available.

Riddelliine is isolated from plants of the genera *Crotalaria*, *Amsinckia*, and *Senecio*. Structurally, riddelliine belongs to a class of toxic pyrrolizidine alkaloids that are esters of unsaturated basic alcohols (necines) and of a necic acid produced by plants growing in climates ranging from temperate to tropical. The plants are unrelated taxonomically, but a characteristic they share is the production of pyrrolizidine alkaloids. The alkaloids occur in different parts of the plants; the highest content is in the seeds and flowering tops. The quantity of the alkaloids varies, depending on the season, climate, and soil constitution (Fu *et al.*, 2001).

The plants from which riddelliine and other pyrrolizidine alkaloids are isolated are found in the rangelands of the western United States (Fu *et al.*, 2001). Cattle, horses, and, less commonly, sheep that ingest these plants succumb to their toxic effects. Riddelliine residues have been found in meat, milk, and honey (Food and Drug Administration, unpublished data). The plants may contaminate human food sources as intact plants, and their seeds may contaminate commercial grains (Fu *et al.*, 2001).

In India, the root of *Crotalaria juncea* is taken as a hemoptysis remedy, and the plant is used to treat impetigo and psoriasis and as an emmenagogue. The

"bush tea" used in Jamaica to treat children for a cold and an herbal tea that is popular in the American Southwest, gordolobo yerba, may contain riddelliine (Huxtable, 1980).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Little is known about the disposition of riddelliine. However, other pyrrolizidine alkaloids have been studied extensively, and results of these studies serve as a background for riddelliine. Studies of [^{3}H]-monocrotaline (Hayashi, 1966), [^{14}C]-lasiocarpine (Culvenor *et al.*, 1969), [^{14}C]-senecionine, and [^{14}C]-seneciphylline (Eastman *et al.*, 1982) showed that 80% of the ingested pyrrolizidine alkaloids are rapidly excreted unchanged in urine and feces, with urine being the more prevalent route. Carbon dioxide expiration is a minor route, accounting for about 10% of the dose excreted. The ingested alkaloids are found mainly in the liver and kidney. Low concentrations of radioactivity are found in other tissues, including the lungs. The metabolism of senecionine shows there are three principal metabolic pathways: hydrolysis of the ester functional groups; oxidation of the necine bases to the corresponding necine-*N*-oxides; and hydroxylation of the necine base, particularly at the C-8 position, to form 8-hydroxynecine derivatives, followed by dehydration to form the corresponding dehydropyrrolizidine (pyrrolic) derivatives (Mattocks, 1968; IARC, 1976). Metabolism is mainly catalyzed by the cytochrome P450 isozymes (Williams *et al.*, 1989; Miranda *et al.*, 1991). The rate of metabolism is higher with liver microsomes of rats pretreated with phenobarbital than with those from untreated rats (Huxtable and Wild, 1994). Hydrolysis can be catalyzed by cytosolic esterases (Dueker *et al.*, 1992). Pretreatment of the animal with phenobarbital increases the rate of metabolic conversion, while chloramphenicol or SKF 525A reduces it (Allen *et al.*, 1972; White *et al.*, 1983).

The pyrrole metabolites (dehydropyrrolizidines) resulting from hydroxylation followed by dehydration have been found to be strong alkylating agents responsible for most of the genotoxic activities of the parent pyrrolizidine alkaloids (Schoch *et al.*, 2000). However, although dehydropyrrolizidines are considered reactive metabolites, the mechanisms by which pyrrolizidine alkaloids induce genotoxicity and carcinogenicity are not clear.

Humans

No information on the absorption, distribution, metabolism, or excretion of riddelliine in humans was found in the literature.

In an NTP study, human liver microsomes were incubated *in vitro* with riddelliine for 30 minutes (Appendix I). The metabolism products contained in the supernatant were separated and analyzed by reverse-phase high-performance liquid chromatography. The metabolites contained in chromatographic peaks eluting at 25.4 and 30.1 minutes were identified as dehydroretronecine and riddelliine-*N*-oxide.

Mitotic Inhibition

The pyrrolizidine alkaloids, as well as their metabolites and synthetic analogues, are potent antimitotic compounds. Administration of heliotrine, lasiocarpine, or lasiocarpine-*N*-oxide reduced mitosis by more than 50% in regenerating rat liver following partial hepatectomy (Peterson, 1965; Downing and Peterson, 1968). Heliotridine added to leukocyte cultures depressed mitosis (Bick *et al.*, 1975). Hincks *et al.* (1991) showed that riddelliine and four other pyrrolizidine alkaloids inhibited colony formation by cultured bovine kidney epithelial cells. The pyrrolizidine alkaloids were not lethal to the cultured cells and only inhibited mitosis. McGrath *et al.* (1975) noted renal glomerular lesions in pigs fed *Crotalaria* seeds and suggested that the primary cause was mitotic inhibition.

Because of the antimitotic property of pyrrolizidine alkaloids, indicine-*N*-oxide was used as a chemotherapeutic agent for cancer (Letendre *et al.*, 1981, 1984). The relationship between the antimitotic activity and tumorigenic activity of the pyrrolizidine alkaloids is not clear. It has been noted that more tumors developed following the cessation of pyrrolizidine alkaloid intake than with continued dosing (Svoboda and Reddy, 1972; Allen *et al.*, 1975). Higher tumor yields were observed in rats fed *Petasites japonicus maxim* or *Symphytum officinale* intermittently than in rats fed continuously (Hirono *et al.*, 1973, 1978). Pyrrolizidine alkaloids allow the cells to go through DNA synthesis and the cell cycle to repeat without undergoing cell division, resulting in megalocyte development (Samuel and Jago, 1975; Mattocks, 1986). Hincks *et al.* (1991) postulated that the antimitotic activity of pyrrolizidine alkaloids is related to their ability to cross-link DNA and thereby inactivate the section of genome that codes for the proteins involved in division (Samuel and Jago, 1975).

TOXICITY

Experimental Animals

No information on the LD_{50} of riddelliine was available in the literature. Most of the documentation of pyrrolizidine toxicity is found in the literature of veterinary medicine and animal husbandry. A few common animal diseases related to pyrrolizidine alkaloid exposure are Missouri River bottom disease of horses, Pictou disease of cattle (Canada), Winton disease of cattle and horses (New Zealand), Schweinsberger disease of horses (Germany), Molteno cattle sickness (South Africa), and various chronic cirrhotic diseases of range animals worldwide. The knowledge among stockmen that pyrrolizidine alkaloid-containing plants such as *Crotalaria* and *Senecio* species are common toxic agents in animal diseases was verified by studies in which the plants were fed whole to cattle and horses (Mattocks, 1986). Clinical findings in poisoned animals include neurological, gastrointestinal (diarrhea), and hematologic (increased blood ammonia, hemolysis) effects. Ascites is often observed.

In studies conducted by the National Toxicology Program (1993), groups of five male and five female F344/N rats and $B6C3F_1$ mice received riddelliine in 0.1 M phosphate buffer by gavage at doses of 0, 0.33, 1.0, 3.3, 10, or 25 mg per kg body weight, 5 days per week, for 2 weeks. Four of five male rats administered 25 mg/kg died or were killed moribund before the end of the study; mean body weight gains of male rats in the 10 and 25 mg/kg groups were depressed. Dose-related increased incidences of hemorrhagic centrilobular hepatic necrosis, hepatocytic karyomegaly and cytologic alterations, pulmonary hemorrhage and edema, splenic extramedullary hematopoiesis, and pancreatic edema occurred in male rats administered 1.0 mg/kg or greater. No early deaths or body weight effects were observed in female rats or male or female mice. Cytologic alteration of the liver, hemorrhage and edema of the lung, and splenic hematopoiesis were also observed in female rats administered 3.3 mg/kg or greater but were generally slightly less severe than in male rats. In mice, the only treatment-related findings included increased liver weights and increased incidences of hepatic cytomegaly in males and females.

The NTP (1993) also conducted 13-week studies in which groups of 20 male and 20 female F344/N rats received 0, 0.1, 0.33, 1.0, 3.3, or 10 mg/kg and $B6C3F_1$ mice received 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg riddelliine in 0.1 M phosphate buffer by gavage, 5 days per week, for 13 weeks. Ten male and ten female rats and mice were evaluated at 13 weeks, five male and five female rats and mice were evaluated after 7 weeks of recovery, and the remaining animals were evaluated after 14 weeks of recovery. In the rat study, 19 of 20 males in the 10 mg/kg group died during the dosing period, and five 10 mg/kg females died during the recovery period. Body weights of rats decreased with increasing dose at 13 weeks, but those of all groups except the 1.0 and 3.3 mg/kg females had returned to values similar to those of the vehicle controls by the end of the recovery period. At 13 weeks, lung and spleen weights of dosed males and females and brain and liver weights of dosed females were increased and heart and liver weights of males were decreased. Clinical pathology findings were indicative of liver damage and erythrocyte and platelet sequestration and included increases in reticulocyte counts and decreases in platelet counts in males and females, increased serum alkaline phosphatase activities in males, and increased sorbitol dehydrogenase activities in females. The most significant treatment-related lesions were observed in the liver of dosed males and females at 13 weeks or after the recovery period and included hepatocyte cytomegaly and karyomegaly, cytoplasmic vacuolization, centrilobular necrosis, mixed inflammatory cell infiltration, foci of cytologic alteration, hyperplastic hepatocytes, and bile duct hyperplasia. Liver lesions initially observed after 13 weeks of dosing often persisted or progressed in severity during the recovery period, particularly in 10 mg/kg female rats. Treatment-related lesions were also observed in the bone marrow, gastrointestinal tract, heart, kidney, lung, lymph nodes, pancreas, and spleen.

No treatment-related deaths occurred in mice in the 13-week NTP (1993) study. Depressed body weight gains of 10 and 25 mg/kg mice (males, 9% and 17% less than those of the vehicle controls; females, 16% and 21% less, respectively) were noted at the end of the dosing period and persisted throughout the recovery period. Dose-related increases in erythrocyte counts in males and reticulocyte counts in females were observed. Centrilobular cytomegaly in the liver of 25 mg/kg mice and forestomach epithelial hyperplasia in the 10 and 25 mg/kg groups were noted after 13 weeks of dosing; the forestomach lesions became less severe during the 14-week recovery period, but cytomegaly in the liver persisted. Bile duct hyperplasia was observed in the liver of 25 mg/kg females at the end of the recovery period.

Molyneux *et al.* (1991) reported that calves fed *Senecio riddellii*, which contains only riddelliine and its *N*-oxide,

for 20 days exhibited weight loss, signs of depression, reduced feed consumption, ataxia of hind limbs, ascites, and edema before death. Microscopic examination revealed hepatocellular necrosis and collapse of lobules, increased numbers of fibroblasts and collagen, portal edema, anisokaryosis of hepatocyte nuclei with some cytomegaly, and bile duct proliferation.

Two stages of reactions are associated with pyrrolizidine poisoning. The first is a primary reaction in which the pyrrolizidine alkaloid or its metabolites act on the liver tissue, inducing acute necrosis and/or a marked enlargement of hepatocytes (megalocytosis). This process takes place even after a single exposure to the pyrrolizidine alkaloid. The second stage develops in response to the initial stage; responses include development of liver fibrosis accentuated by stromal collapse and endothelial cell damage. The latter may lead to thrombosis and occlusion of central veins; eventually, cirrhosis develops. Chronic exposure to pyrrolizidine alkaloids also gives rise to proliferation of bile ductule cells and nodular hyperplasia. The functional capacity of the liver deteriorates; death results from hepatic failure (Schoental, 1976; Peterson and Culvenor, 1983). The toxic metabolites from the liver may affect the heart and lungs. Huxtable (1979) reviewed the toxicity of the pyrrolizidine alkaloids and described the following sequelae of cardiopulmonary damage: endothelial proliferation in the heart and lung, arterial hypertrophy, pulmonary arterial hypertension, right ventricular hypertrophy, and cor pulmonale.

While large single doses of monocrotaline can damage the liver, chronic exposure to lower doses causes pulmonary damage in the absence of hepatotoxicity. Gillis *et al.* (1978) reported that ingestion of 0.4 mg monocrotaline per day by young rats (45 to 50 g) for 21 days led to pulmonary toxicity, manifested by increased lung-to-body-weight ratios and lung protein levels and reduced 5-hydroxytryptamine and noradrenaline removal and metabolism, in the absence of hepatotoxicity. The rats also developed pulmonary hypertension and right ventricular hypertrophy. The reduced ability of monocrotaline-damaged lung endothelial cells to remove 5-hydroxytryptamine probably allowed circulating levels of this and other vasoconstrictors to increase, resulting in increased pulmonary arterial pressure; compensatory hypertrophy of the right ventricular wall developed as the heart attempted to overcome the elevated pulmonary arterial pressure. Monthly doses of monocrotaline administered to young *Macaca* monkeys resulted in significant ultrastructural changes in the right ventricle (Raczniak *et al.*, 1978). These changes, which included mitochondrial hypertrophy, streaming and clumping of Z-band material, degeneration of myofibrils, changes in ribosomes, and proliferation of intercellular collagen fibers, were considered to be indicative of compensation to hypertensive heart disease.

Swick *et al.* (1982) reported that dietary intake of *Senecio* plant material modified mineral metabolism in rats. Copper and iron levels in the liver were markedly increased, even in the absence of supplemental copper. When copper was added to the diet of the rats, an even greater accumulation of copper was observed. Impairment of hematopoiesis and accelerated erythrocyte destruction were also noted.

Humans

No epidemiology studies or reports of health effects related to riddelliine exposure in humans were found in the literature.

Reproductive and Developmental Toxicity

Experimental Animals

In the studies conducted by the NTP (1993), the length of the estrous cycle was increased in 10 mg/kg female rats and 25 mg/kg female mice. In mating trials in which F344/N rats and $B6C3F_1$ mice received riddelliine in corn oil by gavage for 10 weeks (rats) or 10.5 weeks (mice), pups from rat dams administered 1.0 mg/kg and mouse dams administered 25 mg/kg weighed less at birth and during suckling than did those from control dams; the average live litter size, number of pups born alive, and pup survival through day 14 were reduced in the 25 mg/kg mouse group (NTP, 1993; Chan *et al.*, 1994).

Results of studies by Peterson and Jago (1980) indicate that pyrrolizidine alkaloids and their metabolites can cross the placenta. Heliotrine and dehydroheliotridine injected into rat dams on the fourteenth day of pregnancy were recovered in the fetuses. The pyrrolizidine alkaloids induced growth retardation and fetal mortality. Administration of heliotrine to pregnant rats during organogenesis led to fetal growth retardation and musculoskeletal defects, mainly in rib development. Hypoplasia of the lower jaw and cleft palate were common findings; however, there was no liver damage (Green and Christie, 1961). Intraperitoneal administration of fulvine to rat dams on days 9 through 12 of

gestation induced exencephaly, cleft palate, microphthalmia, limb and tail abnormalities, and other defects in offspring (Persaud and Hoyte, 1974).

Schoental (1959) reported that retrorsine administered to lactating rats caused liver lesions in the pups. Pups dying at 18 to 30 days of age had hydropic or fatty vacuolation of liver cells. Weanling rats that died or were killed between 1 and 6 months of age had hemorrhagic necrosis, increased hepatic centrilobular reticulin, thickening of centrilobular veins, hyperplastic nodules, and bile duct proliferation. The occurrence of hepatic lesions only after birth and not in fetuses was probably due to the inability of embryonic liver to activate pyrrolizidine alkaloids (Mattocks, 1986).

Humans

No information on the reproductive or developmental toxicity of riddelliine in humans was found in the literature.

Carcinogenicity

Experimental Animals

Two reports on the carcinogenicity of riddelliine were found in the literature. Liver nodules were observed in all four male and five of 12 female Wistar rats given 0.02 mg/mL riddelliine in drinking water twice weekly for 6 months, followed by three intraperitoneal injections of 25 mg/kg each during the seventh month or by a single intraperitoneal injection of 30 mg/kg one year after initial riddelliine exposure (Schoental and Head, 1957). The International Agency for Research on Cancer (1976) considered the study inconclusive.

In the rat study conducted by the NTP (1993), adenomas were observed in the liver of two 10 mg/kg females after 13 weeks of dosing and in one additional female after 14 weeks of recovery; two of the three females had multiple neoplasms. No adenomas were observed in vehicle control females.

The toxicity and carcinogenicity of many other pyrrolizidine alkaloids have been studied (WHO, 1988). Harris and Chen (1970) reported malignant liver tumors in rats fed 0.5% dried *Senecio longilobus L.* every other week for 1 year. Newberne and Rogers (1973) demonstrated that monocrotaline given intragastrically once weekly for 55 weeks or longer induced liver cell carcinoma in rats. Male and female rats fed 7, 15, or 30 ppm lasiocarpine developed liver angiosarcoma, hepatocellular adenoma, and hepatocellular carcinoma. Female rats also developed hematopoietic neoplasms (NCI, 1978).

In drinking water studies, hepatomas were induced in rats administered 0.03 g/L retrorsine or isatidine 3 days per week (Schoental *et al.*, 1954). Hemangioendothelial sarcoma and hepatic adenoma were induced in rats exposed to 0.01% petasitenine (Hirono *et al.*, 1977). Clivorine administered at a concentration of 0.05 g/L for 340 days induced liver tumors, hemangioendothelial sarcomas, and neoplastic nodules in rats (Kuhara *et al.*, 1980).

Young male Sprague-Dawley rats administered subcutaneous injections of dehydroretronecine every other week for 12 months developed rhabdomyosarcomas at the site of injection (Allen *et al.*, 1975). Peterson *et al.* (1983) reported that rats receiving intraperitoneal injections of dehydroheliotridine developed tumors in the liver, lungs, intestines, and other organs.

Humans

No epidemiology studies of riddelliine exposure in humans were found in the literature.

Genetic Toxicology

Riddelliine has demonstrated genotoxic activity *in vitro* and *in vivo* in some assays under certain testing conditions. Riddelliine induced mutations in *Salmonella typhimurium* strain TA100 when tested with 30% rat or hamster liver S9 activation enzymes (Zeiger *et al.*, 1988); no mutation induction was seen in the absence of S9 or with 10% S9, indicating a specific requirement for metabolic activation. Riddelliine induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells in the presence of S9 (Galloway *et al.*, 1987); sister chromatid exchanges were also significantly increased in the absence of S9. Additional positive results for riddelliine were reported in several abstracts, including induction of mutations in mouse lymphoma L5178Y cells (Rudd *et al.*, 1983), unscheduled DNA synthesis in Fischer 344 rat hepatocytes *in vitro* (Mirsalis *et al.*, 1983), and S-phase and unscheduled DNA synthesis *in vivo* (Mirsalis, 1987). Metabolism, cytotoxicity, and DNA and protein binding studies performed with other pyrrolizidine alkaloids have shown that these compounds are metabolized to reactive pyrroles that covalently bind to liver proteins and DNA (Eastman *et al.*, 1981, 1982) and are potent hepatotoxins (Green *et al.*, 1981; Griffin and Segall,

1986). The liver's apparent sensitivity to the genotoxic action of riddelliine is supported by the observation that riddelliine induced a significant increase in unscheduled DNA synthesis in hepatocytes of rats treated by gavage *in vivo* (Mirsalis, 1987).

In contrast to the effects observed in rat liver *in vivo*, administration of up to 25 mg/kg riddelliine by gavage for 4 or 13 weeks did not result in increased frequencies of micronucleated erythrocytes in peripheral blood of male or female $B6C3F_1$ mice (Witt *et al.*, 2000). Additional studies of the bone marrow erythrocytes of mice in the 4-week study confirmed the absence of an effect (Mirsalis *et al.*, 1993). A small but significant increase in the frequency of micronucleated erythrocytes was observed in male Swiss mice 48 hours after administration of a single intraperitoneal injection of 150 mg/kg riddelliine (MacGregor *et al.*, 1985).

Riddelliine caused DNA cross-linking, DNA-protein cross-linking, and a dose-dependent inhibition of colony formation and megalocytosis in cultured bovine kidney epithelial cells (Hincks *et al.*, 1991; Kim *et al.*, 1993, 1995).

Study Rationale

Riddelliine was nominated by the FDA for toxicity and carcinogenicity testing because of its potential for human exposure and its economic impact on the livestock industry and because the toxicity of other pyrrolizidine alkaloids suggests riddelliine may be carcinogenic. Gavage in phosphate buffer was chosen as the route of administration because the oral route is the major route of exposure in humans and because the quantity of riddelliine available for study was limited.

Based on the findings of hepatocellular neoplasms in female rats in the 13-week studies (NTP, 1993), it was considered important to use a large number and broad range of doses to better characterize the shape of the dose-response curve for carcinogenicity rather than focus on the identification of a carcinogenic response. This would also allow for a more complete comparison of neoplastic responses in relation to the anticipated nonneoplastic effects resulting from the known toxicity of riddelliine. Due to the limited amount of riddelliine available for study, five dosed groups of female rats and only one dosed group of male rats were used, instead of an equal number of dose groups in each sex. The female rats were considered more suitable than male rats for the investigation of low-dose effects, as hepatocellular adenomas were found in female rats in the 13-week studies. The male dosed group, at a dose equal to the highest dose in females, was added for comparison. Because female rats were selected for the investigation of low-dose effects of riddelliine, male mice were used as the counterpart for female rats. Four dosed groups of male mice and one dosed group of female mice, at a dose equal to the highest dose in males, were used. The doses selected for the 2-year studies were 0 and 1 mg/kg for male rats; 0, 0.01, 0.033, 0.1, 0.33, and 1 mg/kg for female rats; 0, 0.1, 0.3, 1, and 3 mg/kg for male mice, and 0 and 3 mg/kg for female mice. The highest doses of 1 mg/kg for rats and 3 mg/kg for mice were selected because in the 13-week studies, no effects on body weights, survival, or histopathology were observed in animals administered these doses. The lower dose levels were selected because the FDA was interested in low-dose effects. Liver tissue from additional groups of female rats that received the same doses of riddelliine as the core study animals for 3 or 6 months was analyzed to identify DNA adducts.

MATERIALS AND METHODS

Procurement and Characterization of Riddelliine

Riddelliine was obtained from the Food and Drug Administration (Rockville, MD) in one lot (8194-110-01). Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC), and the study laboratory. Reports on analyses performed in support of the riddelliine studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white, crystalline solid, was identified as riddelliine by infrared, ultraviolet/visible, and proton nuclear magnetic resonance spectroscopy and by low-resolution mass spectrometry (MS). The purity of lot 8194-110-01 was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC) and gas chromatographic water analysis. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). Purity was confirmed by the study laboratory using HPLC. Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were in agreement with the theoretical values for riddelliine. HPLC revealed a major peak and five minor peaks accounting for 6.3% of the total integrated area by one system and one major peak and two minor peaks accounting for 8.0% of the total integrated area by a second system. By both systems, only one impurity peak was present at greater than 1% of the total peak area. This component was identified by HPLC/MS as retrorsine. Gas chromatography indicated less than 0.1% water. The overall purity determined by the analytical chemistry laboratory was approximately 92%. The study laboratory confirmed a purity of 92.6% using HPLC.

Stability studies were performed by Midwest Research Institute (Kansas City, MO) using HPLC. These studies indicated that riddelliine is stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at approximately 5° C, protected from light. Stability was monitored during the 2-year studies using HPLC. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 4 weeks by mixing riddelliine with 0.1 M sodium phosphate buffer (pH 6.0) (EM Science, Gibbstown, NJ; Fischer Scientific, Fairlawn, NJ) (Table F2). Homogeneity studies of the 0.0020 and 0.30 mg/mL dose formulations were performed by the study laboratory using HPLC. Stability studies of a 0.00204 mg/mL dose formulation were performed by the analytical chemistry laboratory using HPLC. Homogeneity was confirmed, and the stability of the dose formulation was confirmed for at least 35 days at 1° to 3° C or at room temperature when stored in sealed glass containers. Dose formulations were stored at 5° C in amber glass bottles for up to 35 days.

Periodic analyses of the dose formulations of riddelliine were conducted at the study laboratory using HPLC. The formulations were analyzed every 8 to 12 weeks (Table F3). Of the dose formulations used during the 2-year studies, all were within 10% of the target concentrations, with no value greater than 105% (rats) or 109% (mice) of the target concentration; 18 of 20 animal room samples for rats and all 16 animal room samples for mice also were within 10% of the target concentrations.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats were administered riddelliine in sodium phosphate buffer by gavage at doses of 0 or 1 mg riddelliine/kg body weight 5 days per week; additional groups of 50 female rats received 0.01, 0.033, 0.1, or 0.33 mg/kg. Females were dosed for 105 weeks; due to high mortality, male rats were terminated at week 72. Groups of 50 male and 50 female mice were administered 0 or 3 mg/kg riddelliine in sodium phosphate buffer 5 days per week for 105 weeks; additional groups of 50 male mice received 0.1, 0.3, or 1 mg/kg for 105 weeks. The dose ranges for female rats and male mice were selected based on the no-observed-adverse-effect levels (NOAELs) of 0.1 mg/kg for rats and 3.3 mg/kg for mice estimated from the results of the 13-week riddelliine studies (NTP,

1993). Lower dose groups were added to ensure that NOAELs were reached for each species.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 12 or 13 days, respectively, before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix H).

Animal Maintenance

Rats were housed up to three (males) or five (females) per cage. Male mice were housed individually, and female mice were housed five per cage. Feed and water were available *ad libitum*. Animals were given nonirradiated feed from the beginning of the studies until July 21, 1996, and were given irradiated feed thereafter to reduce potential microbial contamination. 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 in Appendix G.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks. Animals were weighed initially, during week 5, and every 4 weeks thereafter.

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

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. 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. A quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the bone marrow, glandular stomach, kidney, liver, lung, mediastinal and mesenteric lymph nodes, spleen, thymus (males only), and thyroid gland (females only) of rats, and the kidney, liver, lung (females only), and spleen of mice.

To gain more information about the morphological characteristics of the glomerulosclerosis observed in dosed mice, a total of 18 kidneys were selected from the male and female vehicle controls, 1 mg/kg males, and 3 mg/kg males and females and stained with Congo red, Masson's trichrome, and periodic acid-Schiff histochemical stains. In addition, each kidney was stained for the presence of immunoglobulin, complement 3, and albumin by immunohistochemistry.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, 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 chairperson 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 or previously rendered diagnoses. 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).

Liver tissue from additional groups of female F344/N rats administered riddelliine in sodium phosphate buffer by gavage at doses of 0, 0.01, 0.033, 0.1, 0.33, or 1 mg/kg daily for 3 or 6 months was collected and sent to the National Center for Toxicological Research (Jefferson, AR) for analysis of DNA adducts. These studies are described in Appendix I.

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Gavage Studies of Riddelliine

Study Laboratory
Southern Research Institute (Birmingham, AL)

Strain and Species
F344/N rats
$B6C3F_1$ mice

Animal Source
Taconic Laboratory Animals and Services (Germantown, NY)

Time Held Before Studies
Rats: 12 days
Mice: 13 days

Average Age When Studies Began
5-6 weeks

Date of First Dose
Rats: March 6, 1996
Mice: March 20, 1996

Duration of Dosing
5 days/week for 72 weeks (male rats) or 105 weeks (mice and female rats)

Date of Last Dose
Rats: July 14-15, 1997 (males), or March 3-5, 1998 (females)
Mice: March 17-23, 1998

Necropsy Dates
Rats: July 15-16, 1997 (males), or March 4-6, 1998 (females)
Mice: March 18-24, 1998

Average Age at Necropsy
Rats: 77 weeks (males) or 110 weeks (females)
Mice: 110-111 weeks

Size of Study Groups
50 males and 50 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
Rats: up to 3 (males) or 5 (females)
Mice: 1 (males) or 5 (females)

Method of Animal Identification
Tail tattoo

Diet
NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*. Animals received nonirradiated feed from the beginning of the studies through July 21, 1996, and irradiated feed thereafter.

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Gavage Studies of Riddelliine

Water
Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages
Polycarbonate (Lab Products, Maywood, NJ), changed at least once (male mice) or twice (rats and female mice) weekly

Bedding
Sani-Chips® (P.J. Murphy Forest Products Corp.; Montville, NJ); nonirradiated prior to July 18, 1996, and irradiated thereafter

Cage Filters
Reemay® (Andico, Birmingham, AL), changed every two weeks

Racks
Stainless steel (Lab Products, Maywood, NJ), changed and rotated every two weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: at least 10/hour

Doses
Rats: 0 or 1 mg/kg per day (males); 0, 0.01, 0.033, 0.1, 0.33, or 1 mg/kg (females). Doses were prepared in 0.1 M sodium phosphate buffer and administered at a volume of 5 mL/kg.
Mice: 0, 0.1, 0.3, 1, or 3 mg/kg per day (males); 0 or 3 mg/kg (females). Doses prepared in 0.1 M sodium phosphate buffer and administered at a volume of 10 mL/kg.

Type and Frequency of Observation
Observed twice daily; animals were weighed initially, during week 4, and every four weeks thereafter; clinical findings were recorded every 4 weeks.

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all animals.

Histopathology
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, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (except male mice), nose, ovary, pancreas, pancreatic islets, 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.

DNA Adduct Characterization
Additional groups of female F344/N rats that had been obtained from Taconic Laboratory Animals and Services received daily gavage doses of 0, 0.01, 0.033, 0.1, 0.33, or 1 mg/kg of riddelliine in sodium phosphate buffer for 3 or 6 months. Six animals from each dose group were sacrificed at each time point, and liver tissue from these animals was collected and sent to the National Center for Toxicological Research for analysis of DNA adducts. These studies are described in Appendix I.

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 from the survival analyses; 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, A5, B1, B5, C1, C5, D1, and D5 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 A3, B3, C3, and D3) 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., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or 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 A3, B3, C3, and D3 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, to 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 $B6C3F_1$ 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

Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

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. Until recently, the NTP historical control database consisted of animals fed the NIH-07 diet. In 1995, the NTP changed the diet fed to animals used in toxicity and carcinogenesis studies conducted by the NTP. This new diet (NTP-2000)

contains less protein and more fiber and fat than the NIH-07 diet previously used (Rao, 1996, 1997). This dietary change was instituted primarily to increase longevity and decrease the incidence and/or severity of some spontaneous neoplastic and nonneoplastic lesions in the rats and mice used in NTP studies. These studies of riddelliine are among the first in which the animals on study were fed the NTP-2000 diet. Because the incidence of some neoplastic and nonneoplastic lesions may be affected by the dietary change, use of the existing historical control database (NIH-07 diet) may not be appropriate for all neoplasm types.

Currently, the database includes 11 (10 for male rats) studies by various routes in which the NTP-2000 diet was used. Based on the extensive NTP historical database using the NIH-07 diet, incidences of the vast majority of spontaneous neoplasms are similar among control groups regardless of the route of administration. There is no reason to expect this to be different with the NTP-2000 diet. For example, control animals from dosed feed and dosed water studies are treated no differently and no differences in incidence of neoplasms are expected. Exceptions exist for some neoplasms/routes, and if comparisons are necessary for these neoplasm types, only studies with similar routes of administration will be used.

Quality Assurance Methods

The 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 riddelliine was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium,* sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, micronucleated erythrocytes in rat/mouse bone marrow, increases in the frequency of micronucleated erythrocytes in mouse peripheral blood, and unscheduled DNA synthesis and S-phase DNA synthesis. 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 (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. 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. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

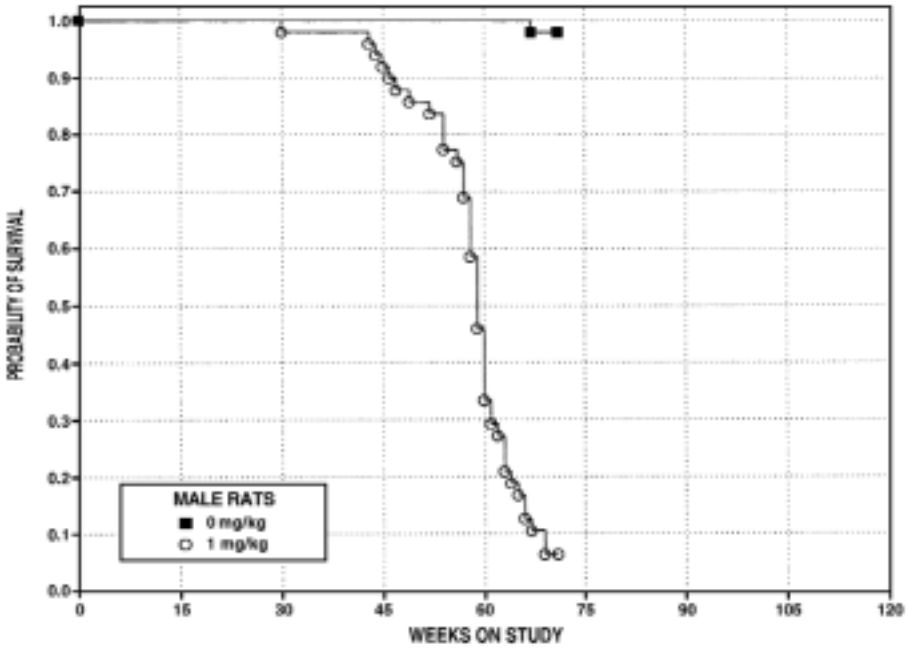
Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 1). All but three 1 mg/kg males died before week 70; due to high mortality in the dosed group, the male rat study was terminated at week 72. All 1 mg/kg females died before week 97 of the study; survival of all other dosed groups of females was similar to that of the vehicle control group.

TABLE 2
Survival of Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Male						
Animals initially in study	50					50
Accidental deaths[a]	0					2
Moribund	1					9
Natural deaths	0					36
Animals surviving to study termination	49					3
Percent probability of survival at end of study[b]	98					6
Mean survival (days)[c]	496					401
Survival analysis[d]						P<0.001
Female						
Animals initially in study	50	50	50	50	50	50
Accidental deaths[a]	2	6	3	2	3	0
Moribund	9	11	13	14	16	19
Natural deaths	6	11	6	12	2	31
Animals surviving to study termination	33	22	28	22	29	0
Percent probability of survival at end of study	69	51	61	46	63	0
Mean survival (days)	683	666	678	666	681	505
Survival analysis	P<0.001	P=0.166	P=0.513	P=0.063	P=0.641	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column (females only), and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns.



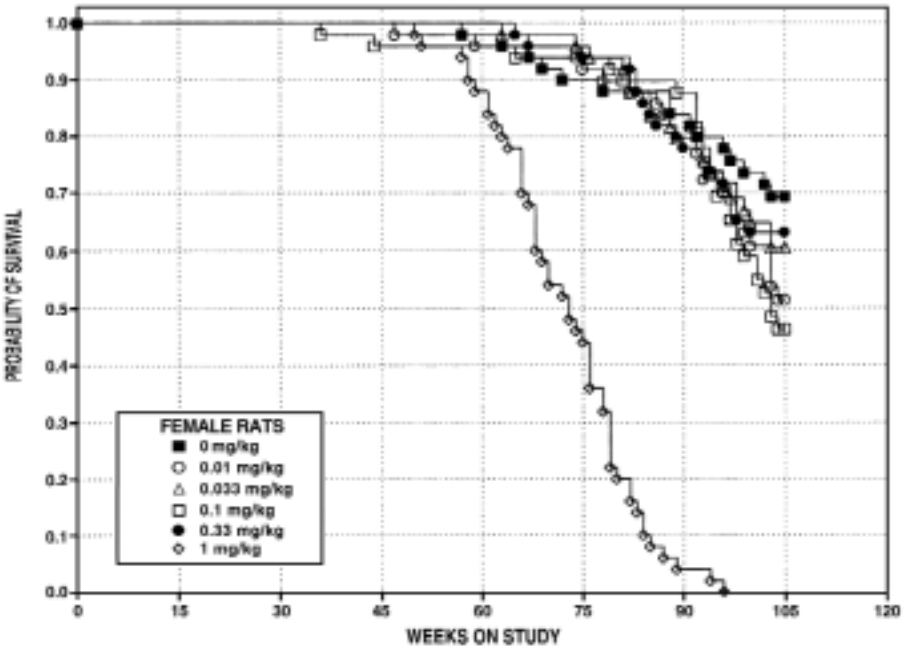


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Riddelliine by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of 1 mg/kg males and females were less than those of the vehicle controls throughout most of the study (Tables 3 and 4; Figure 2). The only clinical finding related to riddelliine administration was a general debilitation, including thinness in males and females, prior to death.

Table 3
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Riddelliine

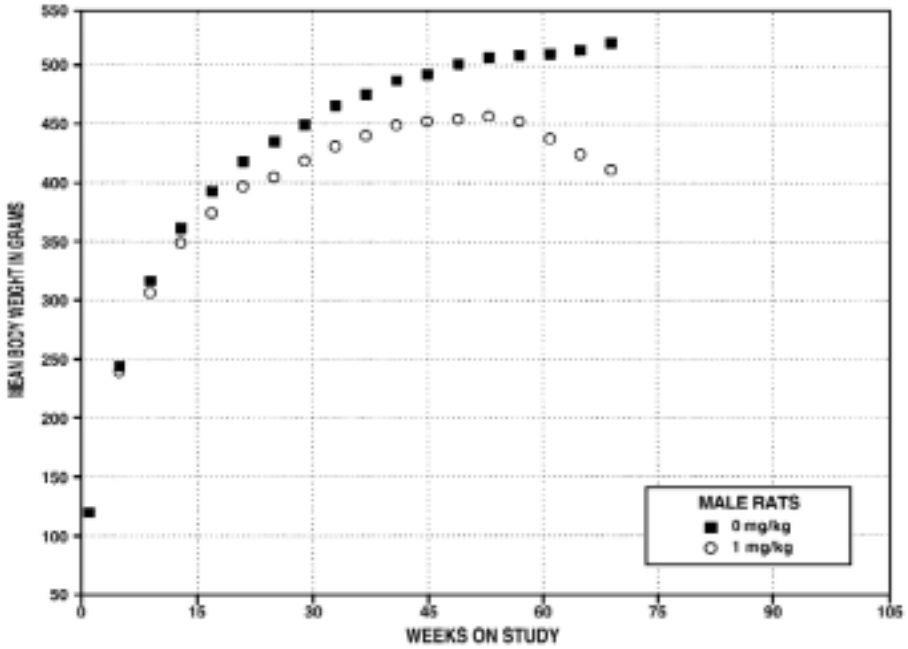
Weeks on Study	Vehicle Control		1 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	120	50	120	100	50
5	244	50	240	98	50
9	316	50	307	97	50
13	361	50	349	97	50
17	393	50	375	95	50
21	418	50	397	95	50
25	435	50	405	93	50
29	449	50	419	93	50
33	465	50	431	93	49
37	475	50	440	93	49
41	487	50	449	92	48
45	492	50	452	92	46
49	501	50	454	91	42
53	507	50	457	90	40
57	509	50	453	89	36
61	510	50	438	86	16
65	513	50	424	83	9
69	520	49	412	79	5
Mean for weeks					
1-13	260		254	98	
14-53	462		428	93	
54-69	513		432	84	

TABLE 4
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Riddelliine

Weeks on Study	Vehicle Control		0.01 mg/kg			0.033 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	95	50	95	100	50	96	101	50
5	155	50	154	99	50	155	100	50
9	182	50	183	100	50	182	100	50
13	196	50	195	99	50	197	100	50
17	203	50	204	101	50	205	101	50
21	214	50	216	101	50	215	100	50
25	218	50	219	100	50	219	101	50
29	226	50	228	101	50	228	101	50
33	233	50	235	101	50	236	101	50
37	238	50	240	101	50	240	101	50
41	247	50	249	101	50	248	100	50
45	251	50	252	100	50	253	101	50
49	259	50	260	100	49	260	100	50
53	268	50	271	101	49	269	100	50
57	277	49	277	100	49	277	100	50
61	281	49	287	102	48	284	101	50
65	292	48	296	101	48	293	101	48
69	304	47	306	101	48	301	99	48
73	307	45	308	101	48	303	99	48
77	309	45	311	101	45	306	99	46
81	310	44	311	100	44	309	99	45
85	315	44	316	100	43	311	99	43
89	316	42	318	101	39	319	101	39
93	319	39	318	100	32	322	101	37
97	320	37	320	100	29	317	99	32
101	322	35	318	99	26	320	99	30
Mean for weeks								
1-13	157		157	100		158	101	
14-53	236		237	100		237	100	
54-101	306		307	100		305	100	

Table 4
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Riddelliine

Weeks on Study	0.1 mg/kg			0.33 mg/kg			1 mg/kg		
	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	95	100	50	95	100	50	95	100	50
5	153	98	50	153	99	50	153	99	50
9	179	98	50	181	100	50	178	98	50
13	194	99	50	195	99	50	189	96	50
17	202	100	50	203	100	50	197	97	50
21	212	99	50	217	101	50	206	96	50
25	217	100	50	219	100	50	208	95	50
29	223	99	50	224	99	50	212	94	50
33	233	100	50	232	100	50	220	94	50
37	236	99	49	236	99	50	224	94	50
41	246	100	49	245	99	50	232	94	50
45	251	100	47	248	99	50	233	93	50
49	255	98	47	256	99	50	239	92	50
53	266	99	47	264	99	50	246	92	48
57	273	98	47	271	98	50	251	91	47
61	279	99	47	277	98	50	254	91	44
65	288	99	46	282	97	50	260	89	39
69	296	97	46	294	97	48	272	90	29
73	300	98	46	297	97	48	270	88	25
77	302	98	46	301	98	47	268	87	18
81	305	98	44	299	97	47	264	85	10
85	310	98	44	304	96	42	285	90	4
89	312	99	43	304	96	41	289	92	2
93	310	97	39	308	96	39	262	82	2
97	312	98	33	309	97	33			
101	316	98	28	313	97	29			
Mean for weeks									
1-13	155	99		156	99		154	98	
14-53	234	99		234	99		222	94	
54-101	300	98		297	97		268	88	



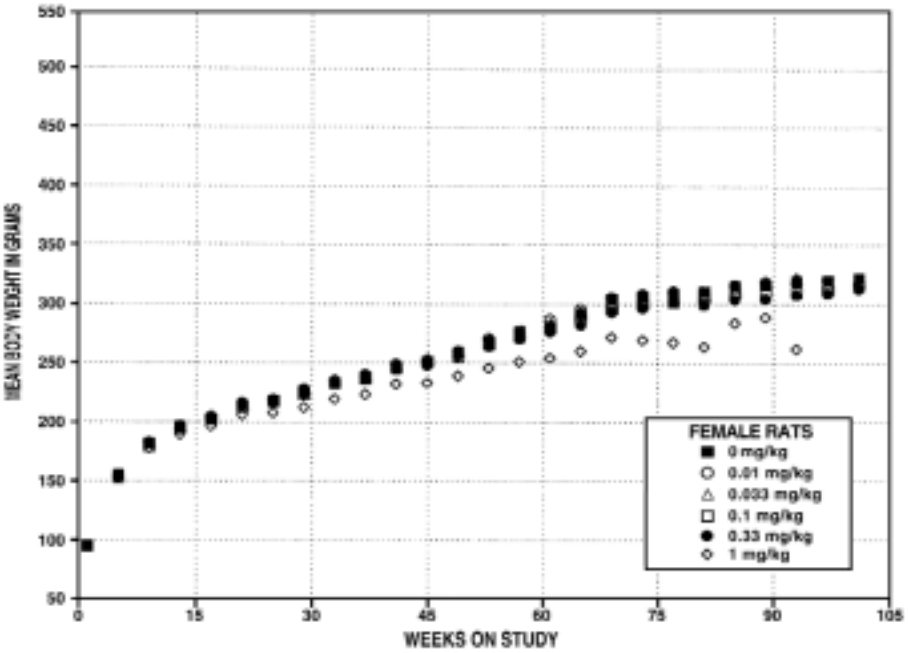


FIGURE 2
Growth Curves for Male and Female Rats Administered Riddelliine by Gavage for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or nonneoplastic lesions of the liver, thyroid gland, mammary gland, spleen, bone marrow, kidney, lung, glandular stomach, and mediastinal and mesenteric lymph nodes. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Liver: The incidences of hemangiosarcoma in 1 mg/kg males and females were significantly greater than those in the vehicle controls; three 0.33 mg/kg females also had this neoplasm (Tables 5, A3, and B3). No neoplasms of this type have been noted in controls (all routes) given NTP-2000 diet or in water gavage controls given NIH-07 diet in NTP studies (Tables A4a and B4a) for 2 years. Hemangiosarcoma was considered the cause of death of 37 males and 32 females administered 1 mg/kg that died early or were sacrificed moribund. Hemangiosarcomas were single or multiple neoplastic masses generally ranging from 0.5 to 1.0 cm or more in diameter, although some of these neoplasms were smaller than 0.5 cm. Most of the hemangiosarcomas consisted of a large central cavity filled with blood and fibrin and surrounded by a variably sized rim of neoplastic tissue (Plate 1). The central portions of the large hemangiosarcomas apparently underwent necrosis and inflammation as the neoplasms grew, leaving a large, blood-filled cavity. The smaller neoplasms often consisted of irregular masses of neoplastic cells without a central cavity. The hemangiosarcomas were generally anaplastic and were composed of elongated, flattened, spindle-shaped, or polyhedral endothelial cells that lined vascular spaces and frequently formed solid sheets (Plate 2). The cells varied considerably in size and had small to abundant amounts of eosinophylic cytoplasm with indistinct borders and variably sized, round to ovoid vesicular nuclei with one or more prominent nucleoli. Invasion and destruction of the hepatic parenchyma, frequently accompanied by prominent necrosis and hemorrhage, were common. In 1 mg/kg rats, approximately half of the hepatic hemangiosarcomas in males and one third of those in females metastasized to the lung (male: 26/50; female, 17/50); metastases also occurred in the mediastinal lymph node (male: 3/50; female: 2/50) and mesentery (male: 4/50; female: 2/50), and in the pancreas (2/50) and spleen (1/50) of males (Tables A1 and B1). Hemangiosarcoma in one 0.33 mg/kg female metastasized to the lung. The metastatic lesions were morphologically similar to the primary neoplasms. A primary hemangiosarcoma was observed in the lung of a single 1 mg/kg female.

The incidences of hepatocellular adenoma in 1 mg/kg males and females and of hepatocellular adenoma or carcinoma (combined) in 1 mg/kg females were significantly greater than those in the vehicle controls (Tables 5, A3, and B3). The incidences of hepatocellular adenoma in 1 mg/kg males and females and of hepatocellular adenoma or carcinoma (combined) in 0.33 and 1 mg/kg females exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 5, A4a, and B4a). Hepatocellular adenomas were typically discrete proliferations of hepatocytes that compressed adjacent tissue. There was loss of normal lobular architecture, and the plates of the neoplastic hepatocytes intersected the surrounding normal hepatocytes at sharp angles rather than merging with them as in the regenerative nodules. Criteria for hepatocellular carcinoma included distinct variable growth pattern (solid, trabecular, or glandular), increased number of mitotic figures, cellular atypia and nuclear pleomorphism, and irregular borders with invasion and/or metastasis.

The incidences of diffuse hepatocytic regenerative hyperplasia were significantly increased in 1 mg/kg males and in 0.33 and 1 mg/kg females (Tables 5, A5, and B5); the toxicity that led to regenerative hyperplasia was the primary cause of death in one male and four female 1 mg/kg rats. These nodules are thought to represent a regenerative response, probably secondary to hepatic damage caused by riddelliine exposure. This lesion consisted of the presence of small to large nodular proliferations of hepatocytes (Plate 3). In severely affected livers, most or all of the parenchyma was involved. The nodules contained increased numbers of small hepatocytes or decreased numbers of large, hypertrophic hepatocytes arranged in normal to irregular hepatic cords. Some of the nodules were large and caused some compression of adjacent parenchyma, but the nodules generally blended with the adjacent parenchyma. Proliferation of the bile ducts and of small oval basophilic cells that resembled oval cells were occasionally seen, especially in the larger nodules.

The incidences of several other nonneoplastic lesions of the liver in 1 mg/kg males and in 0.033 mg/kg or greater females were significantly greater than those in the vehicle controls (Tables 5, A5, and B5). Hepatocyte

Table 5
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Male						
Number Examined						
Microscopically	50					50
Hepatocyte, Hyperplasia, Regenerative[a]	0					49** (3.6)[b]
Hepatocyte, Cytomegaly	0					32** (2.3)
Necrosis, Focal	0					23** (3.2)
Eosinophilic Focus	3					15**
Mixed Cell Focus	3					7*
Basophilic Focus	32					21
Hemorrhage	0					4* (3.5)
Hemangiosarcoma, Multiple	0					24**
Hemangiosarcoma (includes multiple)[c]						
Overall rate[d]	0/50 (0%)					43/50 (86%)
Adjusted rate[e]	0.0%					92.5%
Terminal rate[f]	0/49 (0%)					1/3 (33%)
First incidence (days)	—[h]					307
Poly-3 test[g]						P<0.001
Hepatocellular Adenoma, Multiple	0					1
Hepatocellular Adenoma (includes multiple)[c]						
Overall rate	0/50 (0%)					4/50 (8%)
Adjusted rate	0.0%					13.7%
Terminal rate	0/49 (0%)					0/3 (0%)
First incidence (days)	—					398
Poly-3 test						P=0.033
Female						
Number Examined						
Microscopically	50	50	50	50	50	50
Hepatocyte, Hyperplasia Regenerative	0	0	0	0	8** (2.8)	50** (3.6)
Hepatocyte, Cytomegaly	0	0	7** (1.1)	23** (1.2)	32** (1.9)	29** (2.3)
Necrosis, Focal	4 (1.8)	2 (1.5)	3 (2.3)	4 (1.5)	4 (1.8)	15** (2.7)
Eosinophilic Focus	1	2	6	4	12**	13**
Mixed Cell Focus	8	10	10	11	23**	5
Clear Cell Focus	9	8	9	13	22**	2
Basophilic Focus	45	46	44	42	40	20*
Bile Duct, Hyperplasia	2 (1.0)	1 (1.0)	4 (1.8)	4 (1.5)	3 (2.0)	10** (1.7)
Hemorrhage	0	0	2 (3.5)	0	1 (4.0)	7** (3.0)

Table 5
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Female (continued)						
Hemangiosarcoma, Multiple	0	0	0	0	0	13**
Hemangiosarcoma (includes multiple)[i]						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	38/50 (76%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.0%	89.7%
Terminal rate	0/33 (0%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	—	—	—	—	524	350
Poly-3 test	P<0.001	—[j]	—	—	P=0.118	P<0.001
Hepatocellular Adenoma, Multiple	0	0	0	0	1	0
Hepatocellular Adenoma (includes multiple)[k]						
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	7/50 (14%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	2.4%	32.3%
Terminal rate	1/33 (3%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	729 (T)	—	—	—	729 (T)	426
Poly-3 test	P<0.001	P=0.514N	P=0.506N	P=0.510N	P=0.756	P=0.002
Hepatocellular Carcinoma	0	0	0	0	1	1
Hepatocellular Adenoma or Carcinoma[k]						
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	8/50 (16%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	4.8%	36.1%
Terminal rate	1/33 (3%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	2/29 (7%)	0/0
First incidence (days)	729 (T)	—	—	—	729 (T)	426
Poly-3 test	P<0.001	P=0.514N	P=0.506N	P=0.510N	P=0.493	P<0.001

(T) Terminal sacrifice
* 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] No comparable historical control incidence available because male rats were sacrificed at 72 weeks
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control group incidence (females only) is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for 2-year studies with controls given NTP-2000 diet: 0/659
[j] Value of statistic cannot be computed.
[k] Historical incidence for NTP-2000 diet (mean ± standard deviation): 4/659 (0.7% ± 1.0%), range 0%-2%

cytomegaly consisted of enlarged hepatocytes that had increased amounts of eosinophilic cytoplasm and variably sized, enlarged nuclei (karyomegaly) (Plate 4); these cells were distributed randomly in the hepatic parenchyma or within the areas of hepatic parenchyma between the nodules of regeneration. Increased incidences and severities of focal necrosis were noted in 1 mg/kg males and females. Necrosis was minimal to moderate and consisted of few to many irregular focal areas of coagulative hepatocyte necrosis. The hepatocytes had uniform eosinophilic cytoplasm and pyknotic, fragmented, or lytic nuclei. The necrosis was mostly present in livers in which hemangiosarcoma was also observed, suggesting it was secondary to the presence of these neoplasms. However, a direct effect of riddelliine cannot be excluded. In the 13-week NTP (1993) study, hepatocytic necrosis was noted only in animals receiving 10 mg/kg per day. The incidences of eosinophilic focus in 1 mg/kg males and females and 0.33 mg/kg females, mixed cell focus in 1 mg/kg males and 0.33 mg/kg females, and clear cell focus in 0.33 mg/kg females were significantly increased. The incidences of bile duct hyperplasia in 1 mg/kg females and hemorrhage in 1 mg/kg males and females were also increased.

The incidences of basophilic focus of the liver in 1 mg/kg males and females and of mixed cell focus and clear cell focus in 1 mg/kg females were decreased, and the decrease in the incidence of basophilic focus in 1 mg/kg females was significant (Tables 5, A5, and B5). The low incidences of foci in the 1 mg/kg groups may have been related to the diffuse involvement of the liver with regenerative hyperplasia and neoplasms as well as to the decreased survival in those groups. The foci ranged from small to moderately large lesions composed of hepatocytes arranged in normal hepatic cords that merged imperceptibly with the surrounding normal hepatocytes. The foci usually caused little or no compression of the surrounding tissue and had eosinophilic, basophilic, clear, or mixed cytoplasmic staining.

Mononuclear Cell Leukemia: Mononuclear cell leukemia is one of the most common neoplasms found in F344/N rats. It is a rapidly progressive, lethal, neoplastic disease that develops in the spleen with infiltrates of neoplastic cells occurring in the liver, lung, lymph nodes, bone marrow, and most other organs. The incidences of mononuclear cell leukemia in all dosed groups of rats except the 0.01 mg/kg females were increased; the increases were significant in the 1 mg/kg males and females (Tables 6, A3, and B3). The incidences of mononuclear cell leukemia in the female dosed groups were within or less than the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 6 and B4b). Because the male rat study was terminated at week 72, the mononuclear cell leukemia incidence in this study cannot be compared directly to the 2-year historical control rate. However, data from recent NTP studies show that the observed vehicle control mononuclear cell leukemia incidence in male rats is similar to the rates observed in comparably aged male control F344/N rats sacrificed at approximately 15 months (11/488; Tables 6 and A4b). Moreover, although the observed neoplasm incidence in 1 mg/kg females was similar to that in the vehicle controls, when survival differences are taken into account, the Poly-3 adjusted leukemia incidences are significantly different, and the overall trend is highly significant. Therefore, the increased incidences of mononuclear cell leukemia in male and female rats were considered to be chemical related.

Thyroid Gland (C-cell): The incidence of thyroid gland (C-cell) adenoma was significantly increased in 0.33 mg/kg females (vehicle control, 2/49; 0.01 mg/kg, 4/50; 0.033 mg/kg, 4/49; 0.1 mg/kg, 6/49; 0.33 mg/kg, 11/50; 1 mg/kg, 0/50; Table B3). However, the incidence in the vehicle controls was below the average historical control incidence (12%) in animals given NTP-2000 diet (Table B4c), and the adenoma incidence in 0.33 mg/kg females (22%) was just outside the historical control range of 4% to 21%. No adenomas were seen in 1 mg/kg females; however, survival in this group was reduced, and many deaths occurred fairly early in the study. Hyperplasia, adenoma, and carcinoma of the thyroid gland (C-cell) represent a morphologic and biologic continuum in the F344/N rat, and there was no increase in the incidence of carcinoma or hyperplasia in 0.33 mg/kg females (Tables B1 and B5). The only carcinoma observed in female rats was in a vehicle control animal, bringing the total C-cell neoplasm incidence in that group to 3/49. In addition, the incidence of hyperplasia was not significantly increased in any dosed group (22/49, 9/50, 24/49, 15/49, 20/50, 10/50; Table B5). Because there was no increase in the incidence of malignant neoplasms or hyperplasia, and the significantly increased incidence of adenoma was due, in part, to the low incidence in the vehicle control group, the increase was not considered related to riddelliine administration.

Mammary Gland: The incidence of mammary gland fibroadenoma was significantly decreased in 1 mg/kg

Table 6
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Male						
Mononuclear Cell Leukemia, All Organs[a]						
Overall rate[b]	2/50 (4%)					9/50 (18%)
Adjusted rate[c]	4.0%					28.5%
Terminal rate[d]	2/49 (4%)					0/3 (0%)
First incidence (days)	497 (T)					204
Poly-3 test[e]						P=0.004
Female						
Mononuclear Cell Leukemia, All Organs[f]						
Overall rate	12/50 (24%)	8/50 (16%)	13/50 (26%)	18/50 (36%)	18/50 (36%)	14/50 (28%)
Adjusted rate	27.0%	18.9%	29.9%	40.3%	39.0%	51.6%
Terminal rate	8/33 (24%)	1/22 (5%)	7/28 (25%)	6/22 (27%)	6/29 (21%)	0/0
First incidence (days)	393	513	532	451	463	352
Poly-3 test	P=0.009	P=0.262N	P=0.475	P=0.132	P=0.158	P=0.033

(T) Terminal sacrifice
[a] Historical incidence for 2-year studies (all routes except corn oil gavage) with controls given NIH-07 diet that had scheduled sacrifices at approximately 15 months: (mean ± standard deviation): 11/488 (2.2% ± 6.3%), range 0%-33%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control group incidence (females only) is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.
[f] Historical incidence for 2-year studies with controls given NTP-2000 diet (mean ± standard deviation): 185/659 (29.1% ± 8.4%), range 16%-42%

females (28/50, 21/50, 26/50, 24/50, 23/50, 3/50; Table B3). There is evidence that the incidence of mammary gland fibroadenoma in female F344/N rats is strongly correlated with body weight (Haseman *et al.*, 1997), and 1 mg/kg females had reduced body weights (Table 4). Thus the decreased incidence of fibroadenoma is likely due to a combination of reduced survival and reduced body weight, and possibly other factors.

Other Organs: The incidences of nonneoplastic lesions of the kidney, spleen, bone marrow, lung, glandular stomach, and mediastinal and mesenteric lymph nodes were increased in 1 mg/kg males and/or females (Tables 7, A5, and B5).

Minimal to moderate renal tubule necrosis of the kidney consisted of multiple scattered renal tubules lined by epithelial cells that had undergone coagulative necrosis and were sometimes filled with protein and cell debris. Hyperplasia of the transitional epithelium occurred in the kidney of 1 mg/kg females.

Congestion of the spleen in the 1 mg/kg males and females was considered to be a terminal change related to early death. The incidences of hematopoietic cell proliferation of the spleen were increased in 1 mg/kg males and females. Bone marrow hyperplasia in 1 mg/kg males and females was characterized by an increase in the amount of hematopoietic tissue (both erythroid and myeloid). The changes in the bone marrow and spleen are suggestive of increased hematopoiesis secondary to chemical-related erythrocytic and platelet sequestration as was seen in the 13-week NTP (1993) study of riddelliine.

Table 7
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Male						
Kidney[a]	50					50
Renal Tubule, Necrosis	0[b]					6** (2.2)[c]
Spleen	50					49
Congestion	0					24** (2.7)
Hematopoietic Cell Proliferation	1 (1.0)					23** (2.8)
Bone Marrow	50					49
Hyperplasia	1 (3.0)					36** (3.0)
Lung	50					50
Hemorrhage	1 (1.0)					21** (3.1)
Edema	0					5* (3.2)
Stomach, Glandular	50					50
Erosion	0					10** (2.5)
Ulcer	0					6** (2.8)
Lymph Node, Mediastinal	50					50
Hemorrhage	3 (2.3)					20** (3.6)
Pigmentation	10 (1.4)					20** (2.8)
Lymph Node, Mesenteric	50					50
Hemorrhage	1 (1.0)					8** (2.3)

TABLE 7
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control		0.01 mg/kg		0.033 mg/kg		0.1 mg/kg		0.33 mg/kg		1 mg/kg	
Female												
Kidney	50		50		50		50		50		50	
Renal Tubule, Necrosis	0		0		0		1	(3.0)	1	(2.0)	6**	(2.3)
Transitional Epithelium, Hyperplasia	1	(1.0)	1	(3.0)	1	(2.0)	1	(1.0)	0		5*	(1.4)
Spleen	50		50		50		50		50		50	
Congestion	0		0		0		1	(2.0)	3	(2.3)	7**	(2.6)
Hematopoietic Cell Proliferation	24	(1.8)	33*	(1.5)	25	(2.0)	26	(2.0)	27	(1.9)	34**	(2.9)
Bone Marrow	50		50		50		50		50		50	
Hyperplasia	6	(2.5)	3	(2.7)	8	(2.9)	7	(2.9)	10	(2.2)	32**	(2.6)
Lung	50		50		50		50		50		50	
Hemorrhage	4	(3.3)	7	(2.6)	1	(2.0)	3	(3.0)	5	(3.4)	19**	(2.5)
Stomach, Glandular	50		50		50		49		49		50	
Erosion	0		0		0		2	(2.0)	1	(1.0)	9**	(1.9)
Ulcer	0		0		0		2	(2.5)	0		7**	(2.9)
Lymph Node, Mediastinal	50		50		50		50		50		50	
Hemorrhage	5	(2.2)	8	(2.1)	9	(2.4)	5	(2.0)	7	(2.1)	25**	(3.0)
Pigmentation	23	(1.9)	22	(2.2)	32*	(2.1)	15	(2.0)	16	(2.0)	31**	(2.1)
Lymph Node, Mesenteric	50		50		50		47		49		49	
Hemorrhage	1	(1.0)	2	(1.5)	3	(2.7)	4	(2.3)	0		6**	(1.8)

* 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 except mediastinal lymph node is number of animals necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Hemorrhage of the lung in 1 mg/kg males and females was characterized by alveolar spaces filled with free red blood cells, and edema in 1 mg/kg males was determined by the presence of alveolar spaces filled with homogenous, eosinophilic, proteinaceous fluid. This change was considered related to the presence of metastatic hemangiosarcomas in the lung. Males and females administered 1 mg/kg had increased incidences of focal or multifocal mucosal erosion and ulceration in the glandular stomach.

Hemorrhage in the mediastinal and mesenteric lymph nodes consisted of free red blood cells within the medullary and subcapsular sinuses and was considered to represent a terminal change in 1 mg/kg rats that died early; mediastinal lymph node hemorrhage was secondary to the metastatic hemangiosarcomas in the lung. Pigmentation in the mediastinal lymph node was identified by the presence of brown granular material, apparently hemosiderin, within the macrophages and was considered secondary to the increased incidence of hemorrhage in this organ. However, an association between hemorrhage and pigmentation was not seen in dosed females.

DNA Adducts

Analyses of liver tissue from female rats treated with riddelliine for 3 or 6 months yielded eight DNA adducts; these were the same as DNA adducts formed *in vitro* by the metabolism of riddelliine by human liver microsomes in the presence of calf thymus DNA (Appendix I).

MICE

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 8 and in the Kaplan-Meier survival curves (Figure 3). Survival of 3 mg/kg male and female mice was significantly decreased compared to their respective vehicle control groups.

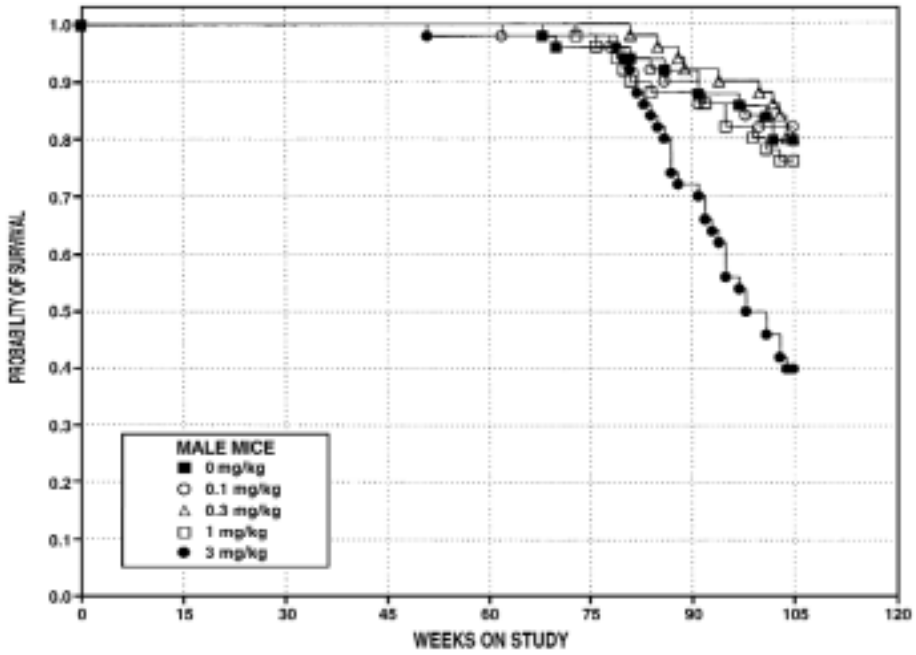
Body Weights and Clinical Findings

Mean body weights of 3 mg/kg males and females were less than those of the vehicle controls throughout most of the study and were 19% (males) and 33% (females) less by the end of the study (Tables 9 and 10; Figure 4). The mean body weights of 1 mg/kg males were less than those of the vehicle controls from week 93, and were 6% less at the end of the study. No clinical findings were attributed to riddelliine administration.

TABLE 8
Survival of Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Male					
Animals initially in study	50	50	50	50	50
Accidental death[a]	1	0	0	0	0
Moribund	4	6	3	6	13
Natural deaths	6	3	7	6	17
Animals surviving to study termination	39	41	40	38	20[e]
Percent probability of survival at end of study[b]	80	82	80	76	40
Mean survival (days)[c]	696	705	716	701	667
Survival analysis[d]	P<0.001	P=0.989N	P=1.000N	P=0.819	P<0.001
Female					
Animals initially in study	50				50
Accidental deaths[a]	4				0
Moribund	9				25
Natural deaths	3				8
Animals surviving to study termination	34				17[f]
Percent probability of survival at end of study	75				34
Mean survival (days)	670				678
Survival analysis					P<0.001

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



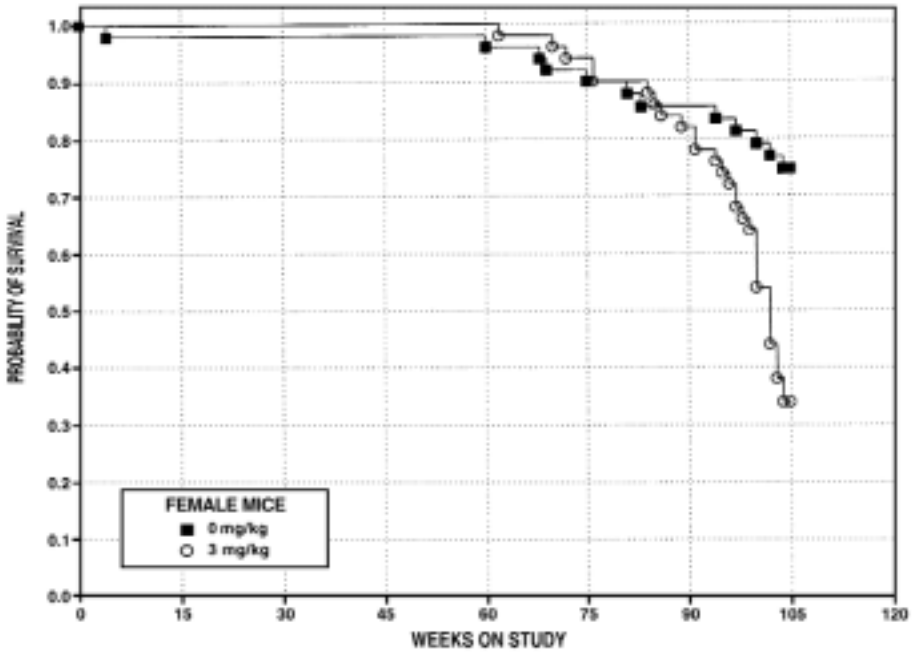


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Administered Riddelliine by Gavage for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Riddelliine

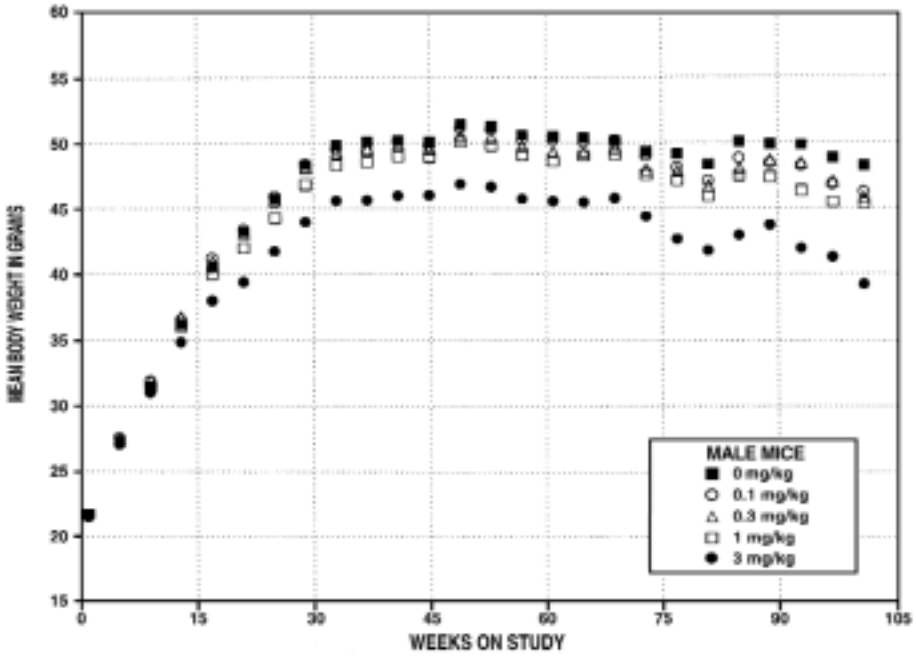
Weeks on Study	Vehicle Control		0.1 mg/kg			0.3 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	21.7	50	21.7	100	50	21.7	100	50
5	27.4	50	27.7	101	50	27.5	100	50
9	31.5	50	31.9	101	50	32.0	102	50
13	36.3	50	36.6	101	50	36.8	101	50
17	40.6	50	41.2	102	50	40.9	101	50
21	43.3	50	43.4	100	50	43.1	100	50
25	45.8	50	46.0	100	50	45.6	100	50
29	48.4	49	48.4	100	50	48.2	100	50
33	49.9	49	49.6	99	50	49.3	99	50
37	50.1	49	49.4	99	50	49.6	99	50
41	50.2	49	50.2	100	50	49.9	99	50
45	50.1	49	49.9	100	50	49.6	99	50
49	51.4	49	51.2	100	50	50.5	98	50
53	51.3	49	51.1	100	50	50.5	98	50
57	50.6	49	50.2	99	50	49.8	98	50
61	50.5	49	50.3	100	50	49.3	98	50
65	50.4	49	50.1	99	49	49.3	98	50
69	50.2	48	50.2	100	49	49.6	99	50
73	49.3	47	49.0	99	49	47.9	97	50
77	49.2	47	48.1	98	49	47.9	97	50
81	48.4	46	47.1	97	48	46.7	97	50
85	50.1	46	48.9	98	46	48.0	96	49
89	49.9	45	48.5	97	45	48.7	98	47
93	49.8	43	48.3	97	43	48.5	97	46
97	48.8	43	46.8	96	43	47.0	96	45
101	48.3	42	46.3	96	41	45.8	95	44
Mean for weeks								
1-13	29.2		29.5	101		29.5	101	
14-53	48.1		48.0	100		47.7	99	
54-101	49.6		48.7	98		48.2	97	

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Riddelliine

Weeks on Study	1 mg/kg			3 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	21.7	100	50	21.5	99	50
5	27.4	100	50	27.1	99	50
9	31.5	100	50	31.0	98	50
13	36.1	99	50	34.9	96	50
17	40.0	99	50	38.0	94	50
21	42.0	97	50	39.4	91	50
25	44.3	97	50	41.8	91	50
26	46.9	97	50	44.0	91	50
33	48.3	97	50	45.6	91	50
37	48.6	97	50	45.7	91	50
41	49.0	98	50	46.0	92	50
45	49.0	98	50	46.1	92	50
49	50.1	98	50	46.9	91	50
53	49.8	97	50	46.7	91	49
57	49.1	97	50	45.8	91	49
61	48.6	96	50	45.6	90	49
65	49.0	97	50	45.5	90	49
69	49.1	98	50	45.8	91	49
73	47.6	97	50	44.4	90	49
77	47.1	96	48	42.7	87	49
81	45.9	95	46	41.8	86	47
85	47.5	95	44	43.0	86	42
89	47.4	95	44	43.7	88	36
93	46.3	93	43	41.9	84	33
97	45.5	93	41	41.3	85	28
101	45.4	94	40	39.2	81	25
Mean for weeks						
1-13	29.2	100		28.6	98	
14-53	46.8	97		44.0	91	
54-101	47.4	96		43.4	88	

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Riddelliine

Weeks on Study	Vehicle Control		3 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	50	18.9	101	50
5	22.5	49	22.2	99	50
9	26.7	49	26.1	98	50
13	29.7	49	28.6	96	50
17	34.6	49	31.5	91	50
21	37.6	49	34.1	91	50
25	41.6	49	36.7	88	50
29	45.8	49	39.3	86	50
33	49.3	49	40.7	83	50
37	52.1	49	40.5	78	50
41	53.9	49	42.2	78	50
45	55.2	49	42.9	78	50
49	56.4	49	44.5	79	50
53	57.3	49	45.0	79	50
57	57.5	49	44.8	78	50
61	58.4	48	44.6	76	50
65	59.1	48	45.8	78	49
69	58.7	47	45.2	77	49
73	59.5	46	45.2	76	47
77	58.3	45	43.4	74	45
81	57.9	41	42.6	74	45
85	58.2	39	42.2	73	44
89	58.1	39	42.4	73	42
93	58.0	39	42.4	73	39
97	56.9	38	40.1	71	36
101	56.3	36	37.7	67	27
Mean for weeks					
1-13	24.4		24.0	98	
14-53	48.4		39.7	82	
54-101	58.1		43.0	74	



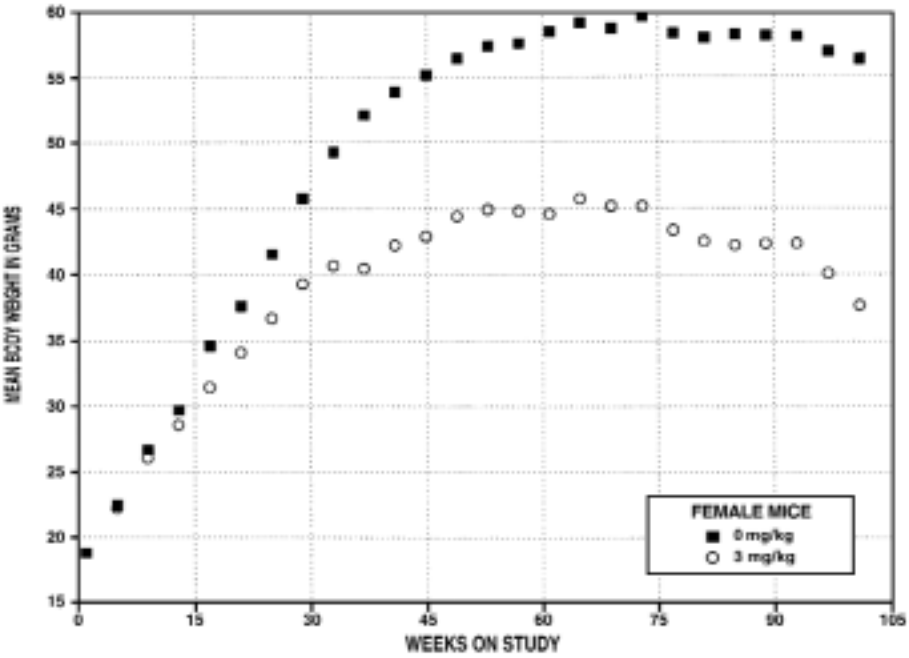


FIGURE 4
Growth Curves for Male and Female Mice Administered Riddelliine by Gavage for 2 Years

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 liver, lung, kidney, artery, adrenal gland, small (duodenum) and large (cecum) intestines, spleen, and skin. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical 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 hemangiosarcoma and of multiple hemangiosarcoma of the liver in 3 mg/kg male mice were significantly greater than those in the vehicle controls and exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 11, C3, and C4). Liver hemangiosarcoma was the primary cause of death in 22 of the 3 mg/kg male mice. Histologically, hemangiosarcoma was characterized by elongated, flattened, spindle-shaped, or pleomorphic cells that formed irregular vascular spaces or solid masses. Large areas of hemorrhage, thrombosis, and necrosis often occurred within the lesions (Plates 5 and 6). One male each in the 1 and 3 mg/kg groups had endothelial cell hyperplasia (Tables 11 and C5), which is considered to be a preneoplastic change. This lesion consisted of increased numbers of flattened to plump endothelial cells forming irregular trabeculae between hepatocytes (Plate 7). Although most hemangiosarcomas occurred in the liver of male mice, hemangiosarcomas also occurred in the bone marrow, epididymis, kidney, mesentery, mesenteric lymph node, and spleen of males (Table C1). These sporadic incidences of hemangiosarcoma were similar to those in the vehicle controls, were within the historical range in controls given NTP-2000 diet (NTP, 2001), or occurred in only one animal in a dosed group; therefore, hemangiosarcomas in tissues other than the liver were not considered to be chemical related. Metastases of the liver hemangiosarcomas were seen in the lung of five 3 mg/kg males. Hemangiosarcoma was also noted in one 3 mg/kg female (Tables 11 and D1). Sporadic incidences of this lesion appear in historical data for controls (all routes) given NTP-2000 diet [3/655 (0.5% ± 1.2%), range 0%-4%], and therefore the single case in females was not considered treatment related. One 3 mg/kg female had a cholangioma.

The incidences of hepatocellular neoplasms occurred with a negative trend in males, and the incidence was significantly decreased in 3 mg/kg females; the incidences in the 3 mg/kg male and female groups were less than the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 11, C3, C4, D3, and D4a).

Incidences of hepatocyte cytomegaly and karyomegaly in males administered 0.3 mg/kg or greater and females given 3 mg/kg were significantly greater than those in the vehicle controls; the severities of these lesions were also increased in the 3 mg/kg groups (Tables 11, C5, and D5). Cytomegaly was characterized by hepatocytes with increased cytoplasmic volume, and karyomegaly was characterized by enlarged nuclei with marginated chromatin. Incidences of minimal to mild bile duct hyperplasia were slightly increased in 3 mg/kg males and significantly increased in 3 mg/kg females (Plate 8). In 3 mg/kg males, the incidence of centrilobular necrosis of hepatocytes was significantly increased, and the incidence of focal hepatocyte coagulative necrosis was slightly increased; the severity of the focal necrosis increased with increasing dose. The necrosis was mostly present in livers that exhibited other lesions, especially hemangiosarcomas. In the 13-week study, administration of doses of up to 25 mg/kg per day was not associated with hepatocellular necrosis (NTP, 1993). Incidences of focal hemorrhage, which was usually concomitant with hemangiosarcoma, were significantly increased in males administered 1 or 3 mg/kg. The incidence of mixed cell cellular infiltration was significantly increased in 3 mg/kg females. A dose-related decrease in the incidence of syncytial (multinucleated) cell alteration was noted in the 3 mg/kg males.

Table 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Male					
Number Examined Microscopically	50	50	50	50	50
Endothelial Cell, Hyperplasia[a]	0	0	0	1 (2.0)[b]	1 (2.0)
Hepatocyte, Cytomegaly	4 (1.5)	4 (1.8)	16** (1.7)	33** (1.7)	43** (2.5)
Hepatocyte, Karyomegaly	4 (1.5)	4 (1.8)	15** (1.7)	33** (1.8)	43** (2.5)
Bile Duct, Hyperplasia	2 (2.0)	0	1 (1.0)	3 (1.7)	6 (2.3)
Hepatocyte, Centrilobular, Necrosis	0	1 (3.0)	3 (3.3)	4 (3.3)	10** (3.0)
Hepatocyte, Necrosis, Focal	18 (1.3)	9* (1.3)	5** (1.8)	6** (2.2)	21 (2.6)
Hemorrhage, Focal	0	2 (2.5)	1 (3.0)	6* (2.3)	21** (2.8)
Syncytial Alteration, Focal	38 (1.2)	30 (1.2)	31 (1.2)	27* (1.0)	0**
Hemangiosarcoma, Multiple	0	0	0	0	17**
Hemangiosarcoma (includes multiple)[c]					
Overall rate[d]	2/50 (4%)	1/50 (2%)	0/50 (0%)	2/50 (4%)	31/50 (62%)
Adjusted rate[e]	4.4%	2.2%	0.0%	4.4%	66.7%
Terminal rate[f]	2/39 (5%)	1/41 (2%)	0/40 (0%)	2/38 (5%)	8/20 (40%)
First incidence (days)	729 (T)	729 (T)	—[h]	729 (T)	550
Poly-3 test[g]	P<0.001	P=0.495	P=0.227N	P=0.694	P<0.001
Hepatocellular Adenoma, Multiple	3	8	2	0	0
Hepatocellular Adenoma (includes multiple)	16	18	14	5**	0**
Hepatocellular Carcinoma, Multiple	16	10	9	5**	0**
Hepatocellular Carcinoma (includes multiple)	23	21	19	20	3**
Hepatocellular Adenoma or Carcinoma[i]					
Overall rate	36/50 (72%)	39/50 (78%)	33/50 (66%)	23/50 (46%)	3/50 (6%)
Adjusted rate	73.4%	80.0%	66.0%	49.2%	7.5%
Terminal rate	26/39 (67%)	32/41 (78%)	24/40 (60%)	19/38 (50%)	2/20 (10%)
First incidence (days)	475	542	567	566	590
Poly-3 test	P<0.001N	P=0.299	P=0.281N	P=0.011N	P<0.001N

Table 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Female					
Number Examined Microscopically	49				50
Hepatocyte, Cytomegaly	0				49** (2.3)
Hepatocyte, Karyomegaly	0				49** (2.3)
Bile Duct, Hyperplasia	0				28** (1.9)
Infiltration Cellular, Mixed Cell	29 (1.4)				41** (1.8)
Cholangioma[j]	0				1
Hemangiosarcoma[k]	0				1
Hepatocellular Adenoma, Multiple	2				0
Hepatocellular Adenoma (includes multiple)	9				0**
Hepatocellular Carcinoma, Multiple	1				0
Hepatocellular Carcinoma (includes multiple)	8				0**
Hepatocellular Adenoma or Carcinoma[l]					
Overall rate	16/49 (33%)				0/50 (0%)
Adjusted rate	36.9%				0.0%
Terminal rate	11/34 (32%)				0/17 (0%)
First incidence (days)	419				—
Poly-3 test					P<0.001N

(T) Terminal sacrifice
* 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 studies with controls given NTP-2000 diet (mean ± standard deviation): 20/659 (3.1% ± 1.1%), range 2%-4%
[d] Number of animals with neoplasm per number of animals with liver 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 (males only) 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 dosed group is indicated by **N**.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for NTP-2000 diet: 304/659 (47.8% ± 12.9%), range 28%-72%
[j] Historical incidence for NTP-2000 diet: 0/655
[k] Historical incidence for NTP-2000 diet: 3/655 (0.5% ± 1.2%), range 0%-4%
[l] Historical incidence for NTP-2000 diet: 143/655 (22.8% ± 9.6%), range 12%-40%

Lung: Compared to those in the vehicle controls, the incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) were significantly increased in 3 mg/kg females, and the incidence of alveolar/bronchiolar carcinoma in this group was increased (Tables 12 and D3). The incidences of these neoplasms in this group exceeded the historical ranges in controls (all routes) given NTP-2000 diet and in water gavage controls given NIH-07 diet (Tables 12 and D4b). Alveolar/bronchiolar adenomas were well demarcated and, histologically, had solid, papillary, or mixed growth patterns. The tumor cells were cuboidal, round, or polygonal with scant intervening fibrous stroma. Alveolar/bronchiolar carcinomas were larger than adenomas and had irregular growth, foci of necrosis, and extensive invasion of the surrounding parenchyma. Neoplastic cells exhibited pleomorphism and nuclear atypia and formed papillary or tubular structures, solid

TABLE 12
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Male					
Number Examined Microscopically	50	50	50	50	50
Alveolar/bronchiolar Adenoma, Multiple[a]	4	1	1	3	0
Alveolar/bronchiolar Adenoma (includes multiple)	12	10	11	8	12
Alveolar/bronchiolar Carcinoma, Multiple	4	2	4	1	4
Alveolar/bronchiolar Carcinoma (includes multiple)	7	8	6	1*	5
Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate[b]	18/50 (36%)	16/50 (32%)	15/50 (30%)	9/50 (18%)	17/50 (34%)
Adjusted rate[c]	39.1%	34.7%	31.1%	19.7%	39.7%
Terminal rate[d]	15/39 (39%)	16/41 (39%)	13/40 (33%)	7/38 (18%)	8/20 (40%)
First incidence (days)	599	729 (T)	567	689	559
Poly-3 test[e]	P=0.424	P=0.413N	P=0.276N	P=0.033N	P=0.564
Female					
Number Examined Microscopically	50				50
Alveolar Epithelium, Hyperplasia	1 (1.0)[f]				6 (1.5)
Alveolar/bronchiolar Adenoma[g]	1				9**
Alveolar/bronchiolar Carcinoma, Multiple	1				1
Alveolar/bronchiolar Carcinoma (includes multiple)	1				4
Alveolar/bronchiolar Adenoma or Carcinoma[h]					
Overall rate	2/50 (4%)				13/50 (26%)
Adjusted rate	4.7%				30.5%
Terminal rate	1/34 (3%)				6/17 (35%)
First incidence (days)	419				587
Poly-3 test					P<0.001

(T) Terminal sacrifice
* 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] Number of animals with neoplasm per number of animals with lung 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 (males only) 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 lower incidence in a dosed group is indicated by **N**.
[f] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[g] Historical incidence for 2-year studies with controls given NTP-2000 diet (mean ± standard deviation): 37/654 (5.4% ± 4.0%), range 0%-12%
[h] Historical incidence for NTP-2000 diet: 53/654 (7.6% ± 4.7%), range 0%-12%

sheets of cells, or a combination of the patterns (Plate 9). Except in the 1 mg/kg group, which had significantly decreased incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined), the incidences of alveolar/bronchiolar neoplasms in dosed males were similar to those in the vehicle controls. In 3 mg/kg females, the incidence of alveolar epithelial hyperplasia was slightly increased.

Kidney: Increased severity of common, age-related nephropathy was observed in 3 mg/kg males and females, and the incidence of this lesion in 3 mg/kg females was significantly greater than that in the vehicle controls (Tables 13, C5, and D5). Nephropathy was considered the primary cause of death in one male and 19 females in the 3 mg/kg groups. The lesions were similar to background nephropathy and included thickening of the tubular basement membranes, glomerular hypercellularity and basement membrane thickening, protein tubular casts, tubular dilation and regeneration, and interstitial inflammation. The incidences of glomerulosclerosis in 1 mg/kg males and 3 mg/kg males and females were significantly increased; the severities of this lesion were also increased. Glomerulosclerosis was characterized by thickening of the glomeruli due to mild to marked deposition of pale, eosinophilic, and somewhat hyaline but faintly fibrillar intercellular material in the tufts, often with complete sclerosis. Masson's trichrome (a connective tissue stain) was used to assess the amount of collagen in the glomeruli. Compared to those of the vehicle controls, the glomeruli from dosed mice had increased amounts of collagen, which was consistent with fibrosis or sclerosis of the glomeruli. Periodic acid-Schiff staining demonstrated increased positivity in the dosed mice, which indicated the presence of carbohydrate-containing substances (plasma glycoproteins or basement membranes). These findings are expected in sclerotic glomeruli. The material did not stain consistently for amyloid (Congo red stain). Immunohistochemical staining with antibodies for albumin, mouse IgG, and C3 suggested that immune-complex-mediated glomerulosclerosis was not the primary mechanism for the accumulation of hyaline material within the glomeruli of the riddelliine-treated mice.

TABLE 13
Incidences of Nonneoplastic Lesions of the Kidney in Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control		0.1 mg/kg		0.3 mg/kg		1 mg/kg		3 mg/kg	
Male										
Number Examined Microscopically	49		49		50		50		50	
Nephropathy[a]	46	(1.3)[b]	48	(1.5)	48	(1.8)	50	(2.1)	50	(2.8)
Glomerulus, Glomerulosclerosis	0		1	(1.0)	0		42**	(1.8)	41**	(2.5)
Renal Tubule, Accumulation, Hyaline Droplet	0		2	(3.0)	1	(3.0)	1	(3.0)	3	(3.0)
Renal Tubule, Karyomegaly	0		0		0		0		12**	(1.4)
Renal Tubule, Dilatation	16	(1.2)	17	(1.1)	24	(1.3)	29**	(1.5)	22	(1.9)
Female										
Number Examined Microscopically	49								50	
Nephropathy	18	(1.3)							47**	(3.4)
Glomerulus, Glomerulosclerosis	0								40**	(2.7)
Renal Tubule, Accumulation, Hyaline Droplet	2	(2.5)							14**	(2.6)
Renal Tubule, Pigmentation	2	(2.0)							27**	(2.8)
Renal Tubule, Karyomegaly	0								1	(2.0)

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

The incidences of renal tubule hyaline droplet accumulation and granular, brown pigment were significantly increased in 3 mg/kg females; the incidence of hyaline droplet accumulation was slightly increased in 3 mg/kg males (Tables 13, C5, and D5). These increased incidences appeared to parallel the increased incidences and/or severity of nephropathy. The hyaline droplets ranged from punctate granules to large droplets located in the cortex and corticomedullary tubules and were bright red with Mallory's Heidenhain stain. The pigment was Prussian blue positive, indicating its hemosiderin nature (Plate 10).

The incidence of renal tubule karyomegaly was significantly increased in 3 mg/kg males, and the incidence of renal tubule dilatation was significantly increased in 1 mg/kg males; mild karyomegaly was observed in one 3 mg/kg female. Karyomegaly was characterized by enlarged hyperchromatic nuclei with marginated or stippled chromatin.

Artery: The incidence of chronic inflammation, confined to the arteries (arteritis), was significantly increased in 3 mg/kg females. Organs most frequently affected were the small (duodenum) and large (cecum) intestines, kidney, mesentery, ovary, spleen, and uterus (Tables 14 and D5). In dosed females, this lesion was also observed sporadically in the meninges of the brain, liver, lung (mediastinum), mesenteric lymph node, pancreas, salivary gland, thymus, urinary bladder, and the subcutis and blood vessels near the spinal ganglion (Table D5). Sporadic cases of chronic arterial inflammation were also observed in the duodenum, heart, kidney, and spleen of dosed males (Table C5). The vascular changes were characterized by all or some of the following features: partial to complete obliteration of the vessel lumina; thickening (hyalinization) or fibrinoid necrosis of the vessel wall; thrombosis; subintimal, transmural, or adventitial infiltrates of mixed inflammatory cells; perivascular fibrosis; and, occasionally, hemorrhage (Plates 11 through 15).

TABLE 14
Selected Incidences of Chronic Arterial Inflammation in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control		3 mg/kg
Small Intestine (Duodenum)[a]	47		46
Artery, Inflammation, Chronic[b]	0		13** (1.9)[c]
Large Intestine (Cecum)	48		47
Artery, Inflammation, Chronic, Focal	0		18** (1.7)
Kidney	49		50
Artery, Inflammation, Chronic	1	(4.0)	16** (2.1)
Mesentery	23		29
Artery, Inflammation, Chronic, Focal	1	(3.0)	19** (2.5)
Ovary	49		48
Artery, Inflammation, Chronic	0		26** (2.6)
Spleen	49		50
Artery, Inflammation, Chronic, Focal	0		6* (2.0)
Uterus	49		50
Artery, Inflammation, Chronic, Focal	0		21** (2.4)

* 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

Other Organs: The incidence and severity of focal cytoplasmic alteration in the adrenal cortex were increased in 3 mg/kg females (Tables 15 and D5). This lesion was characterized by well-demarcated cortical zona fasciculata foci composed of hypertrophied cells with pale or vacuolated cytoplasm.

Edema, hemorrhage, mucosal erosion of the epithelium, and/or necrosis in the cecum and duodenum were observed in 3 mg/kg females (Tables 15 and D5). These changes were thought to be secondary to the primary submucosal vascular changes. The incidences and severities of hematopoietic cell proliferation of the spleen in males increased with increasing dose, and the incidences in 3 mg/kg males and females were significantly increased (Tables 15, C5, and D5). Hematopoietic cell proliferation was characterized by accumulation of myeloid and erythroid precursors in varying proportions admixed with megakaryocytes within the red pulp. This change was considered to be secondary to the dose-related hemorrhage observed in the liver, cecum, and duodenum.

Chemical-related, generalized, subcutaneous edema of the skin was noted in 0.3 mg/kg or greater males, and the incidences in the 1 and 3 mg/kg groups were significantly greater than that in the vehicle controls (Tables 15 and C5). Most mice that exhibited subcutaneous edema also had malignant neoplasms, usually hemangiosarcoma. A single 3 mg/kg female also had subcutaneous edema. Various mechanisms are known to result in edema, including decreased plasma osmotic pressure

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control		0.1 mg/kg		0.3 mg/kg		1 mg/kg		3 mg/kg	
Male										
Spleen[a]	49		49		50		50		49	
Hematopoietic Cell Proliferation[b]	18	(2.3)[c]	16	(2.6)	19	(2.6)	20	(2.7)	33**	(2.8)
Skin (Subcutaneous Tissue)	50		50		50		50		49	
Edema	0		0		2	(2.0)	5*	(2.0)	25**	(2.0)
Female										
Adrenal Cortex	50								50	
Cytoplasmic Alteration, Focal	1	(1.0)							25**	(2.0)
Large Intestine (Cecum)	48								47	
Edema	0								5*	(3.0)
Hemorrhage, Focal	0								6*	(2.3)
Epithelium, Erosion, Focal	0								6*	(1.8)
Small Intestine (Duodenum)	47								46	
Hemorrhage, Focal	0								2	(2.5)
Necrosis, Focal	0								2	(3.0)
Spleen	49								50	
Hematopoietic Cell Proliferation	32	(2.6)							43*	(3.1)
Skin (Subcutaneous Tissue)	50								50	
Edema	0								1	(3.0)

* 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

resulting from hypoproteinemia. This state may be due to renal disease or to decreased synthesis of plasma proteins caused by liver disease, both of which were seen in mice given riddelliine. In addition, hemangiosarcomas may have caused a loss of blood volume, eventually contributing to the development of congestive heart failure with further development of edema (Jones *et al.*, 1996).

Genetic Toxicology

Riddelliine (100 to 5,000 µg/plate) was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of 30% induced rat or hamster liver S9 (Table E1; Zeiger *et al.*, 1988); it was not mutagenic with 10% S9, and results of mutagenicity testing were negative in strains TA98 and TA1535 with and without S9 activation. The small, dose-related increase in mutant colonies observed with strain TA97 in the presence of 30% rat S9 was judged to be equivocal. Riddelliine induced sister chromatid exchanges (SCEs) in cultured Chinese hamster ovary (CHO) cells with and without S9; the response observed in the presence of S9 was very strong (Table E2; Galloway *et al.*, 1987). In the test conducted with S9, only three cells were counted at the highest scorable dose (30 µg/mL) because of the extremely high number of induced SCEs. Riddelliine also induced highly significant increases in aberrations in cultured CHO cells in the presence of S9 at all three doses scored (Table E3; Galloway *et al.*, 1987). No increase in aberrations was observed in the absence of S9.

In single-dose studies in which riddelliine, administered via intraperitoneal injection, was tested for induction of micronucleated erythrocytes in Swiss mice, the increase seen in bone marrow erythrocytes was not dose related and was of insufficient magnitude to be considered positive (Table E4). However, a small but significant dose-related increase in the frequency of micronucleated polychromatic erythrocytes was seen in peripheral blood (Table E5). These single-dose studies were not replicated to confirm the positive response; therefore, these results cannot be considered conclusive. In 4- and 13-week gavage studies, riddelliine did not induce micronucleated erythrocytes in peripheral blood of $B6C3F_1$ mice (Tables E6 and E7; Witt *et al.*, 2000). Several protocol differences between the 4- and 13-week studies and the single-dose study may be implicated in the contrasting test results, including the higher doses used in the single-dose study, differences in riddelliine metabolism arising from the different routes of administration, and the different strains of mice that were used.

In tests to measure unscheduled DNA synthesis (UDS), a measure of DNA damage and repair, and S-phase DNA synthesis in cultured hepatocytes from F344/N rats and $B6C3F_1$ mice following 5 or 30 days of treatment by gavage with riddelliine, an increase in UDS was observed at both time points in at least one dose group each in male rats and in male and female mice (Tables E8 and E9). UDS was only observed in female rats following 5 days of treatment. An increase in S-phase DNA synthesis, an indication of DNA replication, was observed at both time points in at least one group in male and female rats. There was no increase in S-phase synthesis in male or female mice following 5 days of treatment; an increase occurred in only a single dose group (3.3 mg/kg) in male mice following 30 days of treatment. In female mice treated for 5 or 30 days, S-phase synthesis appeared inhibited (i.e., less than vehicle control values) at the highest doses (day 5: 25 mg/kg; day 30: 10 and 25 mg/kg). These data suggest that the hepatotoxicity of riddelliine may be due, in part, to an antimitogenic effect at higher doses, which inhibits compensatory cell proliferation that occurs in response to toxicity. However, the high variability in S-phase DNA synthesis in control female mice confounded interpretation of the results.

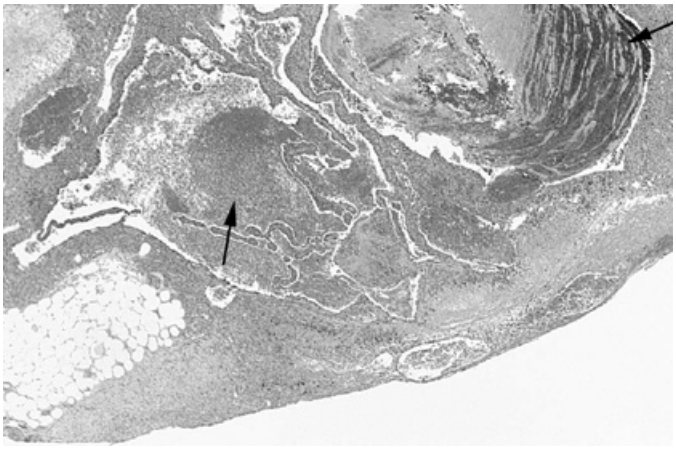


PLATE 1
Liver hemangiosarcoma in a female rat administered 1 mg/kg riddelliine by gavage for 2 years. Multiple hemorrhagic cavities are surrounded by a variably sized rim of neoplastic tissue (arrows). H&E; 6.6x

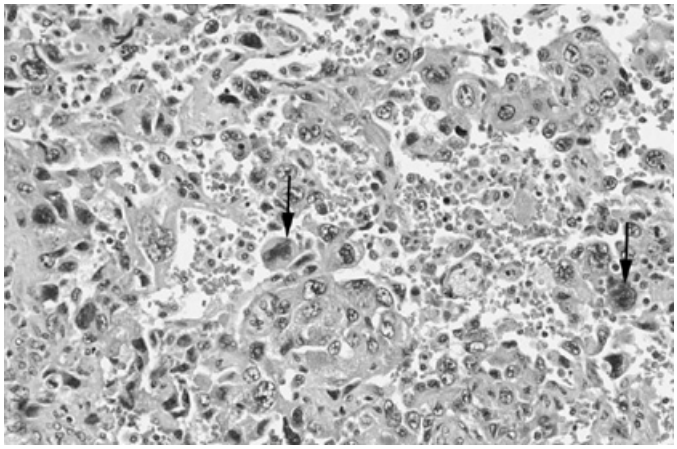


PLATE 2
Liver hemangiosarcoma in a male rat administered 1 mg/kg riddelliine in the 2-year gavage study. Irregular vascular spaces are lined by pleomorphic endothelial cells. H&E; 66x

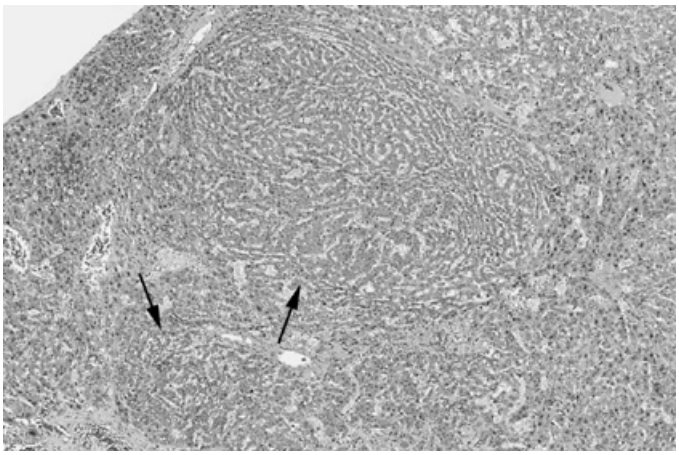


PLATE 3
Hepatocellular regenerative hyperplasia in a male rat administered 1 mg/kg riddelliine in the 2-year gavage study. Note the presence of multiple discrete nodules of hepatocytes (arrows). H&E; 13.2x

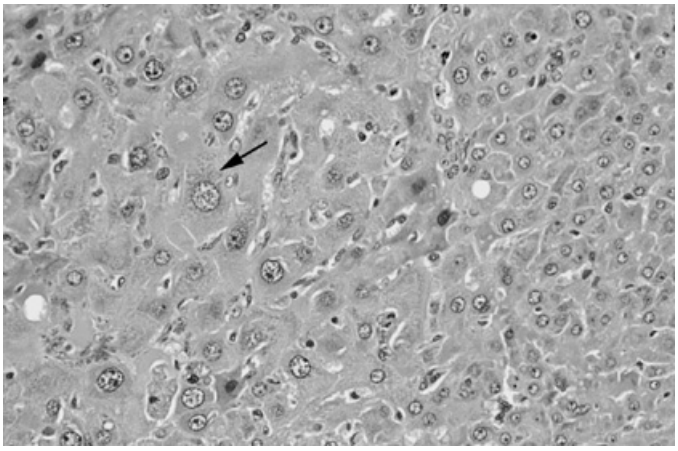


PLATE 4
Hepatocytic enlargement (cytomegaly) associated with enlarged nuclei (karyomegaly) (arrows) in a male rat administered 1 mg/kg riddelliine in the 2-year gavage study. H&E; 66x

PLATE 5
Liver hemangiosarcoma in a male mouse administered 3 mg/kg riddelliine by gavage for 2 years. There are scattered hemorrhagic-neoplastic areas. H&E; 5x

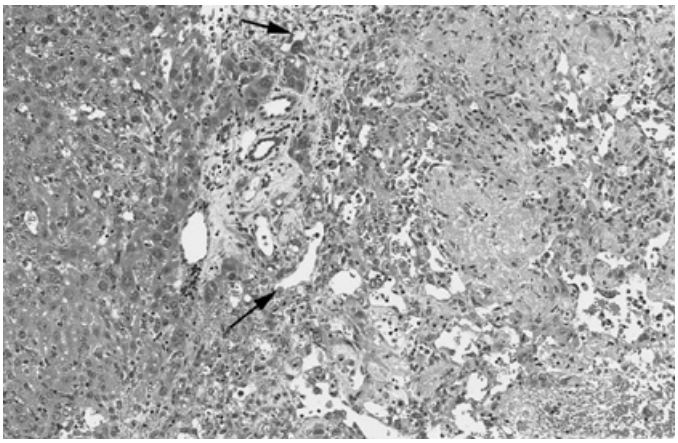


PLATE 6
Liver hemangiosarcoma in a male mouse administered 3 mg/kg riddelliine in the 2-year gavage study. There are irregular vascular spaces (arrows) lined by pleomorphic endothelial cells. H&E; 40x

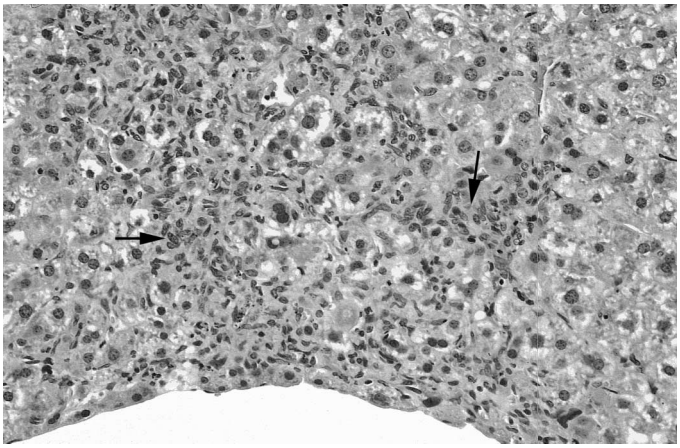


PLATE 7
Liver endothelial hyperplasia in a male mouse administered 3 mg/kg riddelliine in the 2-year gavage study. There is an increased number of flattened to plump endothelial cells, forming irregular trabeculae (arrows) between hepatocytes. H&E; 66x

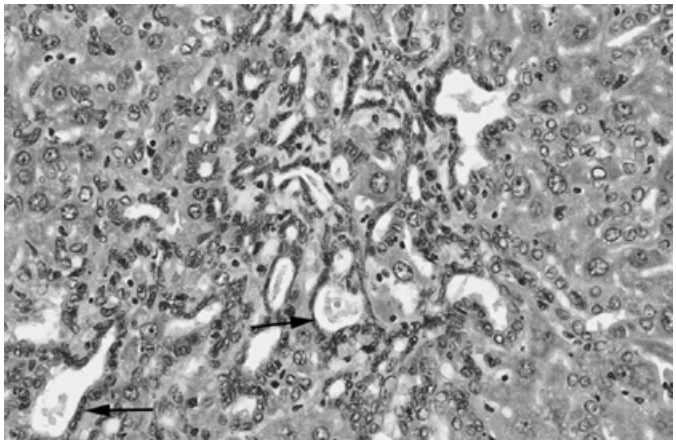


PLATE 8
Liver bile duct hyperplasia in a female mouse administered 3 mg/kg riddelliine in the 2-year gavage study. Some of the ducts are dilated and contain mucoid material (arrows). H&E; 80x

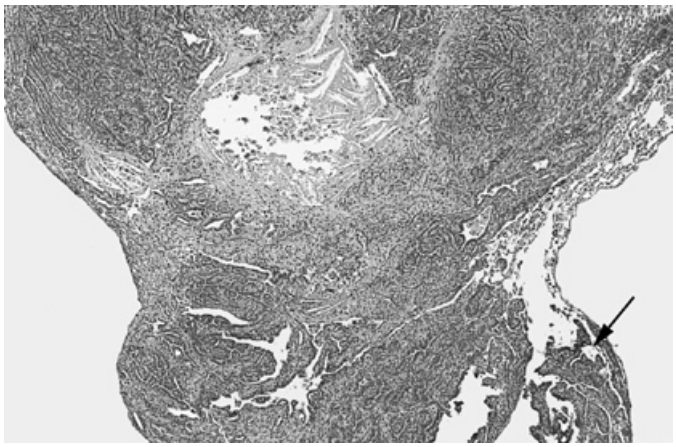


PLATE 9
Pulmonary alveolar/bronchiolar carcinoma in a female mouse administered 3 mg/kg riddelliine in the 2-year gavage study. Note the invasion into the surrounding parenchyma (arrow). H&E; 16x

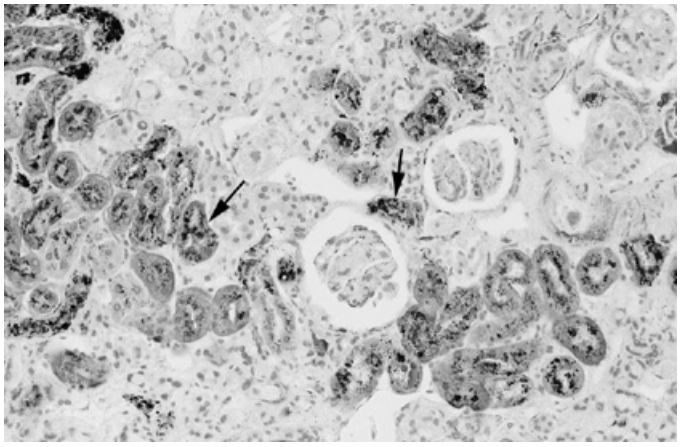


PLATE 10
Renal cortical tubular pigmentation in a female mouse administered 3 mg/kg riddelliine by gavage for 2 years. The granular brown pigment is Prussian blue positive (arrows), indicating its hemosiderin nature. H&E; 66x

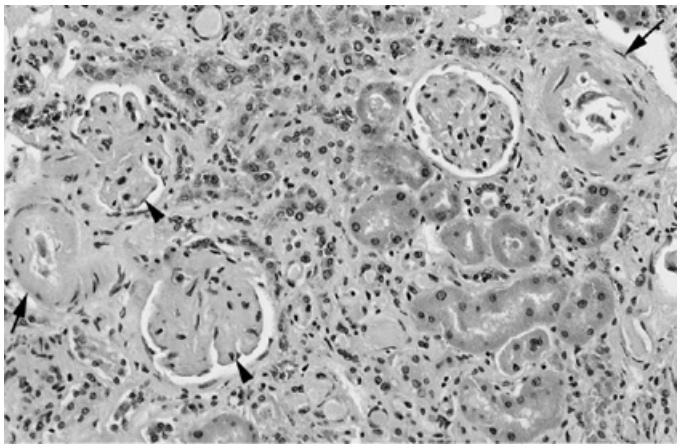


PLATE 11
Chronic arteritis in the kidney of a female mouse administered 3 mg/kg riddelliine in the 2-year gavage study. The glomerular afferent artery lesion is characterized by obliteration of the vessel lumina, thickening of the vessel wall, and adventitial and perivascular fibrosis (arrows). The glomerulus is sclerotic (arrow head). H&E; 66x

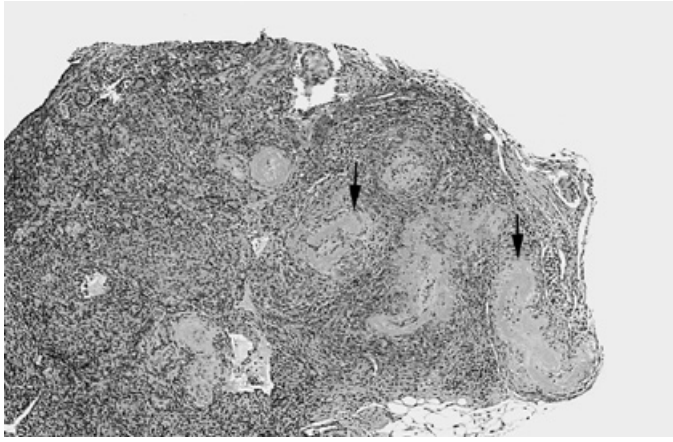


PLATE 12
Chronic arteritis in the ovary of a female mouse administered 3 mg/kg riddelliine in the 2-year gavage study. The vascular region is characterized by obliteration of the vessel lumina and thickening of the vessel wall (arrows). H&E; 20x

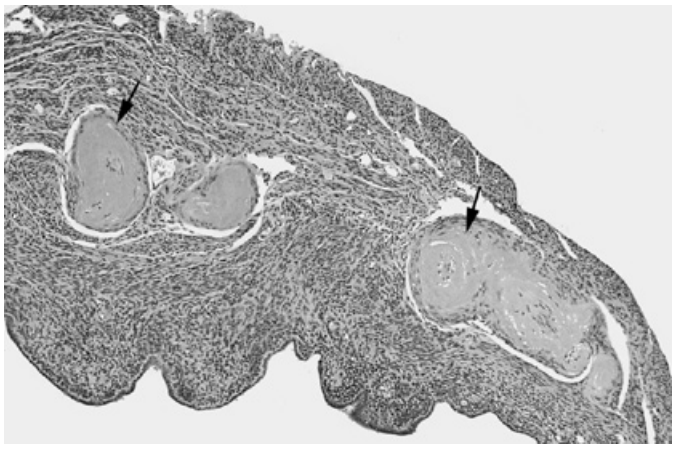


Plate 13
Chronic arteritis in the uterus of a mouse administered 3 mg/kg riddelliine by gavage for 2 years. The vascular lesion is characterized by obliteration of the vessel lumina, thickening of the vessel wall, and adventitial and perivascular fibrosis (arrows). H&E; 25x

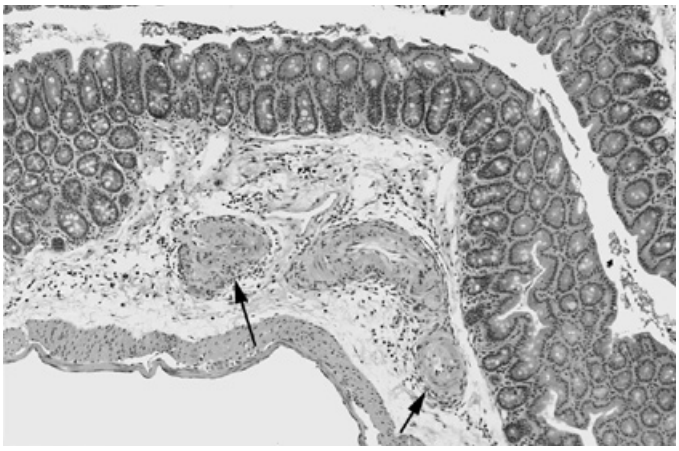


Plate 14
Chronic arteritis in the submucosa of the cecum of a female mouse administered 3 mg/kg riddelliine by gavage for 2 years. The vascular lesion is characterized by obliteration of the vessel lumina and perivascular inflammation fibrosis (arrows). H&E; 25x

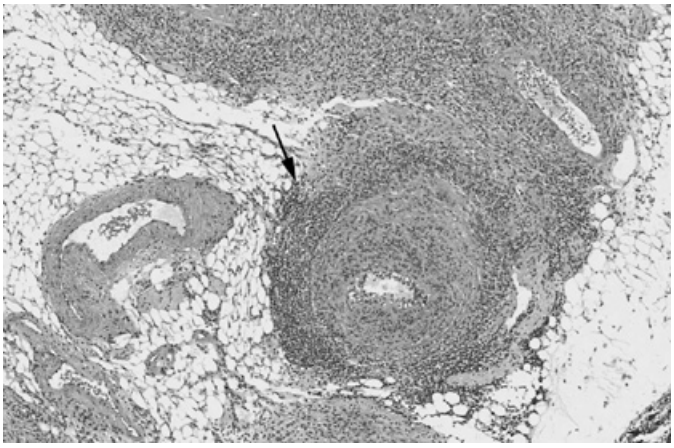


Plate 15
Chronic arteritis in the mesentery (arrow) of a female mouse administered 3 mg/kg riddelliine by gavage for 2 years. The vascular lesion is characterized by prominent transmural and perivascular mononuclear cell infiltration. H&E; 20x

DISCUSSION AND CONCLUSIONS

In the current studies, five groups of female rats were administered riddelliine by gavage at doses of 0.01, 0.033, 0.1, 0.33, or 1 mg riddelliine/kg body weight; four groups of male mice received gavage doses of 0.1, 0.3, 1, or 3 mg/kg. The dose ranges were selected based on the results of 13-week studies reported earlier (NTP, 1993). One dose concentration was selected for male rats and female mice to match the highest dose of the other sex in each species. This study design was chosen to permit a fuller examination of the dose-response relationship for chronic toxicity and the anticipated carcinogenicity in each species and the DNA adduct formation in rats. Judging from the nonneoplastic lesions induced, the lowest dose levels selected for female rats and male mice were very close to their respective no-observed-adverse-effect levels. Neoplasms and nonneoplastic lesions were seen in animals in the higher dose groups.

Administration of riddelliine for 2 years caused hemangiosarcomas (neoplasms of the endothelial cells) in the liver of 1 mg/kg male rats, 0.33 and 1 mg/kg female rats, and 3 mg/kg male mice. No hemangiosarcomas were observed in male or female vehicle control rats or female vehicle control mice. Metastases of the hemangiosarcomas were seen in the lung, mediastinal lymph node, and mesentery of male and female rats and in the pancreas and spleen of male rats. Multiple hemangiosarcomas were observed in the 3 mg/kg male mice; metastases of the liver hemangiosarcomas were seen in the lung of five 3 mg/kg male mice.

The hemangiosarcoma response is consistent with the toxicity of pyrrolizidine alkaloids that has been well described in the literature. The alkaloids are activated by the hepatic P450 enzymes (Mattocks, 1986; Williams *et al.*, 1989; Miranda *et al.*, 1991). The active metabolites cause damage to the endothelial cells. Monocrotaline induces cytotoxicity specifically to endothelial cells (Taylor *et al.*, 1997). Schraufnagel (1990) and Jones and Rabinovitch (1996) also showed that monocrotaline induced endothelial cell apoptosis preceding proliferation of smooth muscle cells in the blood vessels. The right ventricle increased in weight in rats fed crotalaria. In the large muscular pulmonary arteries, the medial and adventitial thickness was increased. The labeling indices of the endothelial cells and the smooth muscle cells in the hilar pulmonary artery were also increased. The data indicated that crotalaria induced endothelial cell injury followed by regeneration (Meyrick and Reid, 1982). Platelet plugs were formed following crotalaria administration, and thrombin activation of platelets caused the release of vascular endothelial growth factor and basic fibroblast growth factor (Verheul and Pinedo, 1998; Waltham *et al.*, 2000) leading to angiogenesis. Hemangiosarcomas have also been observed following the administration of pyrrolizidine alkaloids such as clivorine (Kuhara *et al.*, 1980), lasiocarpine (Svoboda and Reddy, 1972; Rao and Reddy, 1978), petasitenine (Hirono *et al.*, 1977), seneciphylline (Harris and Chen, 1970; Hirono *et al.*, 1983), and senkirkine (Hirono *et al.*, 1979).

The current 2-year studies demonstrated that, like other pyrrolizidine alkaloids, riddelliine was activated by the hepatic P450 enzymes. The active metabolites may cause damage to the endothelial cells, as accumulations of intravascular macrophages were observed in many organs in the 13-week NTP (1993) studies. The macrophages were probably attracted to the intravascular sites to remove damaged or dead endothelial cells in the vascular lesions.

Although the incidence of hemangiosarcoma in 3 mg/kg female mice was not significantly increased, the toxic effects of riddelliine were likely exerted on the arterial walls of female mice. These effects were evidenced by the arteritis seen in multiple organs; one hemangiosarcoma and one cholangioma were also observed in the liver of 3 mg/kg females. Whether the arteritis lesions are reactive or preneoplastic has been debated. According to Fetsch and Weiss (1991) these lesions are not preneoplastic, but arise on a reactive basis, probably secondarily to damage and repair of an artery or vein. The presence of one hemangiosarcoma and one cholangioma may suggest the carcinogenic potential of the toxic effects of riddelliine on the endothelial cells of female mice. The reason for the difference in sensitivity between 3 mg/kg male and female mice may be related

to a sex specificity of P450 isozymes as described by Williams *et al.* (1989) in the metabolism of senecionine by male and female Sprague-Dawley rats.

In the 2-year rat study, hepatocellular neoplasms were also induced in the liver of 1 mg/kg males and females. The incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) occurred with a positive trend in female rats. Dose-response relationships were also seen in the incidences of some nonneoplastic lesions in female rats, i.e. hepatocyte regenerative hyperplasia, hepatocyte cytomegaly, and eosinophilic foci. The hepatocellular regenerative hyperplasia consisted of varying degrees of diffuse to nodular hepatocellular proliferation along with hepatocellular cytomegaly and karyomegaly. There were variable numbers of small basophilic cells resembling oval cells, which are believed to represent stem cells capable of differentiation into hepatocytes or bile duct epithelium. The incidences and severities of hepatic focal necrosis were increased in 1 mg/kg male and female rats. It is unclear whether the necrosis is a primary riddelliine-induced effect or is secondary to the hyperplastic and neoplastic changes in the liver. In the 13-week studies of riddelliine (NTP, 1993), hepatocyte karyomegaly developed in males and females receiving 0.33 mg/kg or greater and cytomegaly developed in males administered 3.3 mg/kg or greater and in 10 mg/kg females. The lesions persisted during the 7- and 14-week recovery periods. The involvement of cytomegaly, karomegaly, focal necrosis, and atypical hyperplasia in hepatocellular neoplasm development is not clearly established (Boorman *et al.*, 1990).

In the 2-year mouse study, riddelliine administration induced dose-related increases in the incidences of hepatocyte cytomegaly, karyomegaly, and centrilobular necrosis in males. Riddelliine administered at 3 mg/kg also induced similar incidences of hepatocyte cytomegaly and karyomegaly in female mice, but a dose-response relationship could not be established in female mice because only one dose concentration was used in the study. In the 13-week studies, liver centrilobular cytomegaly was observed in male and female mice administered 25 mg/kg but not in those administered 3.3 or 10 mg/kg (NTP, 1993). The lesions persisted in the 7-week and 14-week recovery periods in female mice. Cytomegaly apparently developed early when riddelliine was administered at high dose levels; the lesion took longer to develop at low dose levels in male and female mice. The incidence of bile duct hyperplasia was significantly increased in 3 mg/kg female mice but not in 3 mg/kg male mice. Bile duct hyperplasia was also observed in the 7- and 14-week recovery periods in the 13-week studies in females but not in males. Female mice appeared to be more sensitive to the development of bile duct hyperplasia than male mice. In the current study, the incidences of hepatocellular adenoma and of hepatocellular carcinoma occurred with negative trends in male mice, and the incidences of these lesions were significantly decreased in 3 mg/kg female mice. Hepatocellular neoplasms are commonly found in $B6C3F_1$ mice, and the incidences increase with age. The reason for the negative trend in the incidences of hepatocellular neoplasms in the dosed mice is unclear. The known ability of pyrrolizidine alkaloids to inhibit division of hepatocytes may be a contributing factor. The relationship of cytomegaly, karomegaly, centrilobular necrosis, focal necrosis, and bile duct hyperplasia in hepatocellular neoplasm development in mice is also unclear (Maronpot, 1999).

In the current study, significant increases in the incidences of mononuclear cell leukemia (all organs) occurred in male and female rats administered 1 mg/kg (males, P=0.004; females, P=0.033) despite the significant early mortality from liver toxicity in these animals. Mononuclear cell leukemia occurs commonly with increasing incidence in aging F344/N rats. Because the male rat study was terminated at week 72, the incidence of this lesion cannot be compared directly to the 2-year historical control rate. However, the incidence of this lesion in the male vehicle controls was similar to the rate observed in comparably-aged male control F344/N rats sacrificed at approximately 15 months in previous NTP studies. The increased incidences of mononuclear cell leukemia in male and female rats were therefore considered related to riddelliine treatment.

Increased incidences of alveolar/bronchiolar adenoma and of alveolar/bronchiolar adenoma or carcinoma (combined) were noted in the lung of 3 mg/kg female mice in the 2-year study. The incidences of these lesions were high in male vehicle controls, and riddelliine administration at doses ranging from 0.1 to 3 mg/kg did not enhance the development of these neoplasms in male mice. The reason for the sex difference in response may be related to the sex specificity of P450 isozymes in riddelliine metabolism as found in the metabolism of senecionine in Sprague-Dawley rats (Williams *et al.*, 1989).

In the current studies, administration of riddelliine induced renal tubule necrosis in 1 mg/kg rats and

glomerulosclerosis in 1 mg/kg male mice and 3 mg/kg male and female mice. Administration of riddelliine also induced a collection of renal tubule lesions consistent with nephropathy in male and female mice. Glomerulosclerosis in mice was considered a possible response to antigenic factors produced by the liver neoplasms. However, results of immunohistochemical staining of samples of kidney lesions from male and female mice suggested that the primary mechanism for this change was not immune mediated. The presence of increased collagen indicated a chronic scarring process. The pyrrolizidine alkaloid monocrotaline was specifically toxic to the glomerular mesangium and endothelium (Kurozumi *et al.*, 1983). Three weeks after intraperitoneal injection of 50 mg/kg monocrotaline to rats, renal changes consisted of increased glomerular size, increased matrix in the glomerular tuft, thickening of the capillary wall, mesangial hypercellularity, and the presence of exudate in the subendothelial spaces of the capillaries and mesangium. Riddelliine may also exert a direct toxic effect on the glomeruli in mice, leading to glomerulosclerosis.

Other lesions observed in the 1 mg/kg male and female rats included splenic congestion and hematopoietic cell proliferation, bone marrow hyperplasia, lung hemorrhage, glandular stomach erosions and ulcer, and lymph node hemorrhage and pigmentation. These lesions were considered secondary effects. The increased incidence of splenic congestion in riddelliine-treated rats reflected passive pooling of blood in the splenic sinusoids secondary to hepatotoxicity and impaired perfusion of blood within the liver. The increased incidences of bone marrow hyperplasia and splenic hematopoietic cell proliferation were considered responses to the liver neoplasms and associated hemorrhage and necrosis. Lung hemorrhage and edema were primarily associated with metastatic liver tumors. Free red blood cells accompanied by macrophages containing phagocytosed red blood cells were observed in the subcapsular and medullary sinuses of lymph nodes. These changes resulted from increased drainage to the lymph nodes from areas of hemorrhage outside the lymph nodes. Other changes observed in the dosed rats were considered secondary to the hemangiosarcoma burden, including thymus hemorrhage, lymph node hemorrhage and pigmentation, liver Kupffer cell pigmentation, and renal tubular pigmentation. Stomach erosions and ulcers were considered to be nonspecific responses to stress and moribundity resulting from liver neoplasms and toxicity.

In mice, sex-related differences in response to riddelliine administration were observed in the incidences of skin edema, focal cytoplasmic alteration in the adrenal cortex, and focal hemorrhage of the large intestine. On the other hand, there were no sex-related differences in the incidences of other lesions including liver cytomegaly, hepatocellular adenoma and carcinoma, kidney nephropathy, and splenic hematopoietic cell proliferation. Sex-related differences in susceptibility to pyrrolizidine alkaloid activation may be related to sex specificity of P450 isozymes (Mattocks, 1986). Williams *et al.* (1989) indicated that P4502C11 is male specific and P4503A1 is female specific in Sprague-Dawley rats. The enzymes activated senecionine at different rates. Other factors such as DNA repair and the rate of progression from initiated cells to neoplasms may play a role.

In an NTP study, Yang *et al.* (2001) demonstrated that riddelliine incubated aerobically with rat liver microsomes is converted to dehydroretronecine and riddelline-*N*-oxide (Figure 5). The metabolism rate by microsomes from phenobarbital-treated rats was 3.5 times that of microsomes from untreated rats. Because phenobarbital induces CYP2A1, CYP2A2, CYP2B and CYP3A isozymes, the results suggest the isozymes are involved in riddelliine metabolism. In the presence of calf thymus DNA, metabolism of riddelliine *in vitro* resulted in eight DNA adducts. Two of these adducts were identified as epimers of dehydroretronecine-modified 7-deoxyguanosine-N^2-yl adducts; the structures of the other six were not characterized.

Analyses by ^{32}P-postlabeling/high performance liquid chromatography of DNA adducts from the liver of female F344/N rats treated with riddelliine for 3 or 6 months also yielded the same eight DNA adducts (Appendix I). These data suggest that the eight dehydroretronecine-derived DNA adducts may be responsible in part for development of liver neoplasms in female rats. The mechanisms by which riddelliine induces hepatic hemangiosarcoma and hepatocellular adenoma and carcinoma were further investigated. Metabolism of riddelliine by liver microsomes of F344/N female rats *in vitro* generated riddelliine-*N*-oxide and dehydroretronecine (DHR) as major metabolites. DHR was capable of binding to DNA, 3′-dGMP, 5′-dGMP, and 3′-dAMP, which suggested that DHR was an activated metabolite.

A dose-response relationship was obtained between the dose administered to the female rats and the level of the total (eight) DHR-related adducts formed at both time points. A dose-response relationship was also found between the dose administered to the rats and the level of

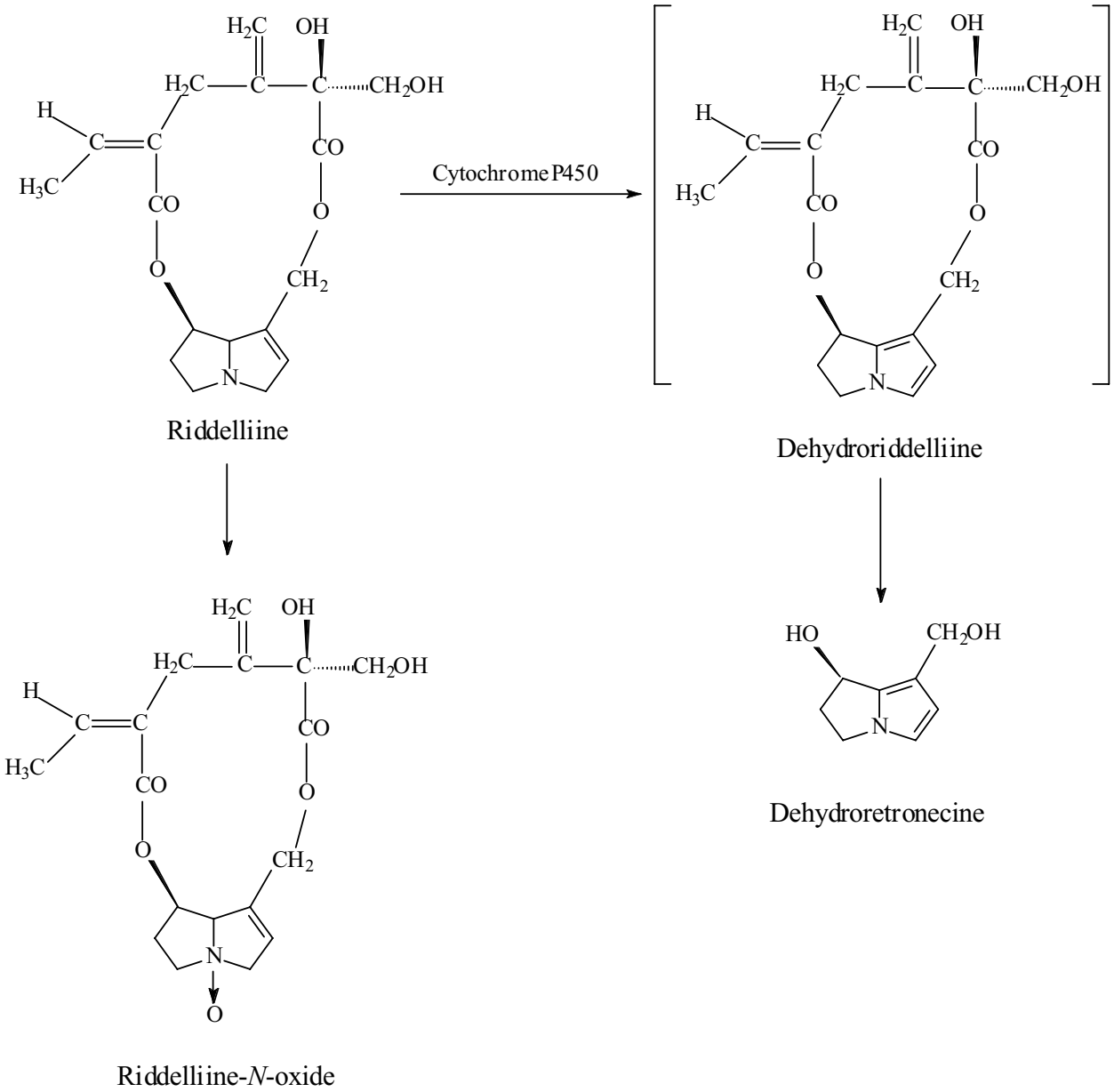


FIGURE 5
Metabolism of Riddelliine and Riddelliine-*N*-oxide by Rat Liver Microsomes
(Yang *et al.*, 2001)

the DHR-3′-dGMP adducts (Figures I15 and I16). The DHR-derived DNA adducts were formed to a higher extent and persisted longer in the target tissues (liver) than in the nontarget tissues (lung and kidney) (data not shown). Thus, the data indicated that the adduct levels were a better reflection of the exposure level than the ultimate neoplasm response in the liver and that the nontarget tissues appeared to be able to remove the adducts faster than the target tissues.

The data suggested that metabolism of riddelliine catalyzed by CYP isozymes first provided 8-hydroxyriddelliine and/or 3-hydroxyriddelliine as the primary metabolites, which upon enzymatic or spontaneous dehydration produced the unstable dehydroriddelliine (Figure 5). Dehydroriddelliine was metabolized by esterase and/or other hepatic enzymes to dehydroretronecine. Both dehydroriddelliine and DHR could covalently bind to DNA for the formation of the eight DHR-derived DNA adducts (Figure 6). The eight DHR-derived DNA adducts, if not repaired prior to DNA synthesis, might produce replication errors and eventually resulted in the development of neoplasms in the dosed rats.

The level of the total DHR-related adducts determined in the liver (Figure I16) represented adducts of both endothelial cells and hepatocytes. Correlation could not be made between the total adduct levels and the liver neoplasm incidences, i.e., hemangiosarcoma and/or hepatocellular adenoma, suggesting other factors were involved, such as DNA repair, cell proliferation, apoptosis, and the rate of progression from initiated cells to neoplasms.

In associated studies, an attempt was made to determine the DHR-derived DNA adduct levels in parenchymal cells and nonparenchymal cells by digesting the liver tissues in collagenase and separating the liver cells in Percoll density gradients (data not shown). Liver tissues were obtained from female rats after 2 weeks of riddelliine administration. It was found that the DHR-derived DNA adduct levels in liver endothelial cells were higher than those in the parenchymal cells, correlating well with the higher incidence of liver hemangiosarcoma than that of hepatocellular adenoma or carcinoma in the male and female F344/N rats. These results suggest that the riddelliine-induced liver hemangioscarcoma and hepatocellular neoplasms in male and female rats involve the formation of the DHR-derived DNA adducts.

Metabolism of riddelliine by human liver microsomes resulted in the formation of DHR and riddelliine-*N*-oxide (Appendix I). The same set of eight DHR-derived DNA adducts was found from metabolism of riddelliine by human male and female liver microsomes in the presence of calf thymus DNA. These results indicate that the same metabolism profile and metabolic activation pathways formed in experimental animals also occur in human liver.

These overall results suggest that riddelliine induces liver neoplasms in rats through a genotoxic mechanism and the eight DHR-derived adducts are responsible in part, if not entirely, for the liver neoplasm development. Hydroxylation of pyrrolizidine alkaloids at the necine base, particularly at the C-8 and C-3 positions to form 8- and 3-hydroxynecine derivatives, followed by dehydration to form the corresponding dehydropyrrolizidine (pyrrolic) derivatives, is a general metabolism pathway (Bull *et al.*, 1968; Mattocks, 1986). Thus, metabolism of riddelliine catalyzed by CYP isozymes first provides 8-hydroxyriddelliine and/or 3-hydroxyriddelliine as the primary metabolites, which upon enzymatic dehydration produced the dehydroriddelliine. Two possible pathways lead to DHR-derived DNA adducts. The first pathway is that dehydroriddelliine, a potent electrophile, covalently binds to cellular DNA (Figure 6) to form dehydroriddelliine-derived DNA adducts, which are subsequently hydrolyzed to DHR-derived DNA adducts. The second pathway is that dehydroriddelliine, like the other dehydropyrrolizidine alkaloids, is unstable and easily hydrolyzed by esterases and/or other hepatic enzymes to form DHR (Mattocks, 1968, 1986; Kim *et al.*, 1999), which subsequently binds to DNA. At present, it is not known from which pathway the eight DHR-derived DNA adducts are formed. Since dehydropyrrolizidine alkaloids (pyrroles) are highly unstable and DHR is the most stable pyrrolic compound (Galloway *et al.*, 1987; Huxtable *et al.*, 1996), more binding should occur through DHR than through dehydroriddelliine.

The data support the hypothesis that riddelliine is metabolically activated by hepatic CYP isozymes. The active metabolites interact with cellular DNA, forming adducts and leading to DNA damage, cytotoxicity, and cellular regeneration. In the process, the unrepaired mutated endothelial cells and hepatocytes eventually develop into neoplasms. Endothelial cells appeared to be more prone to the riddelliine-induced carcinogenic action than

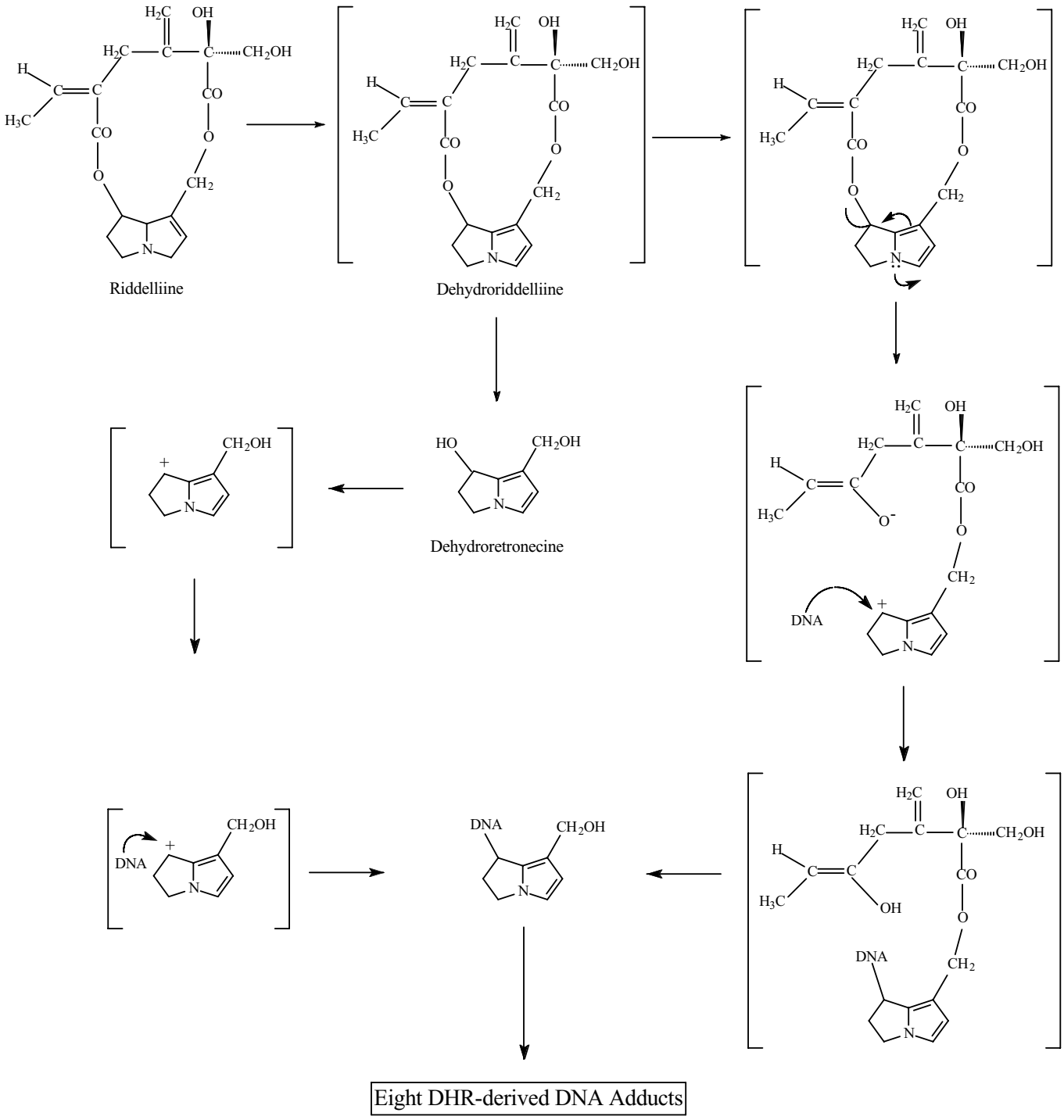


FIGURE 6
Proposed Riddelliine-Derived DNA Adducts

hepatocytes. The early histopathologic changes in the endothelial cells and hepatocytes and the DNA adducts described could be considered as biomarkers of riddelliine toxicity and carcinogenicity. Since other pyrrolizidine alkaloids are likely metabolized and activated by a similar pathway as riddelliine, DNA adduct levels as determined by the process described in the present studies could be useful for assessing the exposure to other pyrrolizidine alkaloids.

CONCLUSIONS

Under the conditions of these studies, there was *clear evidence of carcinogenic activity** of riddelliine in male and female F344/N rats based on increased incidences of hemangiosarcoma in the liver. The increased incidences of hepatocellular adenoma and mononuclear cell leukemia in male and female rats were also considered to be treatment related. There was *clear evidence of carcinogenic activity* of riddelliine in male $B6C3F_1$ mice based on increased incidences of hemangiosarcoma in the liver. There was *clear evidence of carcinogenic activity* in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.

Administration of riddelliine by gavage resulted in nonneoplastic lesions in the liver and kidney of male and female rats; the liver and kidney of male and female mice; and the lung and arteries (multiple tissues) of female mice.

Decreased incidences of hepatocellular neoplasms in male and female mice were related to riddelliine administration.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF RIDDELLIINE

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Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	1 mg/kg
Disposition Summary		
Animals initially in study	50	50
Early deaths		
Accidental deaths		2
Moribund	1	9
Natural deaths		36
Survivors		
Terminal sacrifice	49	3
Animals examined microscopically	50	50
Alimentary System		
Liver	(50)	(50)
Hemangiosarcoma		19 (38%)
Hemangiosarcoma, multiple		24 (48%)
Hepatocellular adenoma		3 (6%)
Hepatocellular adenoma, multiple		1 (2%)
Hepatocholangiocarcinoma		1 (2%)
Mesentery	(8)	(9)
Chordoma, metastatic, mesentery		1 (11%)
Hemangiosarcoma, metastatic, liver		4 (44%)
Pancreas	(50)	(50)
Hemangiosarcoma, metastatic, liver		2 (4%)
Acinus, adenoma	1 (2%)	1 (2%)
Salivary glands	(50)	(50)
Cardiovascular System		
Heart	(50)	(50)
Endocrine System		
Adrenal cortex	(49)	(50)
Adrenal medulla	(49)	(50)
Pheochromocytoma benign	2 (4%)	2 (4%)
Pituitary gland	(50)	(49)
Pars distalis, adenoma	11 (22%)	2 (4%)
Pars distalis, carcinoma	1 (2%)	
Thyroid gland	(50)	(50)
C-cell, adenoma	3 (6%)	3 (6%)
C-cell, carcinoma	1 (2%)	
General Body System		
Peritoneum	(4)	(3)
Genital System		
Epididymis	(50)	(50)
Preputial gland	(50)	(50)
Adenoma	2 (4%)	
Carcinoma		1 (2%)
Seminal vesicle	(50)	(50)
Testes	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (36%)	
Interstitial cell, adenoma	21 (42%)	7 (14%)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Hematopoietic System		
Bone marrow	(50)	(49)
Lymph node	(13)	(39)
Mediastinal, hemangiosarcoma, metastatic, liver		3 (8%)
Lymph node, mandibular	(50)	(50)
Lymph node, mesenteric	(50)	(50)
Spleen	(50)	(49)
Hemangiosarcoma, metastatic, liver		1 (2%)
Thymus	(49)	(49)
Thymoma benign	1 (2%)	
Integumentary System		
Skin	(50)	(50)
Keratoacanthoma	2 (4%)	1 (2%)
Squamous cell papilloma	1 (2%)	
Subcutaneous tissue, fibroma	2 (4%)	
Musculoskeletal System		
Bone	(50)	(50)
Osteosarcoma		1 (2%)
Skeletal muscle	(1)	(5)
Nervous System		
Brain	(50)	(50)
Respiratory System		
Lung	(50)	(50)
Alveolar/bronchiolar adenoma		3 (6%)
Alveolar/bronchiolar carcinoma	1 (2%)	
Chordoma, metastatic, mesentery		1 (2%)
Hemangiosarcoma, metastatic, liver		26 (52%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)
Special Senses System		
Ear	(1)	(1)
Neural crest tumor		1 (100%)
Urinary System		
Kidney	(50)	(50)
Urinary bladder	(50)	(50)
Systemic Lesions		
Multiple organs[b]	(50)	(50)
Leukemia mononuclear	2 (4%)	9 (18%)
Lymphoma malignant		2 (4%)
Mesothelioma malignant	4 (8%)	3 (6%)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Neoplasm Summary		
Total animals with primary neoplasms[c]	47	49
Total primary neoplasms	73	85
Total animals with benign neoplasms	47	15
Total benign neoplasms	64	23
Total animals with malignant neoplasms	9	47
Total malignant neoplasms	9	60
Total animals with metastatic neoplasms	1	30
Total metastatic neoplasms	4	39
Total animals with uncertain neoplasms-benign or malignant		1
Total uncertain neoplasms		2

[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
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	468	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	498	498	498	498	498	498
Carcass ID Number	045	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, peritoneum	X																								
Mesentery	+	+		+	+			+															+		+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																							X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign											X														
Islets, pancreatic		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X				X			X		X	X		X		X	
Pars distalis, carcinoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X							X							
C-cell, carcinoma																									
General Body System																									
Peritoneum	+					+													+			+			

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	
Carcass ID Number	025	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	046	047	048	049	050	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, peritoneum																										1
Mesentery																								+		8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign															X											2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma																	X	X		X				X		11
Pars distalis, carcinoma								X																		1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma															X											3
C-cell, carcinoma						X																				1
General Body System																										
Peritoneum																										4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	468	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	498	498	498	498	498	498
Carcass ID Number	045	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, peritoneum	X																								
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, peritoneum	X																								
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X								X					X					X		X
Interstitial cell, adenoma	X			X		X	X	X	X	X		X		X	X	X			X		X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+			+	+						+			+	+		+				+		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																					X				
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma									X																
Squamous cell papilloma																									
Subcutaneous tissue, fibroma			X																						X
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Mesothelioma malignant, metastatic, peritoneum	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear					+																				

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	
Carcass ID Number	025	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	046	047	048	049	050	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, peritoneum																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, peritoneum																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma		X	X			X			X		X	X			X			X	X	X		X	X		X	18
Interstitial cell, adenoma				X	X		X			X				X		X	X				X					21
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+			+							+						+								+	13
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma benign																										1
Integumentary System																										
Mammary gland	+	+	+	+	M	+	+	+	M	M	+	M	+	M	+	+	+	+	M	+	M	+	+	+	+	42
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																									X	2
Squamous cell papilloma								X																		1
Subcutaneous tissue, fibroma																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Mesothelioma malignant, metastatic, peritoneum																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	468	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	497	498	498	498	498	498	498
Carcass ID Number	045	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X																	
Mesothelioma malignant	X					X													X			X			

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	498	
Carcass ID Number	025	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	046	047	048	049	050	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																	X									2
Mesothelioma malignant																										4

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	204	266	297	307	314	318	321	327	342	364	373	373	375	388	397	397	398	400	400	402	402	403	407	407	408
Carcass ID Number	068	079	061	062	060	082	081	066	074	095	072	086	010	051	056	087	094	083	085	057	092	055	091	099	073
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X	X		X		X	X	X	X	X	X		X			X		X		
Hemangiosarcoma, multiple				X	X			X		X							X			X		X			X
Hepatocellular adenoma																	X								
Hepatocellular adenoma, multiple																							X		
Hepatocholangiocarcinoma																			X						
Mesentery			+												+				+			+			
Chordoma, metastatic, mesentery			X																						
Hemangiosarcoma, metastatic, liver																						X			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver																									
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X																					
General Body System																									
Peritoneum								+											+						
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																	X								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma									X														X		

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	410	411	412	414	414	415	415	418	419	422	424	433	437	440	440	446	449	457	462	463	477	483	497	497	497	
Carcass ID Number	076	097	088	065	078	053	096	075	070	063	093	059	080	084	090	067	077	058	089	071	064	069	052	054	098	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	I	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma												X		X	X	X	X		X		X					19
Hemangiosarcoma, multiple	X	X	X	X	X	X	X	X	X	X	X		X					X		X		X	X			24
Hepatocellular adenoma																	X				X					3
Hepatocellular adenoma, multiple																										1
Hepatocholangiocarcinoma																										1
Mesentery		+			+		+										+				+					9
Chordoma, metastatic, mesentery																										1
Hemangiosarcoma, metastatic, liver					X		X														X					4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, liver														X					X							2
Acinus, adenoma																									X	1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign				X														X								2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma				X															X							2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma						X																			X	3
General Body System																										
Peritoneum																				+						3
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma						X													X			X		X	X	7

Table A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	204	266	297	307	314	318	321	327	342	364	373	373	375	388	397	397	398	400	400	402	402	403	407	407	408
Carcass ID Number	068	079	061	062	060	082	081	086	074	095	072	086	100	051	056	087	094	083	085	057	092	055	091	099	073
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+		+	+	+		+		+	+	+	+		+	+	+		+	+	+	+	+	+		+
Mediastinal, hemangiosarcoma, metastatic, liver										X															
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, liver																		X							
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																								X	
Skeletal muscle			+											+											+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve			+		+					+															
Spinal cord			+		+					+															
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Chordoma, metastatic, mesentery			X																						
Hemangiosarcoma, metastatic, liver				X				X	X	X	X			X	X		X	X			X	X	X		
Hepatocholangiocarcinoma, metastatic, liver																			X						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear														+											
Neural crest tumor														X											
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X								X																
Lymphoma malignant			X																						
Mesothelioma malignant								X											X						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	410	411	412	414	414	415	415	418	419	422	424	433	437	440	440	446	449	457	462	463	477	483	497	497	497	
Carcass ID Number	076	097	088	065	078	053	096	075	070	063	093	059	080	084	090	067	077	058	089	071	064	069	052	054	098	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+			+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	39
Mediastinal, hemangiosarcoma, metastatic, liver										X								X								3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, liver																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	M	M	+	M	+	+	M	+	M	M	M	+	+	+	+	+	+	M	+	+	+	+	+	M	38
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle						+									+											5
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve															+									+		5
Spinal cord															+									+		5
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X										X						X				3
Chordoma, metastatic, mesentery																										1
Hemangiosarcoma, metastatic, liver	X		X	X	X		X	X		X	X		X		X			X	X			X	X			26
Hepatocholangiocarcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Neural crest tumor																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X				X	X		X					X	X				X				9
Lymphoma malignant															X											2
Mesothelioma malignant																				X						3

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Liver: Hemangiosarcoma		
Overall rate[a]	0/50 (0%)	43/50 (86%)
Adjusted rate[b]	0.0%	92.5%
Terminal rate[c]	0/49 (0%)	1/3 (33%)
First incidence (days)	—[e]	307
Poly-3 test[d]		P<0.001
Liver: Hepatocellular Adenoma		
Overall rate	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	13.7%
Terminal rate	0/49 (0%)	0/3 (0%)
First incidence (days)	—	398
Poly-3 test		P=0.033
Lung: Alveolar/bronchiolar Adenoma		
Overall rate	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	10.5%
Terminal rate	0/49 (0%)	0/3 (0%)
First incidence (days)	—	415
Poly-3 test		P=0.088
Lung: Alveolar/bronchiolar Adenoma or Carcinoma		
Overall rate	1/50 (2%)	3/50 (6%)
Adjusted rate	2.0%	10.5%
Terminal rate	1/49 (2%)	0/3 (0%)
First incidence (days)	497 (T)	415
Poly-3 test		P=0.211
Pituitary Gland (Pars Distalis): Adenoma		
Overall rate	11/50 (22%)	2/49 (4%)
Adjusted rate	22.1%	7.2%
Terminal rate	11/49 (22%)	0/3 (0%)
First incidence (days)	497 (T)	414
Poly-3 test		P=0.128N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma		
Overall rate	12/50 (24%)	2/49 (4%)
Adjusted rate	24.1%	7.2%
Terminal rate	12/49 (25%)	0/3 (0%)
First incidence (days)	497 (T)	414
Poly-3 test		P=0.094N
Skin: Squamous Cell Papilloma or Keratoacanthoma		
Overall rate	3/50 (6%)	1/50 (2%)
Adjusted rate	6.0%	3.5%
Terminal rate	3/49 (6%)	0/3 (0%)
First incidence (days)	497 (T)	402
Poly-3 test		P=0.613N
Testes: Adenoma		
Overall rate	39/50 (78%)	7/50 (14%)
Adjusted rate	78.0%	23.6%
Terminal rate	38/49 (78%)	2/3 (67%)
First incidence (days)	468	342
Poly-3 test		P<0.001N

Table A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Thyroid Gland (C-cell): Adenoma		
Overall rate	3/50 (6%)	3/50 (6%)
Adjusted rate	6.0%	10.3%
Terminal rate	3/49 (6%)	1/3 (33%)
First incidence (days)	497 (T)	307
Poly-3 test		P=0.479
Thyroid Gland (C-cell): Adenoma or Carcinoma		
Overall rate	4/50 (8%)	3/50 (6%)
Adjusted rate	8.0%	10.3%
Terminal rate	4/49 (8%)	1/3 (33%)
First incidence (days)	497 (T)	307
Poly-3 test		P=0.592
All Organs: Mononuclear Cell Leukemia		
Overall rate	2/50 (4%)	9/50 (18%)
Adjusted rate	4.0%	28.5%
Terminal rate	2/49 (4%)	0/3 (0%)
First incidence (days)	497 (T)	204
Poly-3 test		P=0.004
All Organs: Malignant Mesothelioma		
Overall rate	4/50 (8%)	3/50 (6%)
Adjusted rate	8.0%	10.3%
Terminal rate	3/49 (6%)	0/3 (0%)
First incidence (days)	468	327
Poly-3 test		P=0.593
All Organs: Benign Neoplasms		
Overall rate	47/50 (94%)	15/50 (30%)
Adjusted rate	94.0%	45.9%
Terminal rate	46/49 (94%)	2/3 (67%)
First incidence (days)	468	307
Poly-3 test		P<0.001N
All Organs: Malignant Neoplasms		
Overall rate	9/50 (18%)	47/50 (94%)
Adjusted rate	18.0%	95.6%
Terminal rate	8/49 (16%)	1/3 (33%)
First incidence (days)	468	204
Poly-3 test		P<0.001
All Organs: Benign or Malignant Neoplasms		
Overall rate	47/50 (94%)	49/50 (98%)
Adjusted rate	94.0%	99.7%
Terminal rate	46/49 (94%)	3/3 (100%)
First incidence (days)	468	204
Poly-3 test		P=0.144

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, 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 dosed group incidence is the P value corresponding to a pairwise comparison between the vehicle controls and the dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in the dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

Table A4a
Historical Incidence of Liver Neoplasms in Control Male F344/N Rats

Study	Incidence in Controls	
	Hemangiosarcoma	Hepatocellular Adenoma
Historical Incidence in Controls Given NTP-2000 Diet[a]		
Citral (feed)	0/100	0/100
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50
Indium phosphide (inhalation)	0/50	0/50
60-Hz Magnetic fields (whole body exposure)	0/100	1/100
Methacrylonitrile (gavage)	0/50	1/50
Naphthalene (inhalation)	0/49	1/49
o-Nitrotoluene (feed)	0/60	2/60
p-Nitrotoluene (feed)	0/50	0/50
Sodium nitrite (drinking water)	0/50	0/50
Vanadium pentoxide (inhalation)	0/50	0/50
Overall Historical Incidence in Controls Given NTP-2000 Diet		
Total (%)	0/609	5/609 (0.8%)
Mean ± standard deviation		0.8% ± 1.2%
Range		0%-3%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]		
Total (%)	0/50	1/50 (2.0%)

[a] Data as of January 17, 2001
[b] Data as of December 21, 1999

Table A4b
Historical Incidence of Mononuclear Cell Leukemia in Control Male F344/N Rats

Study	Incidence in Controls
Historical Incidence in Controls Given NTP-2000 Diet[a]	
Citral (feed)	68/100
p,p′-Dichlorodiphenyl sulfone (feed)	27/50
Indium phosphide (inhalation)	16/50
60-Hz Magnetic fields (whole body exposure)	50/100
Methacrylonitrile (gavage)	20/50
Naphthalene (inhalation)	26/49
o-Nitrotoluene (feed)	30/60
p-Nitrotoluene (feed)	24/50
Sodium nitrite (drinking water)	17/50
Vanadium pentoxide (inhalation)	22/50
Overall Historical Incidence in Controls Given NTP-2000 Diet	
Total (%)	300/609 (49.3%)
Mean ± standard deviation	47.3% ± 10.5%
Range	32%-68%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]	
Total (%)	33/50 (66.0%)
Overall Historical Incidence for 2-Year Studies (All Routes Except Corn Oil Gavage) with Controls Given NIH-07 Diet that had Scheduled Sacrifices at Approximately 15 Months[c]	
Total (%)	11/488 (2.2%)
Mean ± standard deviation	2.2% ± 6.3%
Range	0%-33%

[a] Data as of January 17, 2001; includes data for lymphocytic, monocytic, and undifferentiated leukemia
[b] Data as of December 21, 1999; includes data for lymphocytic, monocytic, and undifferentiated leukemia
[c] Data as of August 29, 2001; includes data for lymphocytic, monocytic, and undifferentiated leukemia

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	1 mg/kg
Disposition Summary		
Animals initially in study	50	50
Early deaths		
Accidental deaths		2
Moribund	1	9
Natural deaths		36
Survivors		
Terminal sacrifice	49	3
Animals examined microscopically	50	50
Alimentary System		
Esophagus	(50)	(50)
Perforation		1 (2%)
Intestine large, cecum	(50)	(49)
Edema	1 (2%)	3 (6%)
Intestine small, duodenum	(50)	(50)
Hemorrhage		1 (2%)
Necrosis		1 (2%)
Liver	(50)	(50)
Basophilic focus	32 (64%)	21 (42%)
Clear cell focus	3 (6%)	5 (10%)
Degeneration, cystic	1 (2%)	3 (6%)
Eosinophilic focus	3 (6%)	15 (30%)
Hematopoietic cell proliferation		2 (4%)
Hemorrhage		4 (8%)
Hepatodiaphragmatic nodule	4 (8%)	4 (8%)
Infiltration cellular, mixed cell	8 (16%)	
Metaplasia, osseous		1 (2%)
Mixed cell focus	3 (6%)	7 (14%)
Necrosis, focal		23 (46%)
Bile duct, hyperplasia	48 (96%)	37 (74%)
Hepatocyte, cytomegaly		32 (64%)
Hepatocyte, hyperplasia, atypical		1 (2%)
Hepatocyte, hyperplasia, regenerative		49 (98%)
Hepatocyte, vacuolization cytoplasmic	30 (60%)	5 (10%)
Kupffer cell, pigmentation		1 (2%)
Mesentery	(8)	(9)
Accessory spleen	1 (13%)	1 (11%)
Fat, necrosis	7 (88%)	3 (33%)
Pancreas	(50)	(50)
Atrophy	7 (14%)	7 (14%)
Acinus, cytoplasmic alteration	1 (2%)	
Acinus, hyperplasia, focal		1 (2%)
Salivary glands	(50)	(50)
Atrophy		1 (2%)
Necrosis		2 (4%)
Stomach, forestomach	(50)	(50)
Edema		2 (4%)
Ulcer		3 (6%)
Stomach, glandular	(50)	(50)
Erosion		10 (20%)
Hemorrhage		1 (2%)
Ulcer		6 (12%)

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

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Cardiovascular System		
Heart	(50)	(50)
Cardiomyopathy	32 (64%)	28 (56%)
Myocardium, hemorrhage		1 (2%)
Endocrine System		
Adrenal cortex	(49)	(50)
Accessory adrenal cortical nodule	8 (16%)	1 (2%)
Degeneration, fatty	5 (10%)	4 (8%)
Hyperplasia, diffuse		1 (2%)
Hyperplasia, focal	3 (6%)	1 (2%)
Hypertrophy	1 (2%)	
Necrosis		1 (2%)
Pituitary gland	(50)	(49)
Pars distalis, cyst	4 (8%)	2 (4%)
Pars distalis, hyperplasia, focal	5 (10%)	4 (8%)
Thyroid gland	(50)	(50)
Ultimobranchial cyst		1 (2%)
C-cell, hyperplasia	4 (8%)	1 (2%)
Follicle, cyst	1 (2%)	
General Body System		
None		
Genital System		
Epididymis	(50)	(50)
Atypia cellular	9 (18%)	9 (18%)
Preputial gland	(50)	(50)
Inflammation, chronic	24 (48%)	16 (32%)
Prostate	(50)	(50)
Inflammation, chronic	18 (36%)	15 (30%)
Testes	(50)	(50)
Atrophy	3 (6%)	6 (12%)
Interstitial cell, hyperplasia	22 (44%)	12 (24%)
Hematopoietic System		
Bone marrow	(50)	(49)
Hyperplasia	1 (2%)	36 (73%)
Myelofibrosis		1 (2%)
Lymph node	(13)	(39)
Bronchial, hemorrhage		1 (3%)
Bronchial, pigmentation		1 (3%)
Iliac, necrosis		1 (3%)
Mediastinal, congestion	3 (23%)	11 (28%)
Mediastinal, hemorrhage	3 (23%)	20 (51%)
Mediastinal, hyperplasia	1 (8%)	
Mediastinal, necrosis		1 (3%)
Mediastinal, pigmentation	10 (77%)	20 (51%)
Pancreatic, hemorrhage		2 (5%)
Pancreatic, pigmentation		2 (5%)
Renal, hemorrhage		3 (8%)
Renal, necrosis		1 (3%)
Renal, pigmentation		3 (8%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Hematopoietic System (continued)		
Lymph node, mandibular	(50)	(50)
Congestion		1 (2%)
Hemorrhage	2 (4%)	1 (2%)
Pigmentation	1 (2%)	1 (2%)
Lymph node, mesenteric	(50)	(50)
Congestion		4 (8%)
Hemorrhage	1 (2%)	8 (16%)
Pigmentation		1 (2%)
Spleen	(50)	(49)
Congestion		24 (49%)
Fibrosis	1 (2%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)	23 (47%)
Hyperplasia, reticulum cell		1 (2%)
Necrosis		1 (2%)
Thymus	(49)	(49)
Hemorrhage		4 (8%)
Integumentary System		
Mammary gland	(42)	(38)
Cyst	1 (2%)	1 (3%)
Hyperplasia	5 (12%)	2 (5%)
Skin	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)
Hyperkeratosis	1 (2%)	
Subcutaneous tissue, fibrosis	1 (2%)	
Musculoskeletal System		
Bone	(50)	(50)
Femur, osteopetrosis		2 (4%)
Skeletal muscle	(1)	(5)
Hemorrhage		3 (60%)
Nervous System		
Brain	(50)	(50)
Compression	2 (4%)	
Hydrocephalus	1 (2%)	
Spinal cord		(5)
Hemorrhage		1 (20%)
Necrosis		1 (20%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	1 mg/kg
Respiratory System		
Lung	(50)	(50)
Edema		5 (10%)
Hemorrhage	1 (2%)	21 (42%)
Infiltration cellular, polymorphonuclear		1 (2%)
Infiltration cellular, histiocyte	42 (84%)	32 (64%)
Metaplasia, osseous	2 (4%)	1 (2%)
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)
Nose	(50)	(50)
Foreign body	5 (10%)	5 (10%)
Hemorrhage	1 (2%)	2 (4%)
Inflammation, chronic	7 (14%)	5 (10%)
Respiratory epithelium, hyperplasia	7 (14%)	6 (12%)
Special Senses System		
None		
Urinary System		
Kidney	(50)	(50)
Glomerulosclerosis		1 (2%)
Hydronephrosis		2 (4%)
Infarct		1 (2%)
Inflammation, suppurative		1 (2%)
Nephropathy	47 (94%)	38 (76%)
Renal tubule, hyaline droplet	1 (2%)	3 (6%)
Renal tubule, necrosis		6 (12%)
Renal tubule, pigmentation	1 (2%)	4 (8%)
Transitional epithelium, hyperplasia		2 (4%)
Urinary bladder	(50)	(50)
Hemorrhage		1 (2%)
Transitional epithelium, hyperplasia		1 (2%)

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

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

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Disposition Summary						
Animals initially in study	50	50	50	50	50	50
Early deaths						
Accidental deaths	2	6	3	2	3	
Moribund	9	11	13	14	16	19
Natural deaths	6	11	6	12	2	31
Survivors						
Terminal sacrifice	33	22	28	22	29	
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, colon	(49)	(50)	(50)	(47)	(50)	(50)
Lipoma		2 (4%)				
Intestine large, rectum	(49)	(50)	(49)	(48)	(48)	(49)
Polyp adenomatous	1 (2%)					
Intestine large, cecum	(50)	(50)	(48)	(47)	(50)	(50)
Intestine small, jejunum	(50)	(43)	(47)	(43)	(50)	(49)
Polyp adenomatous			1 (2%)			
Liver	(50)	(50)	(50)	(50)	(50)	(50)
Cholangiocarcinoma					1 (2%)	
Hemangiosarcoma					3 (6%)	25 (50%)
Hemangiosarcoma, multiple						13 (26%)
Hepatocellular carcinoma					1 (2%)	1 (2%)
Hepatocellular adenoma	1 (2%)					7 (14%)
Hepatocellular adenoma, multiple					1 (2%)	
Mesentery	(10)	(10)	(18)	(14)	(13)	(5)
Hemangiosarcoma, metastatic, liver						2 (40%)
Sarcoma stromal, metastatic, uterus					1 (8%)	
Oral mucosa				(2)		
Squamous cell carcinoma				1 (50%)		
Squamous cell papilloma				1 (50%)		
Pancreas	(50)	(50)	(50)	(46)	(50)	(50)
Acinus, adenoma	1 (2%)					
Stomach, forestomach	(50)	(50)	(50)	(49)	(49)	(50)
Stomach, glandular	(50)	(50)	(50)	(49)	(49)	(50)
Tongue			(2)			
Squamous cell papilloma			2 (100%)			
Cardiovascular System						
Heart	(50)	(50)	(50)	(50)	(50)	(50)
Endocrine System						
Adrenal cortex	(50)	(50)	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		1 (2%)	1 (2%)	
Carcinoma		1 (2%)				
Adrenal medulla	(50)	(49)	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)		1 (2%)			
Pheochromocytoma benign	1 (2%)	5 (10%)	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(46)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	4 (8%)		1 (2%)	
Carcinoma			1 (2%)			

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Endocrine System (continued)						
Pituitary gland	(50)	(48)	(50)	(49)	(50)	(49)
Pars distalis, adenoma	26 (52%)	21 (44%)	21 (42%)	17 (35%)	24 (48%)	7 (14%)
Pars distalis, adenoma, multiple	1 (2%)	1 (2%)				
Pars intermedia, adenoma				1 (2%)		1 (2%)
Thyroid gland	(49)	(50)	(49)	(49)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)					
C-cell, adenoma	1 (2%)	4 (8%)	4 (8%)	6 (12%)	11 (22%)	
C-cell, carcinoma	1 (2%)					
Follicular cell, carcinoma		1 (2%)				1 (2%)
General Body System						
Peritoneum				(1)		
Genital System						
Clitoral gland	(49)	(49)	(49)	(48)	(49)	(50)
Adenoma	5 (10%)	6 (12%)	8 (16%)	9 (19%)	3 (6%)	2 (4%)
Carcinoma	3 (6%)	2 (4%)	4 (8%)	3 (6%)	2 (4%)	
Bilateral, adenoma	2 (4%)	1 (2%)		1 (2%)	1 (2%)	
Ovary	(50)	(49)	(50)	(50)	(50)	(50)
Granulosa cell tumor malignant		1 (2%)	1 (2%)	1 (2%)		
Luteoma	1 (2%)					
Uterus	(50)	(50)	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)					
Deciduoma benign			1 (2%)			
Leiomyoma	1 (2%)					
Leiomyosarcoma		1 (2%)				
Polyp stromal	9 (18%)	14 (28%)	15 (30%)	11 (22%)	9 (18%)	5 (10%)
Polyp stromal, multiple	2 (4%)			1 (2%)		
Sarcoma stromal			2 (4%)	4 (8%)	2 (4%)	
Vagina	(2)	(3)	(3)	(4)	(5)	
Leiomyoma		1 (33%)				
Sarcoma stromal, metastatic, uterus				1 (25%)		
Hematopoietic System						
Bone marrow	(50)	(50)	(50)	(50)	(50)	(50)
Lymph node	(26)	(24)	(36)	(19)	(21)	(40)
Mediastinal, hemangiosarcoma, metastatic, liver						2 (5%)
Lymph node, mandibular	(50)	(49)	(48)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(47)	(49)	(49)
Spleen	(50)	(50)	(50)	(50)	(50)	(50)
Thymus	(48)	(48)	(46)	(48)	(50)	(49)
Thymoma benign		1 (2%)				
Thymoma malignant	1 (2%)					

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Integumentary System						
Mammary gland	(50)	(50)	(50)	(50)	(49)	(49)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Carcinoma	2 (4%)	3 (6%)	2 (4%)		4 (8%)	1 (2%)
Fibroadenoma	16 (32%)	17 (34%)	22 (44%)	17 (34%)	18 (37%)	3 (6%)
Fibroadenoma, multiple	12 (24%)	4 (8%)	4 (8%)	7 (14%)	5 (10%)	
Skin	(50)	(50)	(49)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	1 (2%)			
Basal cell carcinoma	1 (2%)		1 (2%)			
Keratoacanthoma		1 (2%)	1 (2%)			
Myxosarcoma	1 (2%)					
Schwannoma malignant	1 (2%)					
Squamous cell papilloma		1 (2%)				
Subcutaneous tissue, fibroma	1 (2%)		5 (10%)	3 (6%)		1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)					
Subcutaneous tissue, lipoma	1 (2%)					
Musculoskeletal System						
Skeletal muscle		(1)	(1)	(2)		(6)
Rhabdomyosarcoma			1 (100%)			
Nervous System						
Brain	(50)	(50)	(50)	(50)	(50)	(50)
Glioma malignant				1 (2%)		
Granular cell tumor malignant			1 (2%)			
Respiratory System						
Lung	(50)	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)			3 (6%)	2 (4%)	
Alveolar/bronchiolar carcinoma					1 (2%)	
Hemangiosarcoma						1 (2%)
Hemangiosarcoma, metastatic, liver					1 (2%)	17 (34%)
Hepatocellular carcinoma, metastatic, uncertain primary site		1 (2%)				
Special Senses System						
Zymbal's gland		(1)				
Carcinoma		1 (100%)				
Urinary System						
Kidney	(50)	(50)	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(48)	(50)	(50)
Papilloma	1 (2%)					
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	12 (24%)	8 (16%)	13 (26%)	18 (36%)	18 (36%)	14 (28%)
Lymphoma malignant					1 (2%)	1 (2%)
Mesothelioma malignant				1 (2%)		

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Neoplasm Summary						
Total animals with primary neoplasms[c]	47	47	48	47	50	50
Total primary neoplasms	115	102	119	109	113	84
Total animals with benign neoplasms	45	42	45	41	45	22
Total benign neoplasms	90	84	92	80	80	27
Total animals with malignant neoplasms	20	16	21	28	28	47
Total malignant neoplasms	25	18	27	29	33	57
Total animals with metastatic neoplasms		1		2	2	17
Total metastatic neoplasms		1		3	2	21
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 B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	393	437	468	483	498	545	611	611	626	636	640	654	672	678	693	710	720	729	729	729	729	730	730	730	730	730
Carcass ID Number	146	122	102	143	114	142	141	145	112	111	138	104	115	116	131	144	133	117	118	119	120	101	103	105	121
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																				X					
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery							+									+	+	+							+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																								X	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																										
Blood vessel			+																						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X																	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant								X																	
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X	X	X			X		X	X	X	X	X	X	X		X	X	X	X	
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									X
C-cell, adenoma																									
C-cell, carcinoma																									
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	123	124	125	126	127	128	129	130	132	134	135	147	148	149	150	106	107	108	109	110	113	136	137	139	140	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp adenomatous																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma									X																	1
Mesentery						+		+									+					+		+		10
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign																							X			1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X						X								2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X		X	X			X	X			X		X		X		X			X				X	26
Pars distalis, adenoma, multiple																							X			1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, C-cell, adenoma																										1
C-cell, adenoma																				X						1
C-cell, carcinoma							X																			1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	393	437	468	483	498	545	611	611	626	636	640	654	672	678	693	710	720	729	729	729	729	730	730	730	730
Carcass ID Number	146	122	102	143	114	142	141	145	112	111	138	104	115	116	131	144	133	117	118	119	120	101	103	105	121
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma																							X		X
Carcinoma		X																		X					
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Leiomyoma																									
Polyp stromal					X					X		X						X		X					
Polyp stromal, multiple																X									
Vagina													+												
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+		+		+		+	+			+	+	+	+	+	+	+			+			+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X	
Carcinoma							X																X		
Fibroadenoma								X					X			X				X		X			
Fibroadenoma, multiple																	X		X					X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Myxosarcoma					X																				
Schwannoma malignant								X																	
Subcutaneous tissue, fibroma				X																					
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, lipoma																X									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+														+										
Spinal cord	+														+										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	123	124	125	126	127	128	129	130	132	134	135	147	148	149	150	106	107	108	109	110	113	136	137	139	140	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma			X		X																				X	5
Carcinoma							X																			3
Bilateral, adenoma												X			X											2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Luteoma																		X								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma			X																							1
Leiomyoma	X																									1
Polyp stromal					X			X				X										X				9
Polyp stromal, multiple				X																						2
Vagina				+																						2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node				+		+	+		+				+	+			+		+	+		+				26
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymoma malignant																							X			1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																										2
Fibroadenoma							X	X			X	X	X			X		X	X	X	X			X		16
Fibroadenoma, multiple		X		X	X					X				X	X		X						X			12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma								X																		1
Myxosarcoma																										1
Schwannoma malignant																										1
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, fibrosarcoma					X																					1
Subcutaneous tissue, lipoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	3	4	4	4	4	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7
	9	3	6	8	9	4	1	1	2	3	4	5	7	7	9	1	2	2	2	2	2	3	3	3	3	3
	3	7	8	3	8	5	1	1	6	6	0	4	2	8	3	0	0	9	9	9	9	0	0	0	0	0
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	4	2	0	4	1	4	4	4	1	1	3	0	1	1	3	4	3	1	1	1	2	0	0	0	2
	6	2	2	3	4	2	1	5	2	1	8	4	5	6	1	4	3	7	8	9	0	1	3	5	1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Lacrimal gland																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma																										
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X							X					X		X			X				X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	123	124	125	126	127	128	129	130	132	134	135	147	148	149	150	106	107	108	109	110	113	136	137	139	140	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma											X				X											2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Lacrimal gland																+										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma				X																						1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X	X		X				X				X				X									12

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.01 mg/kg

Number of Days on Study	328	407	513	519	520	541	573	600	605	612	613	631	636	636	638	643	644	647	650	672	675	679	693	698	715
Carcass ID Number	197	184	169	198	154	185	161	173	200	181	193	192	183	194	188	176	180	186	153	174	179	163	151	177	165
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	A	+	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery							+									+			+	+					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																							X		
Adrenal medulla	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pars distalis, adenoma					X			X				X		X	X										
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X													X	
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma		X																							
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																					X				
Polyp stromal	X				X		X	X							X				X						X
Vagina							M													+					+
Leiomyoma																									

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.01 mg/kg

Number of Days on Study	718	719	725	729	729	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	156	152	191	162	164	187	189	190	195	155	157	158	159	160	166	167	168	170	171	172	175	178	182	196	199	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma						X													X							2
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery				+	+				+				+				+		+							10
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																		X								1
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign						X				X					X				X							5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																							X	X		2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X			X	X			X	X		X		X	X	X	X	X	X	X	X	X				X	21
Pars distalis, adenoma, multiple																								X		1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma		X										X														4
Follicular cell, carcinoma															X											1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X							X							X		X					X	X		6
Carcinoma						X																				2
Bilateral, adenoma			X																							1
Ovary	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Granulosa cell tumor malignant																			X							1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Polyp stromal	X									X		X							X	X	X			X		14
Vagina					+																					3
Leiomyoma					X																					1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.01 mg/kg

Number of Days on Study	328	407	513	519	520	541	573	600	605	612	613	631	636	636	638	643	644	647	650	672	675	679	693	698	715
Carcass ID Number	197	184	169	198	154	185	161	173	200	181	193	192	183	194	188	176	180	186	153	174	179	163	151	177	165
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+				+	+			+		+	+		+		+	+					+	
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X															X					
Fibroadenoma			X			X							X			X		X	X			X			
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																							X		
Keratoacanthoma																		X							
Squamous cell papilloma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																								+	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																								+	
Spinal cord																								+	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, uncertain primary site													X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Zymbal's gland																	+								
Carcinoma																	X								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X		X									X		X			X		X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.01 mg/kg

Number of Days on Study	718	719	725	729	729	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	156	152	191	162	164	187	189	190	195	155	157	158	159	160	166	167	168	170	171	172	175	178	182	196	199	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+	+	+					+			+	+		+	+		+	+		+	+	+	+	24
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Thymoma benign	X																									1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																				X						1
Carcinoma									X																	3
Fibroadenoma	X			X			X	X			X				X				X		X			X	X	17
Fibroadenoma, multiple		X				X			X											X						4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Keratoacanthoma																										1
Squamous cell papilloma																					X					1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X																8

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.033 mg/kg

Number of Days on Study	437	444	518	532	562	571	572	591	591	616	617	647	647	647	654	658	671	675	688	694	715	715	729	729	729
Carcass ID Number	243	238	230	237	229	221	214	204	231	222	233	206	207	212	205	249	224	202	218	242	215	235	209	210	223
Alimentary System																									
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Polyp adenomatous																							X		
Intestine small, ileum	+	A	+	+	+	A	+	+	+	A	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery			+			+	+									+				+	+	+		+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																					+				
Squamous cell papilloma																					X				
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant							X																		
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X			X	
Carcinoma																									
Parathyroid gland	+	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X			X	X	X							X				X	X	X		X		
Thyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma												X						X							
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X														X				X			
Carcinoma					X																			X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant				X																					
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deciduoma benign		X																							
Polyp stromal			X		X						X					X		X							X
Sarcoma stromal	X																							X	
Vagina								+																+	

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.033 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	
Carcass ID Number	225	236	239	240	244	246	247	248	250	208	211	213	232	234	241	245	201	203	216	217	219	220	226	227	228	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Polyp adenomatous																										1
Intestine small, ileum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery			+			+	+						+		+		+					+	+		+	18
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																	+									2
Squamous cell papilloma																	X									2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign						X				X																2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma							X			X																4
Carcinoma			X																							1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X			X			X				X		X	X			X	X	X	X		X		X		21
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma		X																							X	4
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma			X				X					X		X											X	8
Carcinoma				X																				X		4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor malignant																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deciduoma benign																										1
Polyp stromal				X	X				X				X		X	X		X			X				X	15
Sarcoma stromal																										2
Vagina					+																					3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.033 mg/kg

Number of Days on Study	437	444	518	532	562	571	572	591	591	616	617	647	667	647	654	658	671	675	688	694	715	715	729	729	729
Carcass ID Number	243	238	230	237	229	221	214	204	231	222	233	206	207	212	205	249	224	202	218	242	215	235	209	210	223
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+	+	+				+			+		+	+		+	+	+		+	+	+	+	+
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																						X			
Fibroadenoma					X	X	X							X	X			X	X				X	X	X
Fibroadenoma, multiple								X																	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									X
Basal cell carcinoma																									
Keratoacanthoma																									
Subcutaneous tissue, fibroma													X		X										
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle									+																
Rhabdomyosarcoma									X																
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor malignant																									
Peripheral nerve																				+					
Spinal cord																				+					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X	X							X					X	X				X	X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.033 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	225	236	239	240	244	246	247	248	250	208	211	213	232	234	241	245	201	203	216	217	219	220	226	227	228	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+		+		+	+		+	+	+	+		+	+		+	36
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																		X								1
Carcinoma																		X								2
Fibroadenoma		X	X	X		X	X		X					X			X		X			X	X	X		22
Fibroadenoma, multiple	X																	X		X						4
Skin	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Basal cell adenoma																										1
Basal cell carcinoma											X															1
Keratoacanthoma													X													1
Subcutaneous tissue, fibroma					X											X							X			5
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor malignant									X																	1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X	X								X				X						X	X	13

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	252	293	307	451	552	561	618	638	638	642	646	650	651	658	660	664	668	678	678	680	682	691	707	707	710
Carcass ID Number	286	282	269	278	300	280	277	275	290	276	272	284	274	298	279	259	262	287	296	299	288	261	251	295	285
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	A	+	+	+	+	A	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	A	+	+	+	+	A	A	+	+	+	+	A	+	A	+	+	+	A	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery									+				+		+		+				+				+
Oral mucosa																									
Squamous cell carcinoma																									
Squamous cell papilloma																									
Pancreas	+	A	M	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	A	M	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X									X	X		X	X			X	X		
Pars intermedia, adenoma																									
Thyroid gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																					X				
General Body System																									
Peritoneum			+																						

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	715	718	726	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	265	258	289	252	253	254	255	256	257	260	281	283	263	264	266	267	268	270	271	273	291	292	293	294	297	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery									+				+	+	+		+		+	+					+	14
Oral mucosa										+										+						2
Squamous cell carcinoma										X																1
Squamous cell papilloma																				X						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth										+																1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X																						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign									X																	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X		X	X	X	X								X	X			X			X	X			17
Pars intermedia, adenoma																					X					1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma				X					X	X					X			X								6
General Body System																										
Peritoneum																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	252	293	307	451	552	561	618	638	638	642	646	650	651	658	660	664	668	678	678	680	682	691	707	707	710
Carcass ID Number	286	282	269	278	300	280	277	275	290	276	272	284	274	298	279	259	262	287	296	299	288	261	251	295	285
Genital System																									
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Adenoma													X									X			
Carcinoma																									
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Mesothelioma malignant, metastatic, peritoneum			X																						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal						X					X		X								X				
Polyp stromal, multiple																									
Sarcoma stromal											X										X			X	
Vagina			+						+										+		+				
Sarcoma stromal, metastatic, uterus																					X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+	+		+		+				+	+									+	+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X			
Fibroadenoma								X			X					X					X			X	X
Fibroadenoma, multiple									X			X					X					X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma								X																	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+				+																		
Mesothelioma malignant, metastatic, peritoneum			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant	X																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X											X
Nose	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	715	718	726	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	265	258	289	252	253	254	255	256	257	260	281	283	263	264	266	267	268	270	271	273	291	292	293	294	297	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma				X	X	X		X			X						X							X		9
Carcinoma	X						X		X																	3
Bilateral, adenoma		X																								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor malignant				X																						1
Mesothelioma malignant, metastatic, peritoneum																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal				X							X	X	X	X					X						X	11
Polyp stromal, multiple																				X						1
Sarcoma stromal						X																				4
Vagina	M																									4
Sarcoma stromal, metastatic, uterus																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+	+	+					+		+		+		+					+			+	+	+	19
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Fibroadenoma	X	X				X	X	X				X		X		X		X		X				X		17
Fibroadenoma, multiple											X						X		X							7
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma	X						X																			3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Mesothelioma malignant, metastatic, peritoneum																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X																	3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	252	293	307	451	552	561	618	638	638	642	646	650	651	658	660	664	668	678	678	680	682	691	707	707	710
Carcass ID Number	286	282	269	278	300	280	277	275	290	276	272	284	274	298	279	259	262	287	296	299	288	261	251	295	285
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X		X	X	X	X	X			X	X			X	X		X			X		
Mesothelioma malignant			X																						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	715	718	726	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	265	258	289	252	253	254	255	256	257	260	281	283	263	264	266	267	268	270	271	273	291	292	293	294	297	Total Tissues/ Tumors
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X				X		X						X					X			X	18
Mesothelioma malignant																										1

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.33 mg/kg

Number of Days on Study	451	463	524	570	576	581	587	590	602	619	630	654	654	656	657	658	671	680	686	686	697	729	729	729	729
Carcass ID Number	312	335	301	307	329	326	305	309	350	344	319	304	341	342	320	337	338	331	324	345	321	308	311	313	314
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																									
Hemangiosarcoma			X														X								
Hepatocellular carcinoma																									
Hepatocellular adenoma, multiple																									
Mesentery		+			+		+		+	+								+	+		+			+	
Sarcoma stromal, metastatic, uterus																								X	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																	X			X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X				X	X				X	X						X	X	X		X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X		X							X		X	X									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Carcinoma																									
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X						X											X			X		
Sarcoma stromal		X																						X	
Vagina	+	+					+			+														+	

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.33 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	315	327	328	330	332	333	334	346	347	348	349	302	303	306	310	336	339	340	343	316	317	318	322	323	325	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma																X										1
Hemangiosarcoma																						X				3
Hepatocellular carcinoma																					X					1
Hepatocellular adenoma, multiple										X																1
Mesentery			+					+					+										+			13
Sarcoma stromal, metastatic, uterus																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X									1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																					X					3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																									X	1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma						X			X			X		X		X	X	X	X	X	X	X	X		X	24
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma		X		X			X			X											X				X	11
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma				X																				X		3
Carcinoma		X			X																					2
Bilateral, adenoma										X																1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal											X	X		X	X									X		9
Sarcoma stromal																										2
Vagina																										5

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.33 mg/kg

Number of Days on Study	451	463	524	570	576	581	587	590	602	619	630	654	554	656	657	658	671	680	686	686	697	729	729	729	729
Carcass ID Number	312	335	301	307	329	326	305	309	350	344	319	304	341	442	320	337	338	331	324	345	321	308	311	313	314
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+			+			+	+	+	+									+	+	+	+	+	+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																				X					
Fibroadenoma				X	X			X				X			X			X		X					X
Fibroadenoma, multiple														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+			+																					
Spinal cord	+			+																					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Hemangiosarcoma, metastatic, liver			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter		+																							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X				X	X		X	X	X	X	X				X		X	X	X	X			
Lymphoma malignant	X																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 0.33 mg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	315	327	328	330	332	333	334	346	347	348	349	302	303	306	310	336	339	340	343	316	317	318	322	323	325	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+						+		+					+	+		+					+	+	+	21
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma														X												1
Carcinoma							X							X							X					4
Fibroadenoma	X				X	X		X					X		X	X					X		X	X		18
Fibroadenoma, multiple			X						X		X								X							5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X							X											2
Alveolar/bronchiolar carcinoma																		X								1
Hemangiosarcoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X	X			X	X							X									18
Lymphoma malignant																										1

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	350	352	393	400	405	407	423	426	434	440	447	459	460	461	461	469	470	472	475	476	478	487	488	503	506
Carcass ID Number	368	373	363	365	396	395	358	378	399	389	375	374	382	355	362	356	381	370	391	366	388	386	351	357	379
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	M	+	+	+	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X						X	X	X			X		X		X		X	X		X	X		X
Hemangiosarcoma, multiple	X		X		X		X							X		X								X	
Hepatocellular carcinoma																									
Hepatocellular adenoma								X																	
Mesentery																									
Hemangiosarcoma, metastatic, liver																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																		X							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma									X																
Pars intermedia, adenoma																					X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma												X													
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal											X				X										

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	509	516	522	527	528	528	528	541	542	548	552	552	552	552	556	569	573	581	587	587	591	608	618	658	668	
Carcass ID Number	390	380	360	369	354	372	387	359	385	392	352	383	384	394	397	393	364	353	361	400	377	371	376	398	367	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma			X	X		X			X		X	X			X	X	X	X	X			X	X			25
Hemangiosarcoma, multiple	X	X			X			X												X				X		13
Hepatocellular carcinoma																	X									1
Hepatocellular adenoma		X	X						X							X					X		X			7
Mesentery			+					+			+			+						+						5
Hemangiosarcoma, metastatic, liver											X									X						2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma									X			X		X			X					X			X	7
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																								X		2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal									X		X													X		5

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	350	352	393	400	405	407	423	426	434	440	447	459	460	461	461	469	470	472	475	476	478	487	488	503	506
Carcass ID Number	368	373	363	365	396	395	358	378	399	389	375	374	382	355	362	356	381	370	391	366	388	386	351	357	379
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+		+	+	+	+	+	+	+	+	+	+	+		+	+				+	+	+	+
Mediastinal, hemangiosarcoma, metastatic, liver										X															
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma											X														
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																	+			+		+	+		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve						+	+											+							
Spinal cord						+	+											+							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hemangiosarcoma, metastatic, liver		X			X		X		X	X			X										X	X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																			+						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X		X		X				X	X			X		X							
Lymphoma malignant																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	509	516	522	527	528	528	528	541	442	448	552	552	552	552	556	569	573	581	587	587	591	608	618	658	668	
Carcass ID Number	390	380	360	369	354	372	387	359	385	392	352	383	384	394	397	393	364	353	361	400	377	371	376	398	367	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+		+	+	+	+	+		+	+	+	+			+	+	+	+	+	+	+	+	+	+	40
Mediastinal, hemangiosarcoma, metastatic, liver						X																				2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	49
Carcinoma													X													1
Fibroadenoma							X							X												3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle								+				+														6
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve				+														+								5
Spinal cord				+														+								5
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma										X																1
Hemangiosarcoma, metastatic, liver		X		X	X	X					X								X	X		X	X			17
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X	X	X				X			X											X	14
Lymphoma malignant				X																						1

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Adrenal Medulla: Benign Pheochromocytoma						
Overall rate[a]	1/50 (2%)	5/49 (10%)	2/50 (4%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate[b]	2.3%	12.7%	4.8%	2.5%	7.1%	5.5%
Terminal rate[c]	1/33 (3%)	4/22 (18%)	2/28 (7%)	1/22 (5%)	1/29 (3%)	0/0
First incidence (days)	729 (T)	715	729 (T)	729 (T)	671	472
Poly-3 test[d]	P=0.538	P=0.082	P=0.490	P=0.750	P=0.301	P=0.552
Adrenal Medulla: Benign or Malignant Pheochromocytoma						
Overall rate	2/50 (4%)	5/49 (10%)	3/50 (6%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.6%	12.7%	7.1%	2.5%	7.1%	5.5%
Terminal rate	1/33 (3%)	4/22 (18%)	2/28 (7%)	1/22 (5%)	1/29 (3%)	0/0
First incidence (days)	611	715	572	729 (T)	671	472
Poly-3 test	P=0.469N	P=0.178	P=0.488	P=0.521N	P=0.490	P=0.666
Clitoral Gland: Adenoma						
Overall rate	7/49 (14%)	7/49 (14%)	8/49 (16%)	10/48 (21%)	4/49 (8%)	2/50 (4%)
Adjusted rate	16.7%	17.7%	19.1%	24.7%	9.6%	10.8%
Terminal rate	7/33 (21%)	5/22 (23%)	5/28 (18%)	7/22 (32%)	3/29 (10%)	0/0
First incidence (days)	729 (T)	719	532	651	686	503
Poly-3 test	P=0.149N	P=0.569	P=0.501	P=0.263	P=0.263N	P=0.430N
Clitoral Gland: Carcinoma						
Overall rate	3/49 (6%)	2/49 (4%)	4/49 (8%)	3/48 (6%)	2/49 (4%)	0/50 (0%)
Adjusted rate	7.0%	5.0%	9.6%	7.5%	4.8%	0.0%
Terminal rate	2/33 (6%)	1/22 (5%)	3/28 (11%)	2/22 (9%)	2/29 (7%)	0/0
First incidence (days)	437	407	562	715	729 (T)	—[e]
Poly-3 test	P=0.220N	P=0.526N	P=0.486	P=0.632	P=0.514N	P=0.341N
Clitoral Gland: Adenoma or Carcinoma						
Overall rate	10/49 (20%)	9/49 (18%)	12/49 (24%)	13/48 (27%)	6/49 (12%)	2/50 (4%)
Adjusted rate	23.4%	22.3%	28.2%	32.1%	14.4%	10.8%
Terminal rate	9/33 (27%)	6/22 (27%)	8/28 (29%)	9/22 (41%)	5/29 (17%)	0/0
First incidence (days)	437	407	532	651	686	503
Poly-3 test	P=0.072N	P=0.555N	P=0.397	P=0.258	P=0.218N	P=0.245N
Liver: Hemangiosarcoma						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	38/50 (76%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.0%	89.7%
Terminal rate	0/33 (0%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	—	—	—	—	524	350
Poly-3 test	P<0.001	—[f]	—	—	P=0.118	P<0.001
Liver: Hepatocellular Adenoma						
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	7/50 (14%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	2.4%	32.3%
Terminal rate	1/33 (3%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	729 (T)	—	—	—	729 (T)	426
Poly-3 test	P<0.001	P=0.514N	P=0.506N	P=0.510N	P=0.756	P=0.002
Liver: Hepatocellular Adenoma or Carcinoma						
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	8/50 (16%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	4.8%	36.1%
Terminal rate	1/33 (3%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	2/29 (7%)	0/0
First incidence (days)	729 (T)	—	—	—	729 (T)	426
Poly-3 test	P<0.001	P=0.514N	P=0.506N	P=0.510N	P=0.493	P<0.001

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Lung: Alveolar/bronchiolar Adenoma						
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	4.7%	0.0%	0.0%	7.3%	4.8%	0.0%
Terminal rate	2/33 (6%)	0/22 (0%)	0/28 (0%)	1/22 (5%)	2/29 (7%)	0/0
First incidence (days)	729 (T)	—	—	658	729 (T)	—
Poly-3 test	P=0.538	P=0.253N	P=0.244N	P=0.481	P=0.687	P=0.457N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.7%	0.0%	0.0%	7.3%	7.2%	0.0%
Terminal rate	2/33 (6%)	0/22 (0%)	0/28 (0%)	1/22 (5%)	3/29 (10%)	0/0
First incidence (days)	729 (T)	—	—	658	729 (T)	—
Poly-3 test	P=0.383	P=0.253N	P=0.244N	P=0.481	P=0.490	P=0.457N
Mammary Gland: Fibroadenoma						
Overall rate	28/50 (56%)	21/50 (42%)	26/50 (52%)	24/50 (48%)	23/50 (46%)	3/50 (6%)
Adjusted rate	64.2%	49.2%	58.3%	55.7%	51.5%	15.4%
Terminal rate	24/33 (73%)	12/22 (55%)	18/28 (64%)	12/22 (55%)	15/29 (52%)	0/0
First incidence (days)	611	513	562	638	570	461
Poly-3 test	P=0.006N	P=0.105N	P=0.358N	P=0.269N	P=0.151N	P<0.001N
Mammary Gland: Fibroadenoma or Adenoma						
Overall rate	28/50 (56%)	21/50 (42%)	26/50 (52%)	24/50 (48%)	24/50 (48%)	3/50 (6%)
Adjusted rate	64.2%	49.2%	58.3%	55.7%	53.7%	15.4%
Terminal rate	24/33 (73%)	12/22 (55%)	18/28 (64%)	12/22 (55%)	16/29 (55%)	0/0
First incidence (days)	611	513	562	638	570	461
Poly-3 test	P=0.009N	P=0.105N	P=0.358N	P=0.269N	P=0.208N	P<0.001N
Mammary Gland: Carcinoma						
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.6%	7.4%	4.8%	0.0%	9.5%	5.5%
Terminal rate	1/33 (3%)	1/22 (5%)	1/28 (4%)	0/22 (0%)	3/29 (10%)	0/0
First incidence (days)	611	520	715	—	686	552
Poly-3 test	P=0.334	P=0.473	P=0.681	P=0.250N	P=0.324	P=0.664
Mammary Gland: Adenoma or Carcinoma						
Overall rate	3/50 (6%)	4/50 (8%)	2/50 (4%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.9%	9.8%	4.8%	2.4%	9.5%	5.5%
Terminal rate	2/33 (6%)	2/22 (9%)	1/28 (4%)	0/22 (0%)	3/29 (10%)	0/0
First incidence (days)	611	520	715	691	686	552
Poly-3 test	P=0.497	P=0.468	P=0.517N	P=0.325N	P=0.486	P=0.619N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma						
Overall rate	30/50 (60%)	23/50 (46%)	27/50 (54%)	24/50 (48%)	25/50 (50%)	4/50 (8%)
Adjusted rate	68.2%	52.8%	60.5%	55.7%	56.0%	19.9%
Terminal rate	25/33 (76%)	12/22 (55%)	18/28 (64%)	12/22 (55%)	17/29 (59%)	0/0
First incidence (days)	611	513	562	638	570	461
Poly-3 test	P=0.008N	P=0.094N	P=0.289N	P=0.153N	P=0.157N	P<0.001N
Pancreatic Islets: Adenoma						
Overall rate	2/50 (4%)	2/50 (4%)	4/50 (8%)	0/46 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.7%	5.0%	9.6%	0.0%	2.4%	0.0%
Terminal rate	2/33 (6%)	2/22 (9%)	3/28 (11%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	729 (T)	729 (T)	715	—	729 (T)	—
Poly-3 test	P=0.168N	P=0.669	P=0.323	P=0.255N	P=0.507N	P=0.457N

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Pancreatic Islets: Adenoma or Carcinoma						
Overall rate	2/50 (4%)	2/50 (4%)	5/50 (10%)	0/46 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.7%	5.0%	12.0%	0.0%	2.4%	0.0%
Terminal rate	2/33 (6%)	2/22 (9%)	4/28 (14%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	729 (T)	729 (T)	715	—	729 (T)	—
Poly-3 test	P=0.140N	P=0.669	P=0.203	P=0.255N	P=0.507N	P=0.457N
Pituitary Gland (Pars Distalis): Adenoma						
Overall rate	27/50 (54%)	22/48 (46%)	21/50 (42%)	17/49 (35%)	24/50 (48%)	7/49 (14%)
Adjusted rate	59.7%	54.5%	47.3%	40.4%	53.5%	33.5%
Terminal rate	18/33 (55%)	16/21 (76%)	13/28 (46%)	9/22 (41%)	16/29 (55%)	0/0
First incidence (days)	545	520	518	561	524	434
Poly-3 test	P=0.150N	P=0.393N	P=0.163N	P=0.051N	P=0.349N	P=0.049N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma						
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.3%	7.4%	7.2%	0.0%	0.0%	0.0%
Terminal rate	1/33 (3%)	1/22 (5%)	3/28 (11%)	0/22 (0%)	0/29 (0%)	0/0
First incidence (days)	729 (T)	647	729 (T)	—	—	—
Poly-3 test	P=0.099N	P=0.284	P=0.294	P=0.510N	P=0.504N	P=0.650N
Skin (Subcutaneous Tissue): Fibroma						
Overall rate	1/50 (2%)	0/50 (0%)	5/50 (10%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.3%	0.0%	11.9%	7.3%	0.0%	5.4%
Terminal rate	0/33 (0%)	0/22 (0%)	3/28 (11%)	1/22 (5%)	0/29 (0%)	0/0
First incidence (days)	483	—	647	638	—	447
Poly-3 test	P=0.369N	P=0.517N	P=0.093	P=0.285	P=0.507N	P=0.549
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Myxosarcoma						
Overall rate	3/50 (6%)	0/50 (0%)	5/50 (10%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.8%	0.0%	11.9%	7.3%	0.0%	5.4%
Terminal rate	1/33 (3%)	0/22 (0%)	3/28 (11%)	1/22 (5%)	0/29 (0%)	0/0
First incidence (days)	483	—	647	638	—	447
Poly-3 test	P=0.239N	P=0.138N	P=0.330	P=0.629	P=0.128N	P=0.622N
Thyroid Gland (C-cell): Adenoma						
Overall rate	2/49 (4%)	4/50 (8%)	4/49 (8%)	6/49 (12%)	11/50 (22%)	0/50 (0%)
Adjusted rate	4.8%	9.9%	9.6%	14.7%	25.1%	0.0%
Terminal rate	2/32 (6%)	1/22 (5%)	2/28 (7%)	5/22 (23%)	6/29 (21%)	0/0
First incidence (days)	729 (T)	613	647	682	570	—
Poly-3 test	P=0.169	P=0.321	P=0.337	P=0.123	P=0.008	P=0.452N
Thyroid Gland (C-cell): Adenoma or Carcinoma						
Overall rate	3/49 (6%)	4/50 (8%)	4/49 (8%)	6/49 (12%)	11/50 (22%)	0/50 (0%)
Adjusted rate	7.2%	9.9%	9.6%	14.7%	25.1%	0.0%
Terminal rate	3/32 (9%)	1/22 (5%)	2/28 (7%)	5/22 (23%)	6/29 (21%)	0/0
First incidence (days)	729 (T)	613	647	682	570	—
Poly-3 test	P=0.212	P=0.482	P=0.500	P=0.228	P=0.023	P=0.336N
Uterus: Stromal Polyp						
Overall rate	11/50 (22%)	14/50 (28%)	15/50 (30%)	12/50 (24%)	9/50 (18%)	5/50 (10%)
Adjusted rate	24.9%	32.4%	34.4%	28.5%	20.8%	24.4%
Terminal rate	7/33 (21%)	6/22 (27%)	10/28 (36%)	8/22 (36%)	6/29 (21%)	0/0
First incidence (days)	498	328	518	561	524	447
Poly-3 test	P=0.207N	P=0.291	P=0.227	P=0.445	P=0.423N	P=0.593N

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Uterus: Stromal Sarcoma						
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	4.7%	9.7%	4.7%	0.0%
Terminal rate	0/33 (0%)	0/22 (0%)	1/28 (4%)	1/22 (5%)	1/29 (3%)	0/0
First incidence (days)	—	—	437	646	463	—
Poly-3 test	P=0.504	—	P=0.234	P=0.055	P=0.236	—
Uterus: Stromal Polyp or Stromal Sarcoma						
Overall rate	11/50 (22%)	14/50 (28%)	17/50 (34%)	14/50 (28%)	11/50 (22%)	5/50 (10%)
Adjusted rate	24.9%	32.4%	38.3%	33.2%	25.0%	24.4%
Terminal rate	7/33 (21%)	6/22 (27%)	11/28 (39%)	9/22 (41%)	7/29 (24%)	0/0
First incidence (days)	498	328	437	561	463	447
Poly-3 test	P=0.234N	P=0.291	P=0.126	P=0.268	P=0.591	P=0.593N
All Organs: Hemangiosarcoma						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	39/50 (78%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.0%	90.9%
Terminal rate	0/33 (0%)	0/22 (0%)	0/28 (0%)	0/22 (0%)	1/29 (3%)	0/0
First incidence (days)	—	—	—	—	524	350
Poly-3 test	P<0.001	—	—	—	P=0.118	P<0.001
All Organs: Mononuclear Cell Leukemia						
Overall rate	12/50 (24%)	8/50 (16%)	13/50 (26%)	18/50 (36%)	18/50 (36%)	14/50 (28%)
Adjusted rate	27.0%	18.9%	29.9%	40.3%	39.0%	51.6%
Terminal rate	8/33 (24%)	1/22 (5%)	7/28 (25%)	6/22 (27%)	6/29 (21%)	0/0
First incidence (days)	393	513	532	451	463	352
Poly-3 test	P=0.009	P=0.262N	P=0.475	P=0.132	P=0.158	P=0.033
All Organs: Benign Neoplasms						
Overall rate	45/50 (90%)	42/50 (84%)	45/50 (90%)	41/50 (82%)	45/50 (90%)	22/50 (44%)
Adjusted rate	95.2%	89.4%	93.5%	91.2%	95.2%	72.0%
Terminal rate	32/33 (97%)	21/22 (96%)	27/28 (96%)	22/22 (100%)	29/29 (100%)	0/0
First incidence (days)	483	328	444	561	524	426
Poly-3 test	P<0.001N	P=0.218N	P=0.538N	P=0.342N	P=0.727N	P<0.001N
All Organs: Malignant Neoplasms						
Overall rate	20/50 (40%)	17/50 (34%)	21/50 (42%)	28/50 (56%)	28/50 (56%)	47/50 (94%)
Adjusted rate	43.1%	38.2%	46.4%	59.3%	58.9%	97.8%
Terminal rate	13/33 (39%)	5/22 (23%)	12/28 (43%)	10/22 (46%)	14/29 (48%)	0/0
First incidence (days)	393	407	437	252	451	350
Poly-3 test	P<0.001	P=0.392N	P=0.460	P=0.083	P=0.088	P<0.001

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
All Organs: Benign or Malignant Neoplasms						
Overall rate	47/50 (94%)	47/50 (94%)	48/50 (96%)	47/50 (94%)	50/50 (100%)	50/50 (100%)
Adjusted rate	96.1%	96.0%	96.8%	96.9%	100.0%	100.0%
Terminal rate	32/33 (97%)	21/22 (96%)	27/28 (96%)	22/22 (100%)	29/29 (100%)	0/0
First incidence (days)	393	328	437	252	451	350
Poly-3 test	P=0.080	P=0.710N	P=0.658	P=0.645	P=0.222	P=0.222

(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, liver, lung, pancreatic islets, 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 dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

Table B4a
Historical Incidence of Liver Neoplasms in Control Female F344/N Rats

Study	Incidence in Controls			
	Hemangiosarcoma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]				
Citral (feed)	0/100	0/100	0/100	0/100
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50	0/50	0/50
Indium phosphide (inhalation)	0/50	0/50	0/50	0/50
60-Hz Magnetic fields (whole body exposure)	0/100	0/100	0/100	0/100
Methacrylonitrile (gavage)	0/50	1/50	0/50	1/50
Naphthalene (inhalation)	0/49	0/49	0/49	0/49
o-Nitrotoluene (feed)	0/60	1/60	0/60	1/60
p-Nitrotoluene (feed)	0/50	0/50	0/50	0/50
Riddelliine (gavage)	0/50	1/50	0/50	1/50
Sodium nitrite (drinking water)	0/50	0/50	0/50	0/50
Vanadium pentoxide (inhalation)	0/50	1/50	0/50	1/50
Overall Historical Incidence in Controls Given NTP-2000 Diet				
Total (%)	0/659	4/659 (0.6%)	0/659	4/659 (0.6%)
Mean ± standard deviation		0.7% ± 1.0%		0.7% ± 1.0%
Range		0%-2%		0%-2%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]				
Total (%)	0/50	1/50 (2.0%)	0/50	1/50 (2.0%)

[a] Data as of January 17, 2001
[b] Data as of December 21, 1999

Table B4b
Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/N Rats

Study	Incidence in Controls
Historical Incidence in Controls Given NTP-2000 Diet[a]	
Citral (feed)	24/100
p,p′-Dichlorodiphenyl sulfone (feed)	8/50
Indium phosphide (inhalation)	14/50
60-Hz Magnetic fields (whole body exposure)	20/100
Methacrylonitrile (gavage)	21/50
Naphthalene (inhalation)	16/49
o-Nitrotoluene (feed)	21/60
p-Nitrotoluene (feed)	13/50
Riddelliine (gavage)	12/50
Sodium nitrite (drinking water)	15/50
Vanadium pentoxide (inhalation)	21/50
Overall Historical Incidence in Controls Given NTP-2000 Diet	
Total (%)	185/659 (28.1%)
Mean ± standard deviation	29.1% ± 8.4%
Range	16%-42%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]	
Total (%)	20/50 (40.0%)

[a] Data as of January 17, 2001; includes data for lymphocytic, monocytic, and undifferentiated leukemia
[b] Data as of December 21, 1999; includes data for lymphocytic, monocytic, and undifferentiated leukemia

Table B4c
Historical Incidence of Thyroid Gland (C-cell) Neoplasms in Control Female F344/N Rats

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Citral (feed)			
Untreated controls	8/50	0/50	8/50
Vehicle controls	13/50	0/50	13/50
p,p′-Dichlorodiphenyl sulfone (feed)	5/50	2/50	7/50
Indium phosphide (inhalation)	6/47	3/47	8/47
60-Hz Magnetic fields (whole body exposure)	15/100	5/100	19/100
Methacrylonitrile (gavage)	3/50	2/50	5/50
Naphthalene (inhalation)	4/47	3/47	7/47
o-Nitrotoluene (feed)	9/60	1/60	10/60
p-Nitrotoluene (feed)	6/50	0/50	6/50
Riddelliine (gavage)	2/49	1/49	3/49
Sodium nitrite (drinking water)	7/50	2/50	9/50
Vanadium pentoxide (inhalation)	2/50	1/50	3/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	80/653 (12.3%)	20/653 (3.1%)	98/653 (15.0%)
Mean ± standard deviation	11.1% ± 5.2%	3.2% ± 2.3%	14.1% ± 5.0%
Range	4%-21%	0%-6%	6%-26%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]			
Total (%)	3/50 (6.0%)	2/50 (4.0%)	5/50 (10.0%)

[a] Data as of January 17, 2001
[b] Data as of December 21, 1999

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Disposition Summary						
Animals initially in study	50	50	50	50	50	50
Early deaths						
Accidental deaths	2	6	3	2	3	
Moribund	9	11	13	14	16	19
Natural deaths	6	11	6	12	2	31
Survivors						
Terminal sacrifice	33	22	28	22	29	
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Esophagus	(48)	(50)	(48)	(50)	(49)	(50)
Inflammation, chronic		2 (4%)				
Perforation			1 (2%)			
Intestine large, colon	(49)	(50)	(50)	(47)	(50)	(50)
Diverticulum	1 (2%)					
Intestine large, cecum	(50)	(50)	(48)	(47)	(50)	(50)
Edema					1 (2%)	
Liver	(50)	(50)	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	1 (2%)	2 (4%)		5 (10%)	1 (2%)
Basophilic focus	45 (90%)	46 (92%)	44 (88%)	42 (84%)	40 (80%)	20 (40%)
Clear cell focus	9 (18%)	8 (16%)	9 (18%)	13 (26%)	22 (44%)	2 (4%)
Cyst	1 (2%)					
Degeneration, cystic			1 (2%)		1 (2%)	
Eosinophilic focus	1 (2%)	2 (4%)	6 (12%)	4 (8%)	12 (24%)	13 (26%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	4 (8%)		1 (2%)	3 (6%)
Hemorrhage			2 (4%)		1 (2%)	7 (14%)
Hepatodiaphragmatic nodule	13 (26%)	8 (16%)	9 (18%)	6 (12%)	4 (8%)	5 (10%)
Infiltration cellular, mixed cell	9 (18%)	7 (14%)	9 (18%)	8 (16%)	15 (30%)	3 (6%)
Mixed cell focus	8 (16%)	10 (20%)	10 (20%)	11 (22%)	23 (46%)	5 (10%)
Necrosis, focal	4 (8%)	2 (4%)	3 (6%)	4 (8%)	4 (8%)	15 (30%)
Bile duct, hyperplasia	2 (4%)	1 (2%)	4 (8%)	4 (8%)	3 (6%)	10 (20%)
Hepatocyte, cytomegaly			7 (14%)	23 (46%)	32 (64%)	29 (58%)
Hepatocyte, hyperplasia, atypical					1 (2%)	
Hepatocyte, hyperplasia, focal	1 (2%)					
Hepatocyte, hyperplasia, regenerative					8 (16%)	50 (100%)
Hepatocyte, vacuolization cytoplasmic	4 (8%)	5 (10%)	7 (14%)	4 (8%)	3 (6%)	5 (10%)
Kupffer cell, pigmentation		2 (4%)	3 (6%)	2 (4%)	6 (12%)	4 (8%)
Mesentery	(10)	(10)	(18)	(14)	(13)	(5)
Accessory spleen	2 (20%)		1 (6%)	2 (14%)	1 (8%)	
Fat, necrosis	8 (80%)	10 (100%)	18 (100%)	12 (86%)	9 (69%)	3 (60%)
Pancreas	(50)	(50)	(50)	(46)	(50)	(50)
Atrophy	10 (20%)	3 (6%)	9 (18%)	8 (17%)	4 (8%)	4 (8%)
Acinus, hyperplasia, focal	3 (6%)	1 (2%)			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)	(50)	(50)
Atrophy				1 (2%)		2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(49)	(49)	(50)
Ulcer			2 (4%)			
Epithelium, hyperplasia		1 (2%)	5 (10%)	1 (2%)	1 (2%)	3 (6%)

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

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Alimentary System (continued)						
Stomach, glandular	(50)	(50)	(50)	(49)	(49)	(50)
Erosion				2 (4%)	1 (2%)	9 (18%)
Hemorrhage						1 (2%)
Mineralization						1 (2%)
Ulcer				2 (4%)		7 (14%)
Tooth				(1)		
Malformation				1 (100%)		
Cardiovascular System						
Heart	(50)	(50)	(50)	(50)	(50)	(50)
Cardiomyopathy	12 (24%)	15 (30%)	8 (16%)	13 (26%)	8 (16%)	6 (12%)
Thrombosis		1 (2%)	1 (2%)			
Endocrine System						
Adrenal cortex	(50)	(50)	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	6 (12%)	2 (4%)	6 (12%)	4 (8%)	2 (4%)	7 (14%)
Atrophy					1 (2%)	
Degeneration, fatty	9 (18%)	9 (18%)	12 (24%)	8 (16%)	13 (26%)	3 (6%)
Hyperplasia, diffuse			3 (6%)	1 (2%)		
Hyperplasia, focal	4 (8%)	5 (10%)	6 (12%)	4 (8%)	9 (18%)	3 (6%)
Hypertrophy			1 (2%)	1 (2%)		
Hypertrophy, focal	9 (18%)	5 (10%)	7 (14%)	2 (4%)	4 (8%)	2 (4%)
Necrosis					1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	3 (6%)		3 (6%)	3 (6%)	
Pituitary gland	(50)	(48)	(50)	(49)	(50)	(49)
Pars distalis, angiectasis	6 (12%)	6 (13%)	8 (16%)	8 (16%)	9 (18%)	7 (14%)
Pars distalis, cyst	16 (32%)	11 (23%)	19 (38%)	16 (33%)	17 (34%)	19 (39%)
Pars distalis, hyperplasia	1 (2%)	1 (2%)				
Pars distalis, hyperplasia, focal	10 (20%)	8 (17%)	9 (18%)	11 (22%)	7 (14%)	6 (12%)
Pars intermedia, angiectasis	1 (2%)			1 (2%)	1 (2%)	
Pars intermedia, cyst	1 (2%)		1 (2%)			
Thyroid gland	(49)	(50)	(49)	(49)	(50)	(50)
Ultimobranchial cyst	1 (2%)	2 (4%)			1 (2%)	2 (4%)
C-cell, hyperplasia	22 (45%)	9 (18%)	24 (49%)	15 (31%)	20 (40%)	10 (20%)
Follicle, cyst	1 (2%)	1 (2%)	1 (2%)			
General Body System						
None						
Genital System						
Clitoral gland	(49)	(49)	(49)	(48)	(49)	(50)
Cyst	5 (10%)	2 (4%)	3 (6%)	1 (2%)	4 (8%)	3 (6%)
Hyperplasia	3 (6%)	5 (10%)	4 (8%)	2 (4%)	2 (4%)	
Inflammation, chronic	4 (8%)	5 (10%)	4 (8%)	3 (6%)	4 (8%)	
Ovary	(50)	(49)	(50)	(50)	(50)	(50)
Cyst	11 (22%)	7 (14%)	8 (16%)	9 (18%)	6 (12%)	9 (18%)
Infiltration cellular, mixed cell	1 (2%)					
Uterus	(50)	(50)	(50)	(50)	(50)	(50)
Hydrometra	5 (10%)	7 (14%)		6 (12%)	6 (12%)	6 (12%)
Hyperplasia, cystic	4 (8%)	3 (6%)	7 (14%)	5 (10%)	2 (4%)	9 (18%)
Inflammation, chronic			2 (4%)		1 (2%)	

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Hematopoietic System						
Bone marrow	(50)	(50)	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	3 (6%)	8 (16%)	7 (14%)	10 (20%)	32 (64%)
Inflammation, granulomatous			1 (2%)			
Myelofibrosis		1 (2%)		1 (2%)	1 (2%)	1 (2%)
Lymph node	(26)	(24)	(36)	(19)	(21)	(40)
Iliac, pigmentation		1 (4%)				
Mediastinal, congestion	4 (15%)	3 (13%)	3 (8%)	3 (16%)	1 (5%)	9 (23%)
Mediastinal, hemorrhage	5 (19%)	8 (33%)	9 (25%)	5 (26%)	7 (33%)	25 (63%)
Mediastinal, infiltration cellular, histiocyte	1 (4%)					
Mediastinal, pigmentation	23 (88%)	22 (92%)	32 (89%)	15 (79%)	16 (76%)	31 (78%)
Pancreatic, hemorrhage						2 (5%)
Pancreatic, hyperplasia, histiocytic		1 (4%)	1 (3%)			
Pancreatic, hyperplasia, lymphoid	1 (4%)	1 (4%)	1 (3%)		1 (5%)	
Pancreatic, pigmentation		1 (4%)	2 (6%)		4 (19%)	1 (3%)
Renal, pigmentation			2 (6%)		2 (10%)	1 (3%)
Lymph node, mandibular	(50)	(49)	(48)	(50)	(50)	(50)
Ectasia				3 (6%)		
Hemorrhage	1 (2%)	1 (2%)	1 (2%)		1 (2%)	
Hyperplasia, lymphoid	2 (4%)	3 (6%)	1 (2%)	5 (10%)	5 (10%)	
Pigmentation	5 (10%)	2 (4%)	2 (4%)	11 (22%)	3 (6%)	
Lymph node, mesenteric	(50)	(50)	(50)	(47)	(49)	(49)
Congestion				1 (2%)		1 (2%)
Hemorrhage	1 (2%)	2 (4%)	3 (6%)	4 (9%)		6 (12%)
Hyperplasia					1 (2%)	
Inflammation, granulomatous	1 (2%)					
Pigmentation	3 (6%)	1 (2%)	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Spleen	(50)	(50)	(50)	(50)	(50)	(50)
Congestion				1 (2%)	3 (6%)	7 (14%)
Fibrosis	1 (2%)	1 (2%)	2 (4%)		2 (4%)	
Hematopoietic cell proliferation	24 (48%)	33 (66%)	25 (50%)	26 (52%)	27 (54%)	34 (68%)
Hyperplasia, stromal					1 (2%)	
Necrosis				1 (2%)		
Pigmentation	37 (74%)	41 (82%)	35 (70%)	34 (68%)	35 (70%)	29 (58%)
Thymus	(48)	(48)	(46)	(48)	(50)	(49)
Cyst					1 (2%)	
Hemorrhage		2 (4%)				2 (4%)
Hyperplasia		1 (2%)				
Integumentary System						
Mammary gland	(50)	(50)	(50)	(50)	(49)	(49)
Hyperplasia	48 (96%)	46 (92%)	46 (92%)	45 (90%)	44 (90%)	43 (88%)
Skin	(50)	(50)	(49)	(50)	(50)	(50)
Cyst epithelial inclusion					1 (2%)	
Hemorrhage		1 (2%)				
Hyperkeratosis			1 (2%)			
Inflammation, chronic		1 (2%)				
Ulcer		1 (2%)				
Epidermis, hyperplasia		1 (2%)				
Subcutaneous tissue, edema				2 (4%)		

Table B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.01 mg/kg	0.033 mg/kg	0.1 mg/kg	0.33 mg/kg	1 mg/kg
Musculoskeletal System						
Bone	(50)	(50)	(50)	(50)	(50)	(50)
Cranium, osteopetrosis	10 (20%)	10 (20%)	12 (24%)	6 (12%)	7 (14%)	
Femur, osteopetrosis	12 (24%)	7 (14%)	9 (18%)	15 (30%)	14 (28%)	9 (18%)
Skeletal muscle		(1)	(1)	(2)		(6)
Hemorrhage						4 (67%)
Nervous System						
Brain	(50)	(50)	(50)	(50)	(50)	(50)
Compression	9 (18%)	11 (22%)	15 (30%)	6 (12%)	12 (24%)	2 (4%)
Hemorrhage	1 (2%)	1 (2%)		3 (6%)		
Hydrocephalus	3 (6%)	4 (8%)	8 (16%)	1 (2%)	10 (20%)	3 (6%)
Respiratory System						
Lung	(50)	(50)	(50)	(50)	(50)	(50)
Congestion	4 (8%)	7 (14%)	2 (4%)	8 (16%)	1 (2%)	1 (2%)
Edema					1 (2%)	2 (4%)
Hemorrhage	4 (8%)	7 (14%)	1 (2%)	3 (6%)	5 (10%)	19 (38%)
Infiltration cellular, histiocyte	46 (92%)	41 (82%)	48 (96%)	44 (88%)	49 (98%)	42 (84%)
Inflammation, chronic			1 (2%)			
Inflammation, granulomatous	1 (2%)					
Metaplasia, osseous		2 (4%)				
Alveolar epithelium, hyperplasia	5 (10%)	4 (8%)	10 (20%)	5 (10%)	5 (10%)	7 (14%)
Nose	(50)	(50)	(50)	(49)	(50)	(50)
Foreign body	2 (4%)		1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic	3 (6%)			2 (4%)		1 (2%)
Olfactory epithelium, degeneration			1 (2%)	1 (2%)		
Respiratory epithelium, hyperplasia	1 (2%)		1 (2%)			3 (6%)
Special Senses System						
Eye						(1)
Cataract						1 (100%)
Urinary System						
Kidney	(50)	(50)	(50)	(50)	(50)	(50)
Cyst	1 (2%)				1 (2%)	
Hydronephrosis			1 (2%)		1 (2%)	
Infarct		1 (2%)			1 (2%)	
Inflammation, chronic		1 (2%)				
Nephropathy	32 (64%)	32 (64%)	33 (66%)	30 (60%)	38 (76%)	28 (56%)
Papilla, fibrosis, focal			1 (2%)			
Pelvis, mineralization	1 (2%)					
Renal tubule, cytoplasmic alteration		1 (2%)		1 (2%)		
Renal tubule, dilatation		1 (2%)	2 (4%)		2 (4%)	1 (2%)
Renal tubule, hyaline droplet	33 (66%)	23 (46%)	30 (60%)	26 (52%)	26 (52%)	9 (18%)
Renal tubule, mineralization						1 (2%)
Renal tubule, necrosis				1 (2%)	1 (2%)	6 (12%)
Renal tubule, pigmentation	38 (76%)	28 (56%)	31 (62%)	31 (62%)	41 (82%)	23 (46%)
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)		5 (10%)
Urinary bladder	(50)	(50)	(50)	(48)	(50)	(50)
Edema		1 (2%)	1 (2%)			
Transitional epithelium, hyperplasia					1 (2%)	

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

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Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death	1				
Moribund	4	6	3	6	13
Natural deaths	6	3	7	6	17
Survivors					
Died last week of study					2
Terminal sacrifice	39	41	40	38	18
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Esophagus	(50)	(50)	(50)	(50)	(50)
Gallbladder	(44)	(43)	(36)	(41)	(38)
Histiocytic sarcoma		1 (2%)			
Intestine large, cecum	(44)	(47)	(44)	(48)	(39)
Carcinoma			1 (2%)		
Histiocytic sarcoma				1 (2%)	
Serosa, hepatoblastoma, metastatic, liver				1 (2%)	
Intestine small, duodenum	(45)	(48)	(47)	(47)	(40)
Polyp adenomatous				1 (2%)	
Intestine small, jejunum	(45)	(47)	(44)	(47)	(39)
Carcinoma			1 (2%)	2 (4%)	
Intestine small, ileum	(44)	(49)	(44)	(47)	(41)
Histiocytic sarcoma		1 (2%)			
Liver	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)		2 (4%)	14 (28%)
Hemangiosarcoma, multiple					17 (34%)
Hepatoblastoma	3 (6%)	2 (4%)		1 (2%)	
Hepatocellular carcinoma	7 (14%)	11 (22%)	10 (20%)	15 (30%)	3 (6%)
Hepatocellular carcinoma, multiple	16 (32%)	10 (20%)	9 (18%)	5 (10%)	
Hepatocellular adenoma	13 (26%)	10 (20%)	12 (24%)	5 (10%)	
Hepatocellular adenoma, multiple	3 (6%)	8 (16%)	2 (4%)		
Histiocytic sarcoma		3 (6%)	2 (4%)	3 (6%)	
Leiomyosarcoma			1 (2%)		
Sarcoma, metastatic, skeletal muscle					1 (2%)
Mesentery	(12)	(10)	(9)	(14)	(6)
Hemangiosarcoma					1 (17%)
Hemangiosarcoma, metastatic, liver	1 (8%)				
Hepatoblastoma, metastatic, liver				1 (7%)	
Hepatocellular carcinoma, metastatic, liver				2 (14%)	
Histiocytic sarcoma		2 (20%)		3 (21%)	
Leiomyosarcoma			1 (11%)		
Sarcoma, metastatic, skeletal muscle					1 (17%)
Pancreas	(49)	(50)	(50)	(50)	(48)
Hepatoblastoma, metastatic, liver				1 (2%)	
Histiocytic sarcoma		3 (6%)		1 (2%)	
Leiomyosarcoma			1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)	(49)
Leiomyosarcoma			1 (2%)		
Squamous cell papilloma	1 (2%)			1 (2%)	

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Alimentary System (continued)					
Stomach, glandular	(47)	(48)	(49)	(49)	(46)
Leiomyosarcoma			1 (2%)		
Tooth	(24)	(15)	(18)	(4)	(4)
Peridontal tissue, fibrosarcoma			1 (6%)		
Cardiovascular System					
Heart	(50)	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)	
Histiocytic sarcoma				1 (2%)	
Endocrine System					
Adrenal cortex	(49)	(49)	(50)	(50)	(49)
Adenoma			1 (2%)	1 (2%)	1 (2%)
Adenoma, multiple				1 (2%)	
Carcinoma				1 (2%)	
Hepatoblastoma, metastatic, liver				1 (2%)	
Histiocytic sarcoma		1 (2%)		1 (2%)	
Capsule, adenoma	3 (6%)	7 (14%)	3 (6%)	1 (2%)	1 (2%)
Capsule, leiomyosarcoma			1 (2%)		
Adrenal medulla	(49)	(49)	(49)	(50)	(48)
Pheochromocytoma malignant	1 (2%)				
Pheochromocytoma benign					1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)	(48)
Adenoma	3 (6%)				
Pituitary gland	(49)	(47)	(48)	(46)	(47)
Pars distalis, adenoma	1 (2%)				1 (2%)
Pars intermedia, adenoma		1 (2%)			
Thyroid gland	(49)	(50)	(49)	(50)	(50)
Follicular cell, adenoma		2 (4%)	2 (4%)	1 (2%)	
General Body System					
Tissue NOS		(2)		(1)	(1)
Genital System					
Coagulating gland				(1)	
Histiocytic sarcoma				1 (100%)	
Epididymis	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)		
Histiocytic sarcoma				1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)	(50)
Adenoma	1 (2%)				
Prostate	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)	
Testes	(50)	(50)	(50)	(50)	(50)
Interstitial cell, adenoma					2 (4%)

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)				1 (2%)
Histiocytic sarcoma				2 (4%)	
Sarcoma		1 (2%)			
Lymph node	(2)	(3)	(4)	(7)	(4)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung					1 (25%)
Bronchial, hepatoblastoma, metastatic, liver				1 (14%)	
Inguinal, histiocytic sarcoma		1 (33%)			
Mediastinal, histiocytic sarcoma		2 (67%)		1 (14%)	
Renal, histiocytic sarcoma		2 (67%)		1 (14%)	
Lymph node, mandibular	(49)	(48)	(49)	(49)	(44)
Histiocytic sarcoma				1 (2%)	
Lymph node, mesenteric	(49)	(48)	(48)	(50)	(50)
Hemangiosarcoma		1 (2%)			
Hepatoblastoma, metastatic, liver				1 (2%)	
Histiocytic sarcoma		3 (6%)		4 (8%)	
Leiomyosarcoma			1 (2%)		
Spleen	(49)	(49)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)		3 (6%)	1 (2%)	2 (4%)
Histiocytic sarcoma		2 (4%)	1 (2%)	2 (4%)	
Thymus	(42)	(46)	(45)	(43)	(43)
Histiocytic sarcoma		1 (2%)	1 (2%)		
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(49)
Subcutaneous tissue, fibrosarcoma, multiple			1 (2%)		
Subcutaneous tissue, sarcoma		1 (2%)			
Subcutaneous tissue, sarcoma, metastatic, skeletal muscle					1 (2%)
Musculoskeletal System					
Skeletal muscle				(2)	(1)
Hemangiosarcoma				1 (50%)	
Sarcoma, multiple					1 (100%)
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Meninges, histiocytic sarcoma		1 (2%)			
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	8 (16%)	9 (18%)	10 (20%)	5 (10%)	12 (24%)
Alveolar/bronchiolar adenoma, multiple	4 (8%)	1 (2%)	1 (2%)	3 (6%)	
Alveolar/bronchiolar carcinoma	3 (6%)	6 (12%)	2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma, multiple	4 (8%)	2 (4%)	4 (8%)	1 (2%)	4 (8%)
Carcinoma, metastatic, harderian gland	2 (4%)				
Carcinoma, metastatic, uncertain primary site			1 (2%)		
Fibrosarcoma, metastatic, uncertain primary site					1 (2%)
Hemangiosarcoma, metastatic, liver				1 (2%)	5 (10%)

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Respiratory System (continued)					
Lung (continued)	(50)	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)	
Hepatocellular carcinoma, metastatic, liver	6 (12%)	6 (12%)	4 (8%)	8 (16%)	
Histiocytic sarcoma		2 (4%)	1 (2%)	2 (4%)	
Sarcoma, metastatic, skeletal muscle					1 (2%)
Mediastinum, histiocytic sarcoma		1 (2%)		1 (2%)	
Nose	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)	
Special Senses System					
Harderian gland	(6)	(1)	(7)	(8)	(3)
Adenoma	4 (67%)	1 (100%)	6 (86%)	8 (100%)	2 (67%)
Carcinoma	2 (33%)		1 (14%)	1 (13%)	1 (33%)
Urinary System					
Kidney	(49)	(49)	(50)	(50)	(50)
Carcinoma					1 (2%)
Hepatoblastoma, metastatic, liver				1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)				
Histiocytic sarcoma		1 (2%)	1 (2%)	4 (8%)	
Capsule, hemangiosarcoma					1 (2%)
Renal tubule, adenoma	1 (2%)				
Urinary bladder	(50)	(50)	(50)	(50)	(50)
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma		3 (6%)	3 (6%)	4 (8%)	
Lymphoma malignant	3 (6%)	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	44	45	47	42	47
Total primary neoplasms	85	79	82	64	69
Total animals with benign neoplasms	32	27	28	23	17
Total benign neoplasms	42	39	37	27	20
Total animals with malignant neoplasms	29	31	30	33	41
Total malignant neoplasms	43	40	45	37	49
Total animals with metastatic neoplasms	8	6	5	10	7
Total metastatic neoplasms	10	6	5	20	11
Total animals with malignant neoplasms of uncertain primary site			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 C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	188	475	486	561	559	633	637	675	706	713	713	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	046	029	039	019	033	013	040	032	045	004	025	006	008	009	010	012	016	020	022	028	030	035	041	042	047
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	A	A	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatoblastoma									X														X	X	
Hepatocellular carcinoma			X	X	X		X							X							X				
Hepatocellular carcinoma, multiple						X		X	X		X	X				X								X	
Hepatocellular adenoma		X		X						X			X		X		X		X				X		
Hepatocellular adenoma, multiple												X													
Mesentery													+	+		+					+	+			
Hemangiosarcoma, metastatic, liver																									
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth									+	+				+				+	+	+		+	+		+
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																					X		X		
Adrenal medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant							X																		
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Parathyroid gland	+	M	+	+	+	M	A	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	729	729	730	730	730	730	730	730	730	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734
Carcass ID Number	048	049	001	003	014	018	026	037	038	002	011	015	031	044	005	007	017	021	023	024	027	034	036	043	050	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	45
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma											X		X													2
Hepatoblastoma																										3
Hepatocellular carcinoma			X																							7
Hepatocellular carcinoma, multiple						X					X	X	X					X			X	X	X		X	16
Hepatocellular adenoma	X				X						X			X		X										13
Hepatocellular adenoma, multiple										X							X									3
Mesentery											+	+	+						+	+	+	+				12
Hemangiosarcoma, metastatic, liver													X													1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																X										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tooth		+	+	+	+	+		+	+		+	+		+	+	+			+	+			+			24
Cardiovascular System																										
Blood vessel												+														1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Capsule, adenoma			X																							3
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma										X										X						3
Parathyroid gland	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma																X										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	188	475	486	561	559	633	637	657	706	713	713	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	046	029	039	019	033	013	040	032	045	004	025	006	008	009	010	012	016	020	022	028	030	035	041	042	047
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph node																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	M	M	M	+	I	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X													X		X	
Alveolar/bronchiolar adenoma, multiple												X			X										
Alveolar/bronchiolar carcinoma														X											
Alveolar/bronchiolar carcinoma, multiple					X					X						X									
Carcinoma, metastatic, harderian gland											X														
Hepatocellular carcinoma, metastatic, liver					X		X	X			X			X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland											+														+
Adenoma																									X
Carcinoma											X														
Zymbal's gland																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	729	729	730	730	730	730	730	730	730	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	048	049	001	003	014	018	026	037	038	002	011	015	031	044	005	007	017	021	023	024	027	034	036	043	050	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																							X			1
Lymph node			+						+																	2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																							X			1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	42
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X						X								X		X	X								8
Alveolar/bronchiolar adenoma, multiple					X							X														4
Alveolar/bronchiolar carcinoma																			X			X				3
Alveolar/bronchiolar carcinoma, multiple												X														4
Carcinoma, metastatic, harderian gland																			X							2
Hepatocellular carcinoma, metastatic, liver																					X					6
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland						+													+				+	+		6
Adenoma						X																	X	X		4
Carcinoma																			X							2
Zymbal's gland																									+	1

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	1	4	4	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	8	7	8	6	9	3	3	7	0	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	8	5	6	1	9	3	7	5	6	3	3	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	2	3	1	3	1	4	3	4	0	2	0	0	0	1	1	1	2	2	2	3	3	4	4	4	4
	6	9	9	9	3	3	0	2	5	4	5	6	8	9	0	2	6	0	2	8	0	5	1	2	7	
Urinary System																										
Kidney	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hepatocellular carcinoma, metastatic, liver					X																					
Renal tubule, adenoma														X												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymphoma malignant		X																								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	729	729	730	730	730	730	730	730	730	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	048	049	001	003	014	018	026	037	038	002	011	015	031	044	005	007	017	021	023	024	027	034	036	043	050	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic, liver																										1
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant					X				X																	3

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	434	542	566	587	596	633	634	681	696	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	088	084	079	060	058	092	086	059	097	054	056	063	066	069	073	080	085	089	090	068	070	077	081	083	091
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	A	+	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X																				
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatoblastoma																	X								
Hepatocellular carcinoma		X	X	X		X																	X		X
Hepatocellular carcinoma, multiple							X		X		X						X		X						
Hepatocellular adenoma								X		X			X								X	X		X	
Hepatocellular adenoma, multiple												X			X	X									
Histiocytic sarcoma					X			X																	
Mesentery	+						+	+										+							
Histiocytic sarcoma								X																	
Oral mucosa								+																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X			X																	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+			+						+		+			+		+		+	+			
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Histiocytic sarcoma																									
Capsule, adenoma										X			X								X			X	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pars intermedia, adenoma																					X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
Tissue NOS																									

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	052	055	057	061	072	074	075	082	096	100	051	053	062	064	065	067	071	076	078	087	093	094	095	098	099	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	M	+	+	+	+	+	I	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	43
Histiocytic sarcoma													X													1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																					X					1
Hepatoblastoma													X													2
Hepatocellular carcinoma							X								X	X				X					X	11
Hepatocellular carcinoma, multiple			X	X					X												X	X				10
Hepatocellular adenoma	X										X								X				X			10
Hepatocellular adenoma, multiple		X			X					X		X						X								8
Histiocytic sarcoma													X													3
Mesentery					+		+					+	+	+		+										10
Histiocytic sarcoma													X													2
Oral mucosa																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth	+					+							+	+	+		+				+					15
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma													X													1
Capsule, adenoma				X												X	X									7
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																			X		X					2
General Body System																										
Tissue NOS																		+			+					2

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	434	542	566	587	596	633	634	681	696	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	088	084	079	060	058	092	086	059	097	054	056	063	066	069	073	080	085	089	090	068	070	077	081	083	091
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																										
Lymph node					+			+										+							
Inguinal, histiocytic sarcoma								X																	
Mediastinal, histiocytic sarcoma					X			X																	
Renal, histiocytic sarcoma					X			X																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+
Hemangiosarcoma																										
Histiocytic sarcoma					X			X																	
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X			X																	
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meninges, histiocytic sarcoma																										
Peripheral nerve	+																									
Spinal cord	+																									
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X	X								X	X				X
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma													X												
Alveolar/bronchiolar carcinoma, multiple																X									X
Hepatocellular carcinoma, metastatic, liver						X			X								X								
Histiocytic sarcoma								X																	
Mediastinum, histiocytic sarcoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	052	055	057	061	072	074	075	082	096	010	051	053	062	064	065	067	071	076	078	087	093	094	095	098	099	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma													X													1
Lymph node																										3
Inguinal, histiocytic sarcoma																										1
Mediastinal, histiocytic sarcoma																										2
Renal, histiocytic sarcoma																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																	X									1
Histiocytic sarcoma													X													3
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										2
Thymus	I	+	+	M	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, sarcoma													X													1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meninges, histiocytic sarcoma													X													1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X			X					X		X								9
Alveolar/bronchiolar adenoma, multiple												X														1
Alveolar/bronchiolar carcinoma									X	X		X								X				X		6
Alveolar/bronchiolar carcinoma, multiple																										2
Hepatocellular carcinoma, metastatic, liver			X																		X				X	6
Histiocytic sarcoma													X													2
Mediastinum, histiocytic sarcoma													X													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	434	542	566	587	596	633	634	681	696	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	088	084	079	060	058	092	086	059	097	054	056	063	066	069	073	080	085	089	090	068	070	077	081	083	091
Special Senses System																									
Eye	+																								
Harderian gland									+																
Adenoma									X																
Urinary System																									
Kidney	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X			X																	
Lymphoma malignant							X																		

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.1 mg/kg

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	052	055	057	061	072	074	075	082	096	100	051	053	062	064	065	067	071	076	078	087	093	094	095	098	099	Total Tissues/ Tumors
Special Senses System																										
Eye																										1
Harderian gland																										1
Adenoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma													X													1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													3
Lymphoma malignant																							X			2

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.3 mg/kg

Number of Days on Study	567	592	614	619	657	700	713	720	722	728	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730
Carcass ID Number	122	102	111	137	110	142	134	106	148	139	103	105	107	113	118	120	125	130	131	135	144	145	108	117	119
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	A	A	M	+	M	A	A	+	A	+	+	+	+	I	+	+	+	+	+	+	+	+	M	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	A	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																										
Intestine small, duodenum	+	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	A	A	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																X									
Intestine small, ileum	A	A	A	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma				X	X	X				X							X						X		
Hepatocellular carcinoma, multiple		X	X				X	X										X							
Hepatocellular adenoma	X											X		X						X				X	
Hepatocellular adenoma, multiple																			X						X
Histiocytic sarcoma					X		X																		
Leiomyosarcoma			X																						
Mesentery			+			+		+			+					+			+						
Leiomyosarcoma			X																						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma			X																						
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma			X																						
Tooth		+	+				+			+				+	+		+	+			+				
Peridontal tissue, fibrosarcoma																									
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Capsule, adenoma																					X				
Capsule, leiomyosarcoma			X																						
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								X	
General Body System																									
None																									

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.3 mg/kg

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	121	123	124	143	104	109	112	115	126	128	129	136	140	147	150	101	114	116	127	132	133	138	141	146	149	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	36
Intestine large, colon	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Carcinoma			X																							1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma						X	X	X	X																	10
Hepatocellular carcinoma, multiple													X	X		X					X					9
Hepatocellular adenoma		X									X	X						X	X				X	X		12
Hepatocellular adenoma, multiple																										2
Histiocytic sarcoma																										2
Leiomyosarcoma																										1
Mesentery												+					+						+			9
Leiomyosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma																										1
Tooth	+				+						+		+				+				+	+	+	+		18
Peridontal tissue, fibrosarcoma																	X									1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Capsule, adenoma																						X			X	3
Capsule, leiomyosarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	M	M	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Follicular cell, adenoma			X																							2
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.3 mg/kg

Number of Days on Study	567	592	614	619	657	700	713	720	722	728	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730
Carcass ID Number	122	102	111	137	110	142	134	106	148	139	103	105	107	113	118	120	125	130	131	135	144	145	108	117	119
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma														X												
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+												+													
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma			X																							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X						X																		
Histiocytic sarcoma					X																					
Thymus	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X													
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma, multiple																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																										
Respiratory System																										
Larynx																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X				
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma									X																	
Alveolar/bronchiolar carcinoma, multiple	X																						X			
Carcinoma, metastatic, uncertain primary site																										
Hepatocellular carcinoma, metastatic, liver				X						X								X								
Histiocytic sarcoma					X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.3 mg/kg

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	121	123	124	143	104	109	112	115	126	128	129	136	140	147	150	101	114	116	127	132	133	138	141	146	149	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node											+										+					4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyosarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma					X																					3
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	M	+	+	I	+	+	45
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma, multiple			X																							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																						M				
Respiratory System																										
Larynx					+																					1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X	X			X					X			X	X				X	X		X			10
Alveolar/bronchiolar adenoma, multiple		X																								1
Alveolar/bronchiolar carcinoma																							X			2
Alveolar/bronchiolar carcinoma, multiple														X							X					4
Carcinoma, metastatic, uncertain primary site															X											1
Hepatocellular carcinoma, metastatic, liver									X																	4
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.3 mg/kg

Number of Days on Study	567	592	614	619	657	700	713	720	722	728	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730
Carcass ID Number	122	102	111	137	110	142	134	106	148	139	103	105	107	113	118	120	125	130	131	135	144	145	108	117	119
Special Senses System																										
Eye																							+		
Harderian gland			+													+					+				
Adenoma			X													X					X				
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X																				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X		X						X												
Lymphoma malignant																								X	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 0.3 mg/kg

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734		
Carcass ID Number	121	123	124	143	104	109	112	115	126	128	129	136	140	147	150	101	114	116	127	132	133	138	141	146	149	Total Tissues/ Tumors
Special Senses System																										
Eye																										1
Harderian gland	+				+											+				+						7
Adenoma					X											X				X						6
Carcinoma	X																									1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										3
Lymphoma malignant																										1

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	507	531	552	554	566	582	639	661	661	689	707	720	729	729	729	729	729	729	729	729	730	730	730	730	730
Carcass ID Number	159	187	197	155	178	168	157	165	194	188	156	186	151	154	158	162	172	180	184	190	198	160	164	176	191
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	A	+	+	A	A	A	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Serosa, hepatoblastoma, metastatic, liver			X																						
Intestine small, duodenum	+	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, jejunum	+	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																X								X	
Intestine small, ileum	+	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Hepatoblastoma			X																						
Hepatocellular carcinoma							X	X					X				X		X		X				
Hepatocellular carcinoma, multiple					X					X				X				X							X
Hepatocellular adenoma					X																				
Histiocytic sarcoma	X					X			X																
Mesentery		+	+			+	+		+	+		+				+	+	+					+		
Hepatoblastoma, metastatic, liver			X																						
Hepatocellular carcinoma, metastatic, liver							X			X															
Histiocytic sarcoma						X			X														X		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver			X																						
Histiocytic sarcoma																							X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																								X	
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth									+															+	
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver			X																						
Histiocytic sarcoma																							X		

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	192	196	200	152	153	166	174	175	185	189	199	161	163	167	169	170	171	173	177	179	181	182	183	193	195	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Serosa, hepatoblastoma, metastatic, liver																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Polyp adenomatous										X																1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma																										2
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma															X											2
Hepatoblastoma																										1
Hepatocellular carcinoma				X		X		X			X					X					X		X	X	X	15
Hepatocellular carcinoma, multiple																										5
Hepatocellular adenoma		X		X											X			X								5
Histiocytic sarcoma																										3
Mesentery			+					+																	+	14
Hepatoblastoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, liver																										2
Histiocytic sarcoma																										3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth												+							+							4
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Histiocytic sarcoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	507	531	552	554	566	582	639	661	661	689	707	720	729	729	729	729	729	729	729	729	730	730	730	730	730
Carcass ID Number	159	187	197	155	178	168	157	165	194	188	156	186	151	154	158	162	172	180	184	190	198	160	164	176	191
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adenoma, multiple											X														
Carcinoma														X											
Hepatoblastoma, metastatic, liver			X																						
Histiocytic sarcoma						X																			
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma	X																								
General Body System																									
Tissue NOS												+													
Genital System																									
Coagulating gland																							+		
Histiocytic sarcoma																							X		
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X					X																			
Lymph node			+									+											+		
Bronchial, hepatoblastoma, metastatic, liver			X																						
Mediastinal, histiocytic sarcoma																							X		
Renal, histiocytic sarcoma																							X		
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X		
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver			X																						
Histiocytic sarcoma	X					X			X														X		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														
Histiocytic sarcoma	X								X																
Thymus	+	+	+	+	+	M	+	+	+	+	M	+	+	M	+	I	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	192	196	200	152	153	166	174	175	185	189	199	161	163	167	169	170	171	173	177	179	181	182	183	193	195	Total Tissues/ Tumors
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																									X	1
Adenoma, multiple																										1
Carcinoma																										1
Hepatoblastoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Capsule, adenoma														X												1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Pituitary gland	+	+	I	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	46
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
Tissue NOS																										1
Genital System																										
Coagulating gland																										1
Histiocytic sarcoma																										1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymph node			+		+			+								+										7
Bronchial, hepatoblastoma, metastatic, liver																										1
Mediastinal, histiocytic sarcoma																										1
Renal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Histiocytic sarcoma																										4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										2
Thymus	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	43
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	507	531	552	554	566	682	639	661	661	689	707	720	729	729	729	729	729	729	729	729	729	730	730	730	730
Carcass ID Number	159	187	197	155	178	168	157	165	194	188	156	186	151	154	158	162	172	180	184	190	198	160	164	176	191
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+														
Hemangiosarcoma											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve												+													
Spinal cord												+													
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X						X			
Alveolar/bronchiolar adenoma, multiple										X	X									X					
Alveolar/bronchiolar carcinoma, multiple																									
Hemangiosarcoma, metastatic, liver																			X						
Hepatoblastoma, metastatic, liver			X																						
Hepatocellular carcinoma, metastatic, liver					X					X				X				X	X						
Histiocytic sarcoma	X					X																			
Mediastinum, histiocytic sarcoma																							X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland				+					+	+		+				+									
Adenoma				X					X	X		X				X									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver			X																						
Histiocytic sarcoma	X					X			X														X		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X					X			X														X		
Lymphoma malignant												X													

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 1 mg/kg

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	192	196	200	152	153	166	174	175	185	189	199	161	163	167	169	170	171	173	177	179	181	182	183	193	195	Total Tissues/ Tumors
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle			+																							2
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X										X			X								5
Alveolar/bronchiolar adenoma, multiple																										3
Alveolar/bronchiolar carcinoma, multiple																	X									1
Hemangiosarcoma, metastatic, liver																										1
Hepatoblastoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, liver																X					X			X		8
Histiocytic sarcoma																										2
Mediastinum, histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland								+											+		+					8
Adenoma								X											X		X					8
Carcinoma																			X							1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Histiocytic sarcoma																										4
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										4
Lymphoma malignant			X					X																		3

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	356	550	559	566	568	572	577	587	590	598	603	603	605	614	633	639	644	647	652	660	661	664	675	681	685
Carcass ID Number	206	208	241	237	211	226	236	201	249	229	233	234	239	225	212	203	240	221	238	214	250	204	227	213	243
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	A	+	A	+	+	+	+	+	+	+	M	A	+	A	A	+	+	+	+	+	+	A	+
Intestine large, colon	+	+	A	+	+	A	+	+	+	+	A	A	+	A	+	A	+	+	+	+	+	+	+	A	+
Intestine large, rectum	+	+	A	+	+	A	+	+	+	+	A	A	+	A	+	+	+	A	+	+	+	+	+	A	+
Intestine large, cecum	+	+	A	+	+	A	+	+	+	+	A	A	+	A	+	A	A	A	+	+	+	+	+	A	+
Intestine small, duodenum	+	+	A	+	+	A	+	+	+	+	A	A	+	A	+	A	A	A	+	+	+	+	+	A	+
Intestine small, jejunum	+	+	A	+	+	A	+	+	+	+	A	A	+	A	+	A	A	A	+	+	+	+	+	A	+
Intestine small, ileum	+	+	A	+	+	A	+	+	+	+	A	A	+	A	+	A	+	A	+	+	+	+	+	A	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X	X	X				X								X		X		X			
Hemangiosarcoma, multiple		X									X	X	X	X		X	X				X		X	X	X
Hepatocellular carcinoma									X																
Sarcoma, metastatic, skeletal muscle	X																								
Mesentery	+				+				+						+										
Hemangiosarcoma					X																				
Sarcoma, metastatic, skeletal muscle	X																								
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	A	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X																		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS															+										
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																							X		

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	705	705	716	720	727	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	734	734	734	734	734	
Carcass ID Number	209	232	244	248	202	219	222	224	228	231	245	247	210	215	242	207	216	218	230	235	205	217	220	223	246	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	A	M	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	38
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Intestine small, jejunum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma	X			X	X				X							X								X	X	14
Hemangiosarcoma, multiple		X	X			X		X				X											X			17
Hepatocellular carcinoma										X					X											3
Sarcoma, metastatic, skeletal muscle																										1
Mesentery			+				+																			6
Hemangiosarcoma																										1
Sarcoma, metastatic, skeletal muscle																										1
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Tooth						+								+				+					+			4
Cardiovascular System																										
Blood vessel													+													1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Capsule, adenoma																				X						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Pheochromocytoma benign																		X								1
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	45
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																					X					2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	356	550	559	566	568	572	577	587	590	598	603	603	605	614	633	639	644	647	652	660	661	664	675	681	685
Carcass ID Number	206	208	241	237	211	226	236	201	249	229	233	234	239	225	212	203	240	221	238	214	250	204	227	213	243
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								X	
Lymph node			+												+						+				
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung																					X				
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								X	
Thymus	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma, metastatic, skeletal muscle	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Sarcoma, multiple	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve										+															
Spinal cord										+															
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X							X	X	X				X							X		
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple								X													X				
Fibrosarcoma, metastatic, uncertain primary site	X																								
Hemangiosarcoma, metastatic, liver		X															X						X		
Sarcoma, metastatic, skeletal muscle	X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear			+																						
Eye																									
Harderian gland																			+						
Adenoma																									
Carcinoma																			X						

Table C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	705	705	716	720	727	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	734	734	734	734	734	
Carcass ID Number	209	232	244	248	202	219	222	224	228	231	245	247	210	215	242	207	216	218	230	235	205	217	220	223	246	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph node																							+			4
Bronchial, alveolar/bronchiolar, carcinoma, metastatic, lung																										1
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	I	+	+	+	+	+	+	+	+	+	44
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma														X												2
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	43
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, sarcoma, metastatic, skeletal muscle																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Sarcoma, multiple																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X					X				X		X			X									X	12
Alveolar/bronchiolar carcinoma																							X			1
Alveolar/bronchiolar carcinoma, multiple						X		X																		4
Fibrosarcoma, metastatic, uncertain primary site																										1
Hemangiosarcoma, metastatic, liver												X				X										5
Sarcoma, metastatic, skeletal muscle																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye																					+					1
Harderian gland																		+			+					3
Adenoma																		X			X					2
Carcinoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	356	550	559	566	568	572	577	587	590	598	603	603	605	614	633	639	644	647	652	660	661	664	675	681	685
Carcass ID Number	206	208	241	237	211	226	236	201	249	229	233	234	239	225	212	203	240	221	238	214	250	204	227	213	243
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Capsule, hemangiosarcoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant															X										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	705	705	716	720	727	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	734	734	734	734	734	
Carcass ID Number	209	232	244	248	202	219	222	224	228	231	245	247	210	215	242	207	216	218	230	235	205	217	220	223	246	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																X										1
Capsule, hemangiosarcoma											X															1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant													X													2

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Adrenal Cortex: Adenoma					
Overall rate[a]	3/49 (6%)	7/49 (14%)	4/50 (8%)	3/50 (6%)	2/49 (4%)
Adjusted rate[b]	6.7%	15.5%	8.4%	6.6%	5.1%
Terminal rate[c]	3/39 (8%)	7/40 (18%)	4/40 (10%)	2/38 (5%)	1/20 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	707	660
Poly-3 test[d]	P=0.207N	P=0.159	P=0.532	P=0.656N	P=0.560N
Harderian Gland: Adenoma					
Overall rate	4/50 (8%)	1/50 (2%)	6/50 (12%)	8/50 (16%)	2/50 (4%)
Adjusted rate	8.8%	2.2%	12.5%	17.2%	5.0%
Terminal rate	4/39 (10%)	0/41 (0%)	5/40 (13%)	4/38 (11%)	2/20 (10%)
First incidence (days)	729 (T)	696	614	554	729 (T)
Poly-3 test	P=0.510N	P=0.173N	P=0.405	P=0.187	P=0.402N
Harderian Gland: Adenoma or Carcinoma					
Overall rate	6/50 (12%)	1/50 (2%)	7/50 (14%)	8/50 (16%)	3/50 (6%)
Adjusted rate	13.2%	2.2%	14.6%	17.2%	7.5%
Terminal rate	5/39 (13%)	0/41 (0%)	6/40 (15%)	4/38 (11%)	2/20 (10%)
First incidence (days)	713	696	614	554	652
Poly-3 test	P=0.499N	P=0.053N	P=0.542	P=0.402	P=0.311N
Liver: Hemangiosarcoma					
Overall rate	2/50 (4%)	1/50 (2%)	0/50 (0%)	2/50 (4%)	31/50 (62%)
Adjusted rate	4.4%	2.2%	0.0%	4.4%	66.7%
Terminal rate	2/39 (5%)	1/41 (2%)	0/40 (0%)	2/38 (5%)	8/20 (40%)
First incidence (days)	729 (T)	729 (T)	—[e]	729 (T)	550
Poly-3 test	P<0.001	P=0.495N	P=0.227N	P=0.694	P<0.001
Liver: Hepatocellular Adenoma					
Overall rate	16/50 (32%)	18/50 (36%)	14/50 (28%)	5/50 (10%)	0/50 (0%)
Adjusted rate	34.2%	38.8%	29.0%	10.9%	0.0%
Terminal rate	13/39 (33%)	17/41 (42%)	13/40 (33%)	4/38 (11%)	0/20 (0%)
First incidence (days)	475	681	567	566	—
Poly-3 test	P<0.001N	P=0.400	P=0.375N	P=0.006N	P<0.001N
Liver: Hepatocellular Carcinoma					
Overall rate	23/50 (46%)	21/50 (42%)	19/50 (38%)	20/50 (40%)	3/50 (6%)
Adjusted rate	47.7%	43.2%	38.4%	42.8%	7.5%
Terminal rate	15/39 (39%)	15/41 (37%)	11/40 (28%)	16/38 (42%)	2/20 (10%)
First incidence (days)	486	542	592	566	590
Poly-3 test	P<0.001N	P=0.407N	P=0.237N	P=0.393N	P<0.001N
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	36/50 (72%)	39/50 (78%)	33/50 (66%)	23/50 (46%)	3/50 (6%)
Adjusted rate	73.4%	80.0%	66.0%	49.2%	7.5%
Terminal rate	26/39 (67%)	32/41 (78%)	24/40 (60%)	19/38 (50%)	2/20 (10%)
First incidence (days)	475	542	567	566	590
Poly-3 test	P<0.001N	P=0.299	P=0.281N	P=0.011N	P<0.001N
Liver: Hepatoblastoma					
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.6%	4.3%	0.0%	2.2%	0.0%
Terminal rate	2/39 (5%)	2/41 (5%)	0/40 (0%)	0/38 (0%)	0/20 (0%)
First incidence (days)	706	729 (T)	—	552	—
Poly-3 test	P=0.151N	P=0.494N	P=0.111N	P=0.302N	P=0.146N

Table C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma					
Overall rate	24/50 (48%)	22/50 (44%)	19/50 (38%)	21/50 (42%)	3/50 (6%)
Adjusted rate	49.8%	45.3%	38.4%	44.4%	7.5%
Terminal rate	16/39 (41%)	16/41 (39%)	11/40 (28%)	16/38 (42%)	2/20 (10%)
First incidence (days)	486	542	592	552	590
Poly-3 test	P<0.001N	P=0.407N	P=0.178N	P=0.375N	P<0.001N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma					
Overall rate	36/50 (72%)	40/50 (80%)	33/50 (66%)	24/50 (48%)	3/50 (6%)
Adjusted rate	73.4%	82.0%	66.0%	50.8%	7.5%
Terminal rate	26/39 (67%)	33/41 (81%)	24/40 (60%)	19/38 (50%)	2/20 (10%)
First incidence (days)	475	542	567	552	590
Poly-3 test	P<0.001N	P=0.218	P=0.281N	P=0.016N	P<0.001N
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	12/50 (24%)	10/50 (20%)	11/50 (22%)	8/50 (16%)	12/50 (24%)
Adjusted rate	26.3%	21.7%	23.1%	17.5%	28.5%
Terminal rate	11/39 (28%)	10/41 (24%)	11/40 (28%)	6/38 (16%)	5/20 (25%)
First incidence (days)	706	729 (T)	729 (T)	689	559
Poly-3 test	P=0.356	P=0.391N	P=0.450N	P=0.222N	P=0.505
Lung: Alveolar/bronchiolar Carcinoma					
Overall rate	7/50 (14%)	8/50 (16%)	6/50 (12%)	1/50 (2%)	5/50 (10%)
Adjusted rate	15.2%	17.3%	12.4%	2.2%	12.4%
Terminal rate	5/39 (13%)	8/41 (20%)	4/40 (10%)	1/38 (3%)	3/20 (15%)
First incidence (days)	599	729 (T)	567	729 (T)	587
Poly-3 test	P=0.289N	P=0.503	P=0.463N	P=0.031N	P=0.473N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	18/50 (36%)	16/50 (32%)	15/50 (30%)	9/50 (18%)	17/50 (34%)
Adjusted rate	39.1%	34.7%	31.1%	19.7%	39.7%
Terminal rate	15/39 (39%)	16/41 (39%)	13/40 (33%)	7/38 (18%)	8/20 (40%)
First incidence (days)	599	729 (T)	567	689	559
Poly-3 test	P=0.424	P=0.413N	P=0.276N	P=0.033N	P=0.564
Pancreatic Islets: Adenoma					
Overall rate	3/49 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/48 (0%)
Adjusted rate	6.6%	0.0%	0.0%	0.0%	0.0%
Terminal rate	2/39 (5%)	0/41 (0%)	0/40 (0%)	0/38 (0%)	0/20 (0%)
First incidence (days)	637	—	—	—	—
Poly-3 test	P=0.202N	P=0.116N	P=0.111N	P=0.119N	P=0.153N
Spleen: Hemangiosarcoma					
Overall rate	1/49 (2%)	0/49 (0%)	3/50 (6%)	1/50 (2%)	2/49 (4%)
Adjusted rate	2.2%	0.0%	6.2%	2.2%	5.1%
Terminal rate	1/39 (3%)	0/41 (0%)	1/40 (3%)	0/38 (0%)	1/20 (5%)
First incidence (days)	729 (T)	—	592	707	681
Poly-3 test	P=0.339	P=0.498N	P=0.329	P=0.759N	P=0.452
All Organs: Hemangiosarcoma					
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	3/50 (6%)	33/50 (66%)
Adjusted rate	6.6%	4.3%	8.3%	6.6%	71.0%
Terminal rate	3/39 (8%)	2/41 (5%)	2/40 (5%)	2/38 (5%)	10/20 (50%)
First incidence (days)	729 (T)	729 (T)	592	707	550
Poly-3 test	P<0.001	P=0.493N	P=0.531	P=0.662N	P<0.001

Table C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3.0 mg/kg
All Organs: Histiocytic Sarcoma					
Overall rate	0/50 (0%)	3/50 (6%)	3/50 (6%)	4/50 (8%)	0/50 (0%)
Adjusted rate	0.0%	6.4%	6.3%	8.5%	0.0%
Terminal rate	0/39 (0%)	1/41 (2%)	1/40 (3%)	1/38 (3%)	0/20 (0%)
First incidence (days)	—	596	657	507	—
Poly-3 test	P=0.302N	P=0.124	P=0.129	P=0.064	—[f]
All Organs: Malignant Lymphoma					
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	6.5%	4.3%	2.1%	6.6%	5.0%
Terminal rate	2/39 (5%)	1/41 (2%)	1/40 (3%)	2/38 (5%)	1/20 (5%)
First incidence (days)	475	634	729 (T)	720	633
Poly-3 test	P=0.540	P=0.497N	P=0.294N	P=0.655	P=0.565N
All Organs: Benign Neoplasms					
Overall rate	32/50 (64%)	27/50 (54%)	28/50 (56%)	23/50 (46%)	17/50 (34%)
Adjusted rate	67.7%	58.1%	57.6%	48.2%	39.6%
Terminal rate	27/39 (69%)	25/41 (61%)	26/40 (65%)	16/38 (42%)	8/20 (40%)
First incidence (days)	475	681	567	507	559
Poly-3 test	P=0.006N	P=0.222N	P=0.205N	P=0.039N	P=0.004N
All Organs: Malignant Neoplasms					
Overall rate	29/50 (58%)	31/50 (62%)	31/50 (62%)	33/50 (66%)	41/50 (82%)
Adjusted rate	59.2%	63.0%	62.0%	67.6%	83.8%
Terminal rate	19/39 (49%)	23/41 (56%)	21/40 (53%)	23/38 (61%)	13/20 (65%)
First incidence (days)	475	542	567	507	356
Poly-3 test	P=0.003	P=0.428	P=0.467	P=0.257	P=0.005
All Organs: Benign or Malignant Neoplasms					
Overall rate	44/50 (88%)	45/50 (90%)	47/50 (94%)	42/50 (84%)	47/50 (94%)
Adjusted rate	89.8%	91.4%	94.0%	85.0%	94.0%
Terminal rate	34/39 (87%)	37/41 (90%)	37/40 (93%)	31/38 (82%)	17/20 (85%)
First incidence (days)	475	542	567	507	356
Poly-3 test	P=0.396	P=0.524	P=0.344	P=0.344N	P=0.344

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, and spleen; for other tissues, denominator is number of animals necropsied.
[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 dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

Table C4
Historical Incidence of Liver Neoplasms in Control Male B6C3F$_1$ Mice

Study	Incidence in Controls				
	Hemangiosarcoma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma	Hepatoblastoma
Historical Incidence in Controls Given NTP-2000 Diet[a]					
Acrylonitrile (gavage)	2/50	23/50	14/50	32/50	0/50
Citral (feed)	2/100	20/100	13/100	28/100	0/100
p,p′-Dichlorodiphenyl sulfone (feed)	2/50	6/50	9/50	15/50	0/50
Indium phosphide (inhalation)	2/50	17/50	11/50	26/50	0/50
60-Hz Magnetic fields (whole body exposure)	4/100	30/100	19/100	46/100	2/100
Methacrylonitrile (gavage)	1/49	17/49	13/49	24/49	1/49
o-Nitrotoluene (feed)	1/60	18/60	12/60	27/60	1/60
p-Nitrotoluene (feed)	1/50	14/50	8/50	20/50	0/50
Riddelliine (gavage)	2/50	16/50	23/50	36/50	3/50
Sodium nitrite (drinking water)	2/50	19/50	9/50	24/50	5/50
Vanadium pentoxide (inhalation)	1/50	15/50	14/50	26/50	0/50
Overall Historical Incidence in Controls Given NTP-2000 Diet					
Total (%)	20/659 (3.0%)	195/659 (29.6%)	145/659 (22.0%)	304/659 (46.1%)	12/659 (1.8%)
Mean ± standard deviation	3.1% ± 1.1%	30.4% ± 8.9%	23.1% ± 9.0%	47.8% ± 12.9%	2.0% ± 3.2%
Range	2%-4%	12%-46%	13%-46%	28%-72%	0%-10%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]					
Total (%)	0/50	26/50 (52.0%)	6/50 (12.0%)	30/50 (60.0%)	0/50

[a] Data as of December 20, 2000
[b] Data as of December 23, 1999

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death	1				
Moribund	4	6	3	6	13
Natural deaths	6	3	7	6	17
Survivors					
Died last week of study					2
Terminal sacrifice	39	41	40	38	18
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Gallbladder	(44)	(43)	(36)	(41)	(38)
Cyst	1 (2%)				
Intestine large, cecum	(44)	(47)	(44)	(48)	(39)
Diverticulum		1 (2%)			
Edema		1 (2%)	1 (2%)		1 (3%)
Hemorrhage, focal					1 (3%)
Intestine small, duodenum	(45)	(48)	(47)	(47)	(40)
Artery, inflammation, chronic					1 (3%)
Intestine small, jejunum	(45)	(47)	(44)	(47)	(39)
Inflammation, focal			1 (2%)		
Peyer's patch, hyperplasia, lymphoid			1 (2%)		
Intestine small, ileum	(44)	(49)	(44)	(47)	(41)
Inflammation		1 (2%)			
Peyer's patch, hyperplasia, lymphoid	1 (2%)	2 (4%)			
Liver	(50)	(50)	(50)	(50)	(50)
Angiectasis				6 (12%)	3 (6%)
Atrophy, focal	1 (2%)	1 (2%)			
Clear cell focus	7 (14%)	5 (10%)	4 (8%)	1 (2%)	
Congestion			1 (2%)		
Congestion, focal		3 (6%)	2 (4%)	4 (8%)	
Cyst	1 (2%)				
Eosinophilic focus	6 (12%)	6 (12%)	2 (4%)	1 (2%)	
Fibrosis, focal	1 (2%)				5 (10%)
Hematopoietic cell proliferation				2 (4%)	
Hemorrhage, focal		2 (4%)	1 (2%)	6 (12%)	21 (42%)
Infarct	1 (2%)	2 (4%)	3 (6%)		
Infiltration cellular, mixed cell	33 (66%)	25 (50%)	14 (28%)	14 (28%)	32 (64%)
Inflammation, chronic				1 (2%)	
Inflammation, chronic, focal, suppurative		1 (2%)			
Karyomegaly			1 (2%)		
Metaplasia, focal, lipocyte				1 (2%)	
Mixed cell focus	3 (6%)				
Thrombosis				1 (2%)	5 (10%)
Bile duct, hyperplasia	2 (4%)		1 (2%)	3 (6%)	6 (12%)
Endothelial cell, hyperplasia				1 (2%)	1 (2%)
Endothelial cell, hyperplasia, focal				2 (4%)	

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

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Alimentary System (continued)					
Liver (continued)	(50)	(50)	(50)	(50)	(50)
Hepatocyte, basophilic focus		1 (2%)			
Hepatocyte, cytomegaly	4 (8%)	4 (8%)	16 (32%)	33 (66%)	43 (86%)
Hepatocyte, eosinophilic focus	2 (4%)	1 (2%)	1 (2%)		
Hepatocyte, karyomegaly	4 (8%)	4 (8%)	15 (30%)	33 (66%)	43 (86%)
Hepatocyte, mixed cell focus			1 (2%)		
Hepatocyte, necrosis, focal	18 (36%)	9 (18%)	5 (10%)	6 (12%)	21 (42%)
Hepatocyte, syncytial alteration, focal	38 (76%)	30 (60%)	31 (62%)	27 (54%)	
Hepatocyte, vacuolization cytoplasmic, diffuse	3 (6%)				
Hepatocyte, vacuolization cytoplasmic, focal	18 (36%)	7 (14%)	3 (6%)	5 (10%)	1 (2%)
Hepatocyte, periportal, vacuolization cytoplasmic			1 (2%)		1 (2%)
Hepatocyte, centrilobular, necrosis		1 (2%)	3 (6%)	4 (8%)	10 (20%)
Hepatocyte, centrilobular, vacuolization cytoplasmic				1 (2%)	3 (6%)
Hepatocyte, midzonal, vacuolization cytoplasmic	8 (16%)	14 (28%)	8 (16%)	2 (4%)	
Serosa, fibrosis					1 (2%)
Sinusoid, dilatation, diffuse				1 (2%)	
Mesentery	(12)	(10)	(9)	(14)	(6)
Angiectasis	1 (8%)	1 (10%)			
Inflammation, chronic			1 (11%)		
Artery, inflammation, chronic, focal	2 (17%)	1 (10%)		2 (14%)	1 (17%)
Fat, necrosis	9 (75%)	7 (70%)	8 (89%)	3 (21%)	2 (33%)
Pancreas	(49)	(50)	(50)	(50)	(48)
Fibrosis, focal	1 (2%)				
Necrosis, focal	1 (2%)				
Acinus, atrophy, focal					1 (2%)
Stomach, forestomach	(49)	(50)	(50)	(50)	(49)
Diverticulum			1 (2%)		
Erosion	1 (2%)	1 (2%)			
Inflammation, focal	2 (4%)	1 (2%)	2 (4%)		
Ulcer	1 (2%)		1 (2%)		
Epithelium, hyperplasia	2 (4%)	2 (4%)	2 (4%)		
Stomach, glandular	(47)	(48)	(49)	(49)	(46)
Pigmentation, focal	1 (2%)			1 (2%)	
Glands, degeneration, cystic, focal			1 (2%)	1 (2%)	
Tongue		(1)			
Epithelium, hyperplasia, focal		1			
Tooth	(24)	(15)	(18)	(4)	(4)
Malformation	13 (54%)	3 (20%)	8 (44%)		2 (50%)
Peridontal tissue, inflammation, chronic	16 (67%)	12 (80%)	12 (67%)	4 (100%)	3 (75%)
Cardiovascular System					
Blood vessel	(1)				(1)
Inflammation, chronic	1 (100%)				
Heart	(50)	(50)	(50)	(50)	(50)
Infiltration cellular, mixed cell	2 (4%)	3 (6%)	3 (6%)	3 (6%)	3 (6%)
Inflammation, chronic, focal			1 (2%)		
Mineralization, focal				1 (2%)	4 (8%)
Artery, inflammation, chronic		2 (4%)	1 (2%)	2 (4%)	

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Endocrine System					
Adrenal cortex	(49)	(49)	(50)	(50)	(49)
Accessory adrenal cortical nodule		3 (6%)	1 (2%)	1 (2%)	2 (4%)
Angiectasis		1 (2%)	1 (2%)		
Cyst		2 (4%)	1 (2%)		
Cytoplasmic alteration				2 (4%)	
Cytoplasmic alteration, focal	18 (37%)	7 (14%)	8 (16%)	12 (24%)	18 (37%)
Fibrosis					1 (2%)
Hemorrhage				1 (2%)	
Hyperplasia	1 (2%)				
Hyperplasia, focal	1 (2%)		1 (2%)		
Hypertrophy				2 (4%)	
Inflammation, chronic, focal					1 (2%)
Bilateral, capsule, fibrosis				1 (2%)	
Capsule, fibrosis, focal			1 (2%)		
Capsule, hyperplasia, focal	5 (10%)	7 (14%)	6 (12%)	1 (2%)	
Parathyroid gland	(41)	(50)	(44)	(47)	(45)
Angiectasis				1 (2%)	
Cyst	1 (2%)			1 (2%)	2 (4%)
Pituitary gland	(49)	(47)	(48)	(46)	(47)
Pars distalis, cyst	5 (10%)	4 (9%)	2 (4%)	2 (4%)	3 (6%)
Pars distalis, cytoplasmic alteration, focal	2 (4%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Pars distalis, hyperplasia, focal			2 (4%)		
Pars intermedia, cyst			1 (2%)		
Thyroid gland	(49)	(50)	(49)	(50)	(50)
Degeneration, cystic, focal	8 (16%)	8 (16%)	12 (24%)	7 (14%)	7 (14%)
Follicle, cyst			1 (2%)		
Follicular cell, hyperplasia	1 (2%)				
General Body System					
Tissue NOS		(2)		(1)	(1)
Pelvic, hemorrhage		1 (50%)			
Genital System					
Epididymis	(50)	(50)	(50)	(50)	(50)
Inflammation, chronic					1 (2%)
Preputial gland	(50)	(50)	(50)	(50)	(50)
Atrophy	1 (2%)				
Degeneration, cystic	19 (38%)	19 (38%)	16 (32%)	21 (42%)	19 (38%)
Inflammation, chronic		2 (4%)	1 (2%)	2 (4%)	1 (2%)
Prostate	(50)	(50)	(50)	(50)	(50)
Inflammation, chronic			2 (4%)		
Epithelium, hyperplasia, focal	1 (2%)	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)	(50)
Dilatation	15 (30%)	13 (26%)	8 (16%)	2 (4%)	1 (2%)
Inflammation, chronic	2 (4%)				
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)			1 (2%)
Lymph node	(2)	(3)	(4)	(7)	(4)
Bronchial, hyperplasia, lymphoid					1 (25%)
Bronchial, hyperplasia, plasma cell			1 (25%)		
Iliac, hemorrhage					1 (25%)

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Hematopoietic System (continued)					
Lymph node (continued)	(2)	(3)	(4)	(7)	(4)
Inguinal, inflammation, chronic, focal			1 (25%)		
Mediastinal, hyperplasia, lymphoid				1 (14%)	
Mediastinal, hyperplasia, plasma cell			1 (25%)		
Pancreatic, hyperplasia, lymphoid	1 (50%)			1 (14%)	
Pancreatic, hyperplasia, plasma cell	1 (50%)				
Lymph node, mandibular	(49)	(48)	(49)	(49)	(44)
Hemorrhage			1 (2%)		
Hyperplasia, lymphoid		1 (2%)	1 (2%)		1 (2%)
Hyperplasia, plasma cell		1 (2%)	1 (2%)	2 (4%)	
Lymph node, mesenteric	(49)	(48)	(48)	(50)	(50)
Congestion			1 (2%)		
Hematopoietic cell proliferation					2 (4%)
Hemorrhage			1 (2%)		2 (4%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	1 (2%)	2 (4%)	4 (8%)
Hyperplasia, plasma cell	2 (4%)	3 (6%)	4 (8%)	1 (2%)	
Infiltration cellular, polymorphonuclear			1 (2%)		
Inflammation, chronic					1 (2%)
Inflammation, focal, suppurative			1 (2%)		
Inflammation, granulomatous					1 (2%)
Spleen	(49)	(49)	(50)	(50)	(49)
Angiectasis					1 (2%)
Hematopoietic cell proliferation	18 (37%)	16 (33%)	19 (38%)	20 (40%)	33 (67%)
Hyperplasia, lymphoid		2 (4%)	3 (6%)	3 (6%)	
Hyperplasia, plasma cell	1 (2%)				
Necrosis, focal					1 (2%)
Artery, inflammation, chronic, focal				1 (2%)	
Thymus	(42)	(46)	(45)	(43)	(43)
Cyst	6 (14%)	5 (11%)	4 (9%)	1 (2%)	3 (7%)
Hyperplasia, lymphoid		1 (2%)			
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(49)
Cyst epithelial inclusion		1 (2%)			
Ulcer				1 (2%)	
Subcutaneous tissue, edema			2 (4%)	5 (10%)	25 (51%)
Subcutaneous tissue, lip, hyperplasia, lymphoid		1 (2%)			
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Cranium, hyperostosis	1 (2%)				
Vertebra, hyperostosis, focal	37 (74%)	36 (72%)	37 (74%)	24 (48%)	27 (54%)
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Angiectasis, focal			1 (2%)		
Atrophy, focal					1 (2%)
Hemorrhage, focal				1 (2%)	
Pigmentation, focal			1 (2%)		

Table C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(50)
Congestion		1 (2%)	1 (2%)	2 (4%)	2 (4%)
Hemorrhage		1 (2%)		8 (16%)	3 (6%)
Hyperplasia, focal, histiocytic	1 (2%)			1 (2%)	
Hyperplasia, histiocytic	1 (2%)		4 (8%)	1 (2%)	
Infiltration cellular, mixed cell			1 (2%)		4 (8%)
Metaplasia, focal, osseous				1 (2%)	
Pigmentation, focal				2 (4%)	
Thrombosis					1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	3 (6%)	3 (6%)	6 (12%)	3 (6%)
Interstitium, edema	1 (2%)				
Mediastinum, inflammation, chronic	1 (2%)				
Nose	(50)	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	1 (2%)		
Sinus, foreign body		1 (2%)	1 (2%)		
Sinus, inflammation, chronic, suppurative			1 (2%)		
Special Senses System					
Ear					(1)
Hemorrhage, focal					1 (100%)
Zymbal's gland	(1)				
Cyst, squamous	1 (100%)				
Urinary System					
Kidney	(49)	(49)	(50)	(50)	(50)
Atrophy, focal			1 (2%)		
Congestion			5 (10%)	3 (6%)	1 (2%)
Cyst	8 (16%)	4 (8%)	6 (12%)	7 (14%)	4 (8%)
Cyst, multiple					1 (2%)
Hyperplasia, lymphoid			1 (2%)		
Infarct		1 (2%)			
Inflammation, chronic, focal	1 (2%)		1 (2%)		
Metaplasia, focal, osseous	3 (6%)	3 (6%)		10 (20%)	4 (8%)
Nephropathy	46 (94%)	48 (98%)	48 (96%)	50 (100%)	50 (100%)
Artery, inflammation, chronic			2 (4%)		2 (4%)
Capsule, fibrosis					1 (2%)
Glomerulus, glomerulosclerosis		1 (2%)		42 (84%)	41 (82%)
Papilla, necrosis				1 (2%)	
Pelvis, dilatation		1 (2%)	1 (2%)		1 (2%)
Renal tubule, accumulation, hyaline droplet		2 (4%)	1 (2%)	1 (2%)	3 (6%)
Renal tubule, dilatation	16 (33%)	17 (35%)	24 (48%)	29 (58%)	22 (44%)
Renal tubule, karyomegaly					12 (24%)
Renal tubule, necrosis, focal				1 (2%)	
Renal tubule, pigmentation	2 (4%)		1 (2%)		2 (4%)
Renal tubule, vacuolization cytoplasmic		1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)	(50)
Calculus microscopic observation only			1 (2%)		

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

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Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	3 mg/kg
Disposition Summary		
Animals initially in study	50	50
Early deaths		
Accidental deaths	4	
Moribund	9	25
Natural deaths	3	8
Survivors		
Died last week of study		1
Terminal sacrifice	34	16
Animals examined microscopically	50	50
Alimentary System		
Gallbladder	(45)	(45)
Histiocytic sarcoma	1 (2%)	
Sarcoma	1 (2%)	
Intestine large, colon	(49)	(47)
Intestine large, cecum	(48)	(47)
Histiocytic sarcoma	1 (2%)	1 (2%)
Intestine small, jejunum	(43)	(45)
Histiocytic sarcoma	1 (2%)	
Intestine small, ileum	(46)	(46)
Liver	(49)	(50)
Cholangioma		1 (2%)
Hemangiosarcoma		1 (2%)
Hepatocellular carcinoma	7 (14%)	
Hepatocellular carcinoma, multiple	1 (2%)	
Hepatocellular adenoma	7 (14%)	
Hepatocellular adenoma, multiple	2 (4%)	
Histiocytic sarcoma	1 (2%)	2 (4%)
Sarcoma, multiple	1 (2%)	
Mesentery	(23)	(29)
Carcinoma, metastatic, kidney		1 (3%)
Hemangioma		1 (3%)
Histiocytic sarcoma	1 (4%)	1 (3%)
Sarcoma		1 (3%)
Sarcoma, multiple	1 (4%)	
Pancreas	(49)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)
Sarcoma, multiple	1 (2%)	
Salivary glands	(50)	(50)
Stomach, forestomach	(49)	(49)
Stomach, glandular	(49)	(48)
Histiocytic sarcoma		1 (2%)
Cardiovascular System		
Heart	(50)	(50)

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Endocrine System		
Adrenal cortex	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)
Adrenal medulla	(49)	(49)
Pituitary gland	(48)	(49)
Adenoma		1 (2%)
Pars distalis, adenoma	5 (10%)	1 (2%)
Thyroid gland	(49)	(50)
General Body System		
None		
Genital System		
Clitoral gland	(49)	(49)
Duct, carcinoma	1 (2%)	
Ovary	(49)	(48)
Cystadenoma	1 (2%)	
Granulosa cell tumor benign		1 (2%)
Luteoma		1 (2%)
Uterus	(49)	(50)
Hemangioma		1 (2%)
Histiocytic sarcoma		1 (2%)
Leiomyosarcoma		1 (2%)
Endometrium, adenoma	1 (2%)	
Endometrium, polyp stromal		1 (2%)
Vagina		(2)
Sarcoma		1 (50%)
Hematopoietic System		
Bone marrow	(50)	(50)
Carcinoma, metastatic, kidney		1 (2%)
Histiocytic sarcoma		1 (2%)
Lymph node	(7)	(15)
Iliac, histiocytic sarcoma	1 (14%)	
Mediastinal, histiocytic sarcoma	1 (14%)	1 (7%)
Pancreatic, histiocytic sarcoma	1 (14%)	
Pancreatic, sarcoma	1 (14%)	
Renal, histiocytic sarcoma	1 (14%)	
Lymph node, mandibular	(48)	(47)
Histiocytic sarcoma	1 (2%)	
Lymph node, mesenteric	(48)	(49)
Histiocytic sarcoma	1 (2%)	1 (2%)
Spleen	(49)	(50)
Hemangiosarcoma		1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)
Plasma cell tumor malignant		1 (2%)
Thymus	(49)	(39)
Histiocytic sarcoma		1 (3%)

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Integumentary System		
Mammary gland	(49)	(50)
Adenoma		1 (2%)
Carcinoma		2 (4%)
Skin	(50)	(50)
Subcutaneous tissue, sarcoma	3 (6%)	2 (4%)
Subcutaneous tissue, sarcoma, multiple		1 (2%)
Musculoskeletal System		
Bone	(50)	(50)
Cranium, carcinoma, metastatic, harderian gland	1 (2%)	
Skeletal muscle	(1)	(3)
Histiocytic sarcoma		1 (33%)
Sarcoma, multiple	1 (100%)	
Nervous System		
Brain	(50)	(50)
Respiratory System		
Lung	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	9 (18%)
Alveolar/bronchiolar carcinoma		3 (6%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)	
Histiocytic sarcoma	1 (2%)	1 (2%)
Sarcoma, metastatic, skin		1 (2%)
Sarcoma, metastatic, uncertain primary site	1 (2%)	
Mediastinum, carcinoma, metastatic, kidney		1 (2%)
Nose	(50)	(50)
Special Senses System		
Harderian gland	(4)	(5)
Adenoma	2 (50%)	3 (60%)
Carcinoma	2 (50%)	1 (20%)
Urinary System		
Kidney	(49)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)
Histiocytic sarcoma	1 (2%)	2 (4%)
Osteosarcoma		1 (2%)
Ureter		(1)
Carcinoma, metastatic, kidney		1 (100%)
Urinary bladder	(50)	(48)
Sarcoma	1 (2%)	

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Systemic Lesions		
Multiple organs[b]	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)
Lymphoma malignant	7 (14%)	9 (18%)
Neoplasm Summary		
Total animals with primary neoplasms[c]	31	33
Total primary neoplasms	49	49
Total animals with benign neoplasms	15	17
Total benign neoplasms	19	21
Total animals with malignant neoplasms	21	20
Total malignant neoplasms	30	28
Total animals with metastatic neoplasms	2	3
Total metastatic neoplasms	3	6
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
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	027	419	475	482	524	545	544	545	545	546	577	557	575	696	709	725	735	735	733	735	735	735	735	735	735
Carcass ID Number	261	260	252	281	251	282	283	284	285	265	276	257	272	256	274	264	253	254	255	258	259	262	263	266	267
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Sarcoma											X														
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Intestine small, duodenum	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	M	A	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Intestine small, ileum	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Liver	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma													X		X										
Hepatocellular carcinoma, multiple																							X		
Hepatocellular adenoma		X									X					X								X	
Hepatocellular adenoma, multiple																			X						
Histiocytic sarcoma													X												
Sarcoma, multiple											X														
Mesentery		+	+				+	+			+		+		+	+	+				+		+		+
Histiocytic sarcoma													X												
Sarcoma, multiple											X														
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Sarcoma, multiple											X														
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+											+		+									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
Pars distalis, adenoma																X									
Thyroid gland	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	268	269	270	271	273	275	277	278	279	280	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Histiocytic sarcoma																										1
Sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	43
Histiocytic sarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma			X	X				X	X							X										7
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma													X	X	X											7
Hepatocellular adenoma, multiple																X										2
Histiocytic sarcoma																										1
Sarcoma, multiple																										1
Mesentery		+				+			+			+	+	+		+		+			+		+	+		23
Histiocytic sarcoma																										1
Sarcoma, multiple																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Sarcoma, multiple																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth	+				+															+	+					7
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma		X															X		X						X	5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	027	419	475	482	524	545	545	545	545	566	577	657	675	696	709	725	735	735	733	733	735	735	735	735	735
Carcass ID Number	261	260	252	281	251	282	283	284	285	265	276	257	272	256	274	264	253	254	255	258	259	262	263	266	267
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Duct, carcinoma				X																					
Ovary	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Uterus	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, adenoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+									+	+		+			+									
Iliac, histiocytic sarcoma													X												
Mediastinal, histiocytic sarcoma													X												
Pancreatic, histiocytic sarcoma													X												
Pancreatic, sarcoma											X														
Renal, histiocytic sarcoma													X												
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Lymph node, mesenteric	+	+	A	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma			X								X			X											
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, carcinoma, metastatic, harderian gland																									
Skeletal muscle											+														
Sarcoma, multiple											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma, multiple		X																							
Carcinoma, metastatic, harderian gland																									
Histiocytic sarcoma													X												
Sarcoma, metastatic, uncertain primary site											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	268	269	270	271	273	275	277	278	279	280	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Duct, carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma	X																									1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endometrium, adenoma															X											1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node												+				+										7
Iliac, histiocytic sarcoma																										1
Mediastinal, histiocytic sarcoma																										1
Pancreatic, histiocytic sarcoma																										1
Pancreatic, sarcoma																										1
Renal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, sarcoma																										3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranium, carcinoma, metastatic, harderian gland																							X			1
Skeletal muscle																										1
Sarcoma, multiple																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																					X					1
Alveolar/bronchiolar carcinoma, multiple																										1
Carcinoma, metastatic, harderian gland																							X			1
Histiocytic sarcoma																										1
Sarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	027	419	475	482	524	545	544	545	545	566	577	657	675	696	709	725	735	735	735	735	735	735	735	735	735
Carcass ID Number	261	260	252	281	251	282	283	284	285	265	276	257	272	256	274	264	253	254	255	258	259	262	263	266	267
Special Senses System																									
Eye																									
Harderian gland																	+							+	
Adenoma																								X	
Carcinoma																	X								
Urinary System																									
Kidney	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma											X														
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Lymphoma malignant										X						X		X							

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: Vehicle Control

Number of Days on Study	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	268	269	270	271	273	275	277	278	279	280	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	Total Tissues/ Tumors
Special Senses System																										
Eye																							+			1
Harderian gland																+							+			4
Adenoma																X										2
Carcinoma																							X			2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant	X					X						X				X										7

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	434	489	504	530	532	587	591	596	618	637	637	656	660	669	675	675	681	692	696	699	696	696	700	709	709
Carcass ID Number	350	333	302	338	326	348	329	324	317	315	349	320	322	306	304	344	339	316	315	323	337	340	307	313	318
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Intestine small, duodenum	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangioma																									
Hemangiosarcoma																									
Histiocytic sarcoma				X				X																	
Mesentery	+	+		+		+		+	+	+		+	+	+	+	+		+	+	+		+	+		+
Carcinoma, metastatic, kidney								X																	
Hemangioma												X													
Histiocytic sarcoma				X																					
Sarcoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																					
Cardiovascular System																									
Blood vessel	+								+																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																					
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	I	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																				
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS			+																						

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	709	709	713	716	720	720	722	722	729	729	729	729	729	729	730	730	730	731	734	735	735	735	735	735	735	
Carcass ID Number	336	342	347	310	308	330	319	343	311	312	314	328	341	346	305	309	327	332	345	301	303	321	325	331	334	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	A	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Intestine small, duodenum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangioma											X															1
Hemangiosarcoma	X																									1
Histiocytic sarcoma																										2
Mesentery	+	+			+	+	+	+				+				+					+		+		+	29
Carcinoma, metastatic, kidney																										1
Hemangioma																										1
Histiocytic sarcoma																										1
Sarcoma																X										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Cardiovascular System																										
Blood vessel																										2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	46
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Pars distalis, adenoma																							X			1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
Tissue NOS																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	434	489	504	530	532	587	591	596	618	637	637	656	660	669	675	675	681	692	696	696	696	696	700	709	709
Carcass ID Number	350	333	302	338	326	348	329	324	317	335	349	320	322	306	304	344	339	316	315	323	337	340	307	313	318
Genital System																									
Clitoral gland	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																	X								
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Histiocytic sarcoma				X																					
Leiomyosarcoma																									
Endometrium, polyp stromal																									
Vagina																									+
Sarcoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney								X																	
Histiocytic sarcoma				X																					
Lymph node		+	+	+		+		+		+	+	+		+							+		+	+	
Mediastinal, histiocytic sarcoma				X																					
Lymph node, mandibular	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																					
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																							X		
Histiocytic sarcoma				X																					
Plasma cell tumor malignant								X																	
Thymus	+	I	+	+	+	I	+	I	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	M
Histiocytic sarcoma				X																					
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma											X														
Subcutaneous tissue, sarcoma, multiple																								X	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+			+																					
Histiocytic sarcoma				X																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+																						+		
Spinal cord	+																						+		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	709	709	713	716	720	720	722	722	729	729	729	729	729	729	730	730	730	731	734	735	735	735	735	735	735	
Carcass ID Number	336	342	347	310	308	330	319	343	311	312	314	328	341	346	305	309	327	332	345	301	303	321	325	331	334	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Granulosa cell tumor benign																										1
Luteoma								X																		1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma						X																				1
Histiocytic sarcoma																										1
Leiomyosarcoma																						X				1
Endometrium, polyp stromal														X												1
Vagina																+										2
Sarcoma																X										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Histiocytic sarcoma																										1
Lymph node															+	+					+					15
Mediastinal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Plasma cell tumor malignant																										1
Thymus	+	+	M	+	+	+	M	+	+	I	+	+	+	+	I	+	+	+	I	+	+	+	+	+	+	39
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma										X		X														2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, sarcoma																X										2
Subcutaneous tissue, sarcoma, multiple																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																+										3
Histiocytic sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve	+					+												+							+	6
Spinal cord	+					+												+							+	6

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	434	489	504	530	532	587	591	596	618	637	637	656	660	669	675	675	681	692	696	699	696	696	700	709	709
Carcass ID Number	350	333	302	338	326	348	329	324	317	335	349	320	322	306	304	344	339	316	315	323	337	340	307	313	318
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																				X		X		X	X
Alveolar/bronchiolar carcinoma																	X								
Alveolar/bronchiolar carcinoma, multiple						X																			
Histiocytic sarcoma				X																					
Sarcoma, metastatic, skin											X														
Mediastinum, carcinoma, metastatic, kidney								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pleura		+																							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Harderian gland																									
Adenoma																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland																									
Histiocytic sarcoma				X				X																	
Osteosarcoma																									
Ureter								+																	
Carcinoma, metastatic, kidney								X																	
Urinary bladder	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X				X																	
Lymphoma malignant										X				X			X						X		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Riddelliine: 3 mg/kg

Number of Days on Study	709	709	713	716	720	720	722	722	729	729	729	729	729	729	730	730	730	731	734	735	735	735	735	735	735	
Carcass ID Number	336	342	347	310	308	330	319	343	311	312	314	328	341	346	305	309	327	332	345	301	303	321	325	331	334	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X					X		X							X					X			9
Alveolar/bronchiolar carcinoma												X									X					3
Alveolar/bronchiolar carcinoma, multiple																										1
Histiocytic sarcoma																										1
Sarcoma, metastatic, skin																										1
Mediastinum, carcinoma, metastatic, kidney																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pleura																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																		+							+	2
Harderian gland												+	+	+		+									+	5
Adenoma												X	X	X												3
Carcinoma																									X	1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland												X														1
Histiocytic sarcoma																										2
Osteosarcoma							X																			1
Ureter																										1
Carcinoma, metastatic, kidney																										1
Urinary bladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant											X				X	X				X	X					9

Table D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Harderian Gland: Adenoma		
Overall rate[a]	2/50 (4%)	3/50 (6%)
Adjusted rate[b]	4.8%	7.2%
Terminal rate[c]	2/34 (6%)	3/17 (18%)
First incidence (days)	729 (T)	729 (T)
Poly-3 test[d]		P=0.495
Harderian Gland: Adenoma or Carcinoma		
Overall rate	4/50 (8%)	4/50 (8%)
Adjusted rate	9.5%	9.7%
Terminal rate	4/34 (12%)	4/17 (24%)
First incidence (days)	729 (T)	729 (T)
Poly-3 test		P=0.637
Liver: Hepatocellular Adenoma		
Overall rate	9/49 (18%)	0/50 (0%)
Adjusted rate	20.9%	0.0%
Terminal rate	6/34 (18%)	0/17 (0%)
First incidence (days)	419	—[e]
Poly-3 test		P=0.002N
Liver: Hepatocellular Carcinoma		
Overall rate	8/49 (16%)	0/50 (0%)
Adjusted rate	19.0%	0.0%
Terminal rate	6/34 (18%)	0/17 (0%)
First incidence (days)	675	—
Poly-3 test		P=0.004N
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	16/49 (33%)	0/50 (0%)
Adjusted rate	36.9%	0.0%
Terminal rate	11/34 (32%)	0/17 (0%)
First incidence (days)	419	—
Poly-3 test		P<0.001N
Lung: Alveolar/bronchiolar Adenoma		
Overall rate	1/50 (2%)	9/50 (18%)
Adjusted rate	2.4%	21.5%
Terminal rate	1/34 (3%)	4/17 (24%)
First incidence (days)	729 (T)	696
Poly-3 test		P=0.007
Lung: Alveolar/bronchiolar Carcinoma		
Overall rate	1/50 (2%)	4/50 (8%)
Adjusted rate	2.3%	9.5%
Terminal rate	0/34 (0%)	2/17 (12%)
First incidence (days)	419	587
Poly-3 test		P=0.174
Lung: Alveolar/bronchiolar Adenoma or Carcinoma		
Overall rate	2/50 (4%)	13/50 (26%)
Adjusted rate	4.7%	30.5%
Terminal rate	1/34 (3%)	6/17 (35%)
First incidence (days)	419	587
Poly-3 test		P<0.001

Table D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Mammary Gland: Adenoma or Carcinoma		
Overall rate	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	7.2%
Terminal rate	0/34 (0%)	2/17 (12%)
First incidence (days)	—	656
Poly-3 test		P=0.117
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma		
Overall rate	5/48 (10%)	2/49 (4%)
Adjusted rate	12.3%	4.9%
Terminal rate	4/33 (12%)	1/17 (6%)
First incidence (days)	725	532
Poly-3 test		P=0.208N
Skin (Subcutaneous Tissue): Sarcoma		
Overall rate	3/50 (6%)	3/50 (6%)
Adjusted rate	6.9%	7.2%
Terminal rate	0/34 (0%)	1/17 (6%)
First incidence (days)	475	637
Poly-3 test		P=0.649
All Organs: Hemangioma or Hemangiosarcoma		
Overall rate	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	9.5%
Terminal rate	0/34 (0%)	0/17 (0%)
First incidence (days)	—	656
Poly-3 test		P=0.059
All Organs: Malignant Lymphoma		
Overall rate	7/50 (14%)	9/50 (18%)
Adjusted rate	16.4%	21.3%
Terminal rate	5/34 (15%)	5/17 (29%)
First incidence (days)	566	637
Poly-3 test		P=0.385
All Organs: Benign Neoplasms		
Overall rate	15/50 (30%)	17/50 (34%)
Adjusted rate	34.6%	39.5%
Terminal rate	12/34 (35%)	7/17 (41%)
First incidence (days)	419	532
Poly-3 test		P=0.401
All Organs: Malignant Neoplasms		
Overall rate	21/50 (42%)	20/50 (40%)
Adjusted rate	45.9%	45.1%
Terminal rate	12/34 (35%)	9/17 (53%)
First incidence (days)	419	530
Poly-3 test		P=0.555N

Table D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
All Organs: Benign or Malignant Neoplasms		
Overall rate	31/50 (62%)	33/50 (66%)
Adjusted rate	67.7%	72.2%
Terminal rate	22/34 (65%)	14/17 (82%)
First incidence (days)	419	530
Poly-3 test		P=0.404

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and pituitary 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 dosed group incidence is the P value corresponding to a pairwise comparison between the vehicle controls and the dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in the dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

Table D4a
Historical Incidence of Liver Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls				
	Cholangioma	Hemangiosarcoma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]					
Acrylonitrile (gavage)	0/50	2/50	14/50	7/50	20/50
Citral (feed)	0/99	0/99	8/99	4/99	12/99
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50	4/50	3/50	6/50
Indium phosphide (inhalation)	0/50	0/50	12/50	6/50	18/50
60-Hz Magnetic fields (whole body exposure)	0/98	1/98	17/98	6/98	22/98
Methacrylonitrile (gavage)	0/50	0/50	9/50	2/50	10/50
o-Nitrotoluene (feed)	0/60	0/60	7/60	2/60	9/60
p-Nitrotoluene (feed)	0/49	0/49	6/49	3/49	8/49
Riddelliine (gavage)	0/49	0/49	9/49	8/49	16/49
Sodium nitrite (drinking water)	0/50	0/50	9/50	2/50	10/50
Vanadium pentoxide (inhalation)	0/50	0/50	6/50	6/50	12/50
Overall Historical Incidence in Controls Given NTP-2000 Diet					
Total (%)	0/655	3/655 (0.5%)	101/655 (15.4%)	49/655 (7.5%)	143/655 (21.8%)
Mean ± standard deviation		(0.5% ± 1.2%)	16.0% ± 6.3%	8.0% ± 4.7%	22.8% ± 9.6%
Range		0%-4%	8%-28%	3%-16%	12%-40%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]					
Total (%)	0/51	1/51 (2.0%)	15/51 (29.4%)	8/51 (15.7%)	22/51 (43.1%)

[a] Data as of December 20, 2000
[b] Data as of December 23, 1999

Table D4b
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Controls Given NTP-2000 Diet[a]			
Acrylonitrile (gavage)	4/50	2/50	6/50
Citral (feed)	5/99	6/99	11/99
p,p′-Dichlorodiphenyl sulfone (feed)	0/50	0/50	0/50
Indium phosphide (inhalation)	3/50	1/50	4/50
60-Hz Magnetic fields (whole body exposure)	9/95	2/95	11/95
Methacrylonitrile (gavage)	6/50	1/50	6/50
o-Nitrotoluene (feed)	2/60	3/60	5/60
p-Nitrotoluene (feed)	5/50	1/50	6/50
Riddelliine (gavage)	1/50	1/50	2/50
Sodium nitrite (drinking water)	1/50	0/50	1/50
Vanadium pentoxide (inhalation)	1/50	0/50	1/50
Overall Historical Incidence in Controls Given NTP-2000 Diet			
Total (%)	37/654 (5.7%)	17/654 (2.6%)	53/654 (8.1%)
Mean ± standard deviation	5.4% ± 4.0%	2.3% ± 2.0%	7.6% ± 4.7%
Range	0%-12%	0%-6%	0%-12%
Overall Historical Incidence in Water Gavage Controls Given NIH-07 Diet[b]			
Total (%)	3/51 (5.9%)	1/51 (2.0%)	4/51 (7.8%)

[a] Data as of December 20, 2000
[b] Data as of December 23, 1999

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Riddelliine[a]

	Vehicle Control	3 mg/kg
Disposition Summary		
Animals initially in study	50	50
Early deaths		
Accidental deaths	4	
Moribund	9	25
Natural deaths	3	8
Survivors		
Died last week of study		1
Terminal sacrifice	34	16
Animals examined microscopically	50	50
Alimentary System		
Gallbladder	(45)	(45)
Cyst	1 (2%)	2 (4%)
Epithelium, cytoplasmic alteration		1 (2%)
Intestine large, colon	(49)	(47)
Epithelium, hyperplasia, focal		1 (2%)
Intestine large, rectum	(48)	(47)
Inflammation, chronic, focal		1 (2%)
Intestine large, cecum	(48)	(47)
Edema		5 (11%)
Fibrosis	1 (2%)	
Hemorrhage, focal		6 (13%)
Artery, inflammation, chronic, focal		18 (38%)
Epithelium, erosion, focal		6 (13%)
Intestine small, duodenum	(47)	(46)
Hemorrhage, focal		2 (4%)
Necrosis, focal		2 (4%)
Artery, inflammation, chronic		13 (28%)
Intestine small, ileum	(46)	(46)
Epithelium, cyst	1 (2%)	
Peyer's patch, hyperplasia, lymphoid		1 (2%)
Liver	(49)	(50)
Angiectasis	2 (4%)	2 (4%)
Clear cell focus	1 (2%)	
Congestion, focal		1 (2%)
Cyst		1 (2%)
Eosinophilic focus	2 (4%)	
Fibrosis, focal		2 (4%)
Hemorrhage, focal		2 (4%)
Hyperplasia, focal, lymphoid	2 (4%)	
Infarct		1 (2%)
Infiltration cellular, mixed cell	29 (59%)	41 (82%)
Inflammation, focal, suppurative	1 (2%)	
Mineralization, focal	1 (2%)	
Pigmentation, focal		1 (2%)
Thrombosis		1 (2%)
Artery, inflammation, chronic		1 (2%)
Bile duct, hyperplasia		28 (56%)
Endothelial cell, hyperplasia, focal		1 (2%)

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

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Alimentary System (continued)		
Liver (continued)	(49)	(50)
Hepatocyte, cytomegaly		49 (98%)
Hepatocyte, karyomegaly		49 (98%)
Hepatocyte, mixed cell focus	1 (2%)	
Hepatocyte, necrosis, focal	12 (24%)	19 (38%)
Hepatocyte, vacuolization cytoplasmic, diffuse	8 (16%)	1 (2%)
Hepatocyte, vacuolization cytoplasmic, focal	3 (6%)	
Hepatocyte, centrilobular, necrosis		1 (2%)
Hepatocyte, centrilobular, vacuolization cytoplasmic		3 (6%)
Hepatocyte, midzonal, vacuolization cytoplasmic	1 (2%)	3 (6%)
Mesentery	(23)	(29)
Fibrosis, focal		2 (7%)
Hemorrhage		1 (3%)
Inflammation, chronic		2 (7%)
Inflammation, chronic, focal, suppurative		1 (3%)
Inflammation, suppurative		1 (3%)
Artery, inflammation, chronic, focal	1 (4%)	19 (66%)
Artery, mineralization, focal		1 (3%)
Fat, necrosis	19 (83%)	2 (7%)
Pancreas	(49)	(50)
Amyloid deposition	1 (2%)	
Atrophy, focal	1 (2%)	
Fibrosis, focal		1 (2%)
Necrosis, focal		1 (2%)
Acinus, atrophy, diffuse		1 (2%)
Acinus, atrophy, focal		1 (2%)
Artery, inflammation, chronic		3 (6%)
Salivary glands	(50)	(50)
Artery, inflammation, chronic	1 (2%)	2 (4%)
Stomach, forestomach	(49)	(49)
Inflammation, focal	2 (4%)	
Ulcer	1 (2%)	
Epithelium, cyst	1 (2%)	
Epithelium, hyperplasia	1 (2%)	
Stomach, glandular	(49)	(48)
Cyst		2 (4%)
Tooth	(7)	
Malformation	1 (14%)	
Peridontal tissue, inflammation, chronic	6 (86%)	
Cardiovascular System		
Blood vessel		(2)
Inflammation, chronic		1 (50%)
Aorta, mineralization		1 (50%)
Heart	(50)	(50)
Infiltration cellular, mixed cell	3 (6%)	6 (12%)
Inflammation, chronic, focal		2 (4%)
Mineralization, focal		4 (8%)
Epicardium, infiltration cellular, mixed cell		1 (2%)

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Endocrine System		
Adrenal cortex	(50)	(50)
Accessory adrenal cortical nodule		2 (4%)
Angiectasis		1 (2%)
Cyst	1 (2%)	
Cytoplasmic alteration		1 (2%)
Cytoplasmic alteration, focal	1 (2%)	25 (50%)
Inflammation, chronic, focal		6 (12%)
Thrombosis		1 (2%)
Bilateral, hypertrophy, focal	1 (2%)	
Capsule, fibrosis, focal		1 (2%)
Adrenal medulla	(49)	(49)
Atypia cellular	1 (2%)	
Hyperplasia		1 (2%)
Parathyroid gland	(47)	(46)
Cyst	1 (2%)	1 (2%)
Pituitary gland	(48)	(49)
Angiectasis	4 (8%)	1 (2%)
Pars distalis, cytoplasmic alteration, focal	3 (6%)	3 (6%)
Pars distalis, hemorrhage, focal		1 (2%)
Pars distalis, hyperplasia, focal	1 (2%)	
Rathke's cleft, cyst	1 (2%)	
Thyroid gland	(49)	(50)
Degeneration, cystic, focal	13 (27%)	21 (42%)
Inflammation, chronic, focal	1 (2%)	2 (4%)
Follicle, cyst	1 (2%)	
Follicular cell, cytoplasmic alteration, diffuse		1 (2%)
Follicular cell, hyperplasia	1 (2%)	
General Body System		
Tissue NOS		(1)
Abdominal, inflammation		1 (100%)
Genital System		
Clitoral gland	(49)	(49)
Degeneration, cystic		2 (4%)
Inflammation, chronic	1 (2%)	
Ovary	(49)	(48)
Angiectasis	3 (6%)	1 (2%)
Cyst	15 (31%)	15 (31%)
Cyst, multiple		2 (4%)
Hemorrhage	1 (2%)	1 (2%)
Inflammation, suppurative		1 (2%)
Mineralization	1 (2%)	
Pigmentation, focal	1 (2%)	
Thrombosis		1 (2%)
Artery, inflammation, chronic		26 (54%)
Bilateral, cyst	1 (2%)	2 (4%)
Interstitial cell, hyperplasia	1 (2%)	1 (2%)

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Genital System (continued)		
Uterus	(49)	(50)
Angiectasis	1 (2%)	2 (4%)
Cyst		1 (2%)
Hemorrhage		1 (2%)
Hydrometra	27 (55%)	19 (38%)
Inflammation, suppurative	2 (4%)	
Artery, inflammation		2 (4%)
Artery, inflammation, chronic, focal		21 (42%)
Endometrium, hyperplasia, cystic	43 (88%)	41 (82%)
Hematopoietic System		
Bone marrow	(50)	(50)
Hyperplasia	2 (4%)	6 (12%)
Lymph node	(7)	(15)
Hemorrhage		1 (7%)
Hyperplasia, histiocytic		1 (7%)
Hyperplasia, lymphoid	1 (14%)	
Bronchial, hyperplasia, lymphoid		1 (7%)
Bronchial, hyperplasia, plasma cell		1 (7%)
Deep cervical, hyperplasia, lymphoid		1 (7%)
Iliac, hemorrhage	1 (14%)	
Iliac, hyperplasia, plasma cell		1 (7%)
Mediastinal, hyperplasia, lymphoid	1 (14%)	1 (7%)
Mediastinal, hyperplasia, plasma cell		2 (13%)
Renal, angiectasis		1 (7%)
Renal, hemorrhage		2 (13%)
Renal, hyperplasia, plasma cell		1 (7%)
Renal, inflammation, suppurative		1 (7%)
Lymph node, mandibular	(48)	(47)
Fibrosis		1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)
Hyperplasia, plasma cell	1 (2%)	1 (2%)
Lymph node, mesenteric	(48)	(49)
Angiectasis		1 (2%)
Fibrosis		1 (2%)
Hematopoietic cell proliferation		4 (8%)
Hemorrhage		4 (8%)
Hyperplasia, histiocytic		2 (4%)
Hyperplasia, lymphoid	2 (4%)	2 (4%)
Hyperplasia, plasma cell	3 (6%)	1 (2%)
Artery, inflammation, chronic		1 (2%)
Spleen	(49)	(50)
Fibrosis, focal		1 (2%)
Hematopoietic cell proliferation	32 (65%)	43 (86%)
Hyperplasia, lymphoid	9 (18%)	4 (8%)
Hyperplasia, plasma cell	1 (2%)	
Pigmentation	1 (2%)	
Artery, inflammation, chronic, focal		6 (12%)
Thymus	(49)	(39)
Angiectasis	1 (2%)	
Atrophy		2 (5%)
Cyst		1 (3%)
Hyperplasia, histiocytic		1 (3%)
Hyperplasia, lymphoid	3 (6%)	
Artery, inflammation, chronic		1 (3%)

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Integumentary System		
Mammary gland	(49)	(50)
Ectasia		2 (4%)
Skin	(50)	(50)
Inflammation, chronic, focal	1 (2%)	
Artery, subcutaneous tissue, inflammation, chronic		1 (2%)
Dermis, edema, focal		1 (2%)
Subcutaneous tissue, edema		1 (2%)
Subcutaneous tissue, inflammation, focal, suppurative	1 (2%)	
Musculoskeletal System		
Bone	(50)	(50)
Cranium, fibrous osteodystrophy		1 (2%)
Vertebra, hyperostosis, focal	41 (82%)	33 (66%)
Nervous System		
Brain	(50)	(50)
Atrophy, focal		1 (2%)
Gliosis		1 (2%)
Hemorrhage, focal		4 (8%)
Artery, meninges, inflammation, chronic		1 (2%)
Peripheral nerve		(6)
Spinal, ganglion, inflammation, chronic		1 (17%)
Spinal cord		(6)
Hemorrhage, focal		1 (17%)
Meninges, inflammation, chronic		1 (17%)
Respiratory System		
Lung	(50)	(50)
Congestion	3 (6%)	2 (4%)
Foreign body	4 (8%)	
Hemorrhage	1 (2%)	4 (8%)
Hyperplasia, focal, mast cell		1 (2%)
Hyperplasia, histiocytic	2 (4%)	9 (18%)
Hyperplasia, lymphoid	2 (4%)	
Infiltration cellular, mixed cell		3 (6%)
Pigmentation, focal	1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)	6 (12%)
Artery, mediastinum, inflammation, chronic		2 (4%)
Interstitium, edema	1 (2%)	
Mediastinum, inflammation, chronic		1 (2%)
Mediastinum, inflammation, suppurative		1 (2%)
Nose	(50)	(50)
Nasolacrimal duct, inflammation	2 (4%)	1 (2%)
Pleura		(1)
Inflammation, suppurative		1 (100%)
Special Senses System		
Eye	(1)	(2)
Atrophy	1 (100%)	1 (50%)
Inflammation, chronic	1 (100%)	

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Riddelliine

	Vehicle Control	3 mg/kg
Urinary System		
Kidney	(49)	(50)
Atrophy	1 (2%)	
Cyst	3 (6%)	2 (4%)
Inflammation, chronic		1 (2%)
Metaplasia, focal, osseous	1 (2%)	8 (16%)
Mineralization, focal	1 (2%)	1 (2%)
Nephropathy	18 (37%)	47 (94%)
Artery, inflammation, chronic	1 (2%)	16 (32%)
Artery, mineralization		1 (2%)
Glomerulus, glomerulosclerosis		40 (80%)
Pelvis, dilatation		1 (2%)
Renal tubule, accumulation, hyaline droplet	2 (4%)	14 (28%)
Renal tubule, casts protein	2 (4%)	
Renal tubule, dilatation	2 (4%)	2 (4%)
Renal tubule, hemorrhage	1 (2%)	
Renal tubule, karyomegaly		1 (2%)
Renal tubule, necrosis	1 (2%)	
Renal tubule, pigmentation	2 (4%)	27 (54%)
Urinary bladder	(50)	(48)
Edema	1 (2%)	
Artery, inflammation, chronic		1 (2%)

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1988). Riddelliine was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It 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. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of riddelliine. The high dose was limited by toxicity. All positive trials were repeated under the conditions that elicited the positive response.

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.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al*. (1987). Riddelliine was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of riddelliine. The highest dose was limited by toxicity to 600 µg/mL. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with riddelliine in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2.5 hours after culture initiation. After 26 hours, the medium containing riddelliine was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with riddelliine, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no riddelliine. Incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind, and those from a single test were read by the same person. Up to 50 second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because the 100 µg/mL dose induced significant cell cycle delay in the presence of S9, incubation time was lengthened in an attempt to obtain a sufficient number of scorable (second-division metaphase) cells; however, this dose was completely cytostatic, and no cells were scored.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with riddelliine for 8.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed,

and stained with Giemsa. For the Abs test with S9, cells were treated with riddelliine and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 18.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test: if cell cycle delay was anticipated, the incubation period was extended.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level in the absence of S9; with S9, fewer cells were scored because of the extremely high numbers of aberrations observed. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Mouse Bone Marrow and Peripheral Blood Micronucleus Test Protocols

Single-Dose Peripheral Blood and Bone Marrow Studies: Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by riddelliine. Male Swiss mice received a single intraperitoneal injection of riddelliine dissolved in sodium phosphate buffer. Solvent control animals received buffer only. The positive control animals received an injection of urethane. Bone marrow smears were prepared 24 hours after treatment; peripheral blood smears were prepared 48 hours after treatment. Blood was obtained from the tail vein, and bone marrow was extracted from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of up to eight animals per dose group, per tissue. In addition, the percentage of PCEs among the total erythrocyte population was scored for each dose group as a measure of 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 PCEs was analyzed by the Cochran armitage trend test, and individual dose groups were compared to the concurrent solvent control by Kastenbaum-Bowman's (Kastenbaum and Bowman, 1970) binomial test. The percentage of PCEs among total erythrocytes was analyzed by an analysis of variance on ranks and individual dose groups were compared with the concurrent solvent control using a *t*-test on ranks.

4- and 13-Week Peripheral Blood Studies: A detailed discussion of this assay is presented by Witt *et al.* (2000). Riddelliine, dissolved in sodium phosphate buffer, was administered by gavage to male and female mice 5 days per week for 4 or 13 weeks. Peripheral blood samples were obtained at the end of the studies. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/ pyronin Y (MacGregor *et al.,* 1983) and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 PCEs (13-week study only) and 10,000 normochromatic erythrocytes (NCEs) in each of up to 5 (4-week study) or 10 (13-week study) animals per dose group. The criteria of Schmid (1976) were used in defining micronuclei, with the additional requirement that micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm illumination and orange with 540 nm ultraviolet illumination); the minimum size limit was approximately one-twentieth the diameter of the NCE. In addition, the percentage of PCEs among the total erythrocyte population was determined.

The frequency of micronucleated cells among NCEs and PCEs 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 (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In

the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 13-week study were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Unscheduled DNA Synthesis and S-Phase DNA Synthesis Test Protocols

Livers from male and female F344/N rats (0 to 3.3 mg/kg) and B6C3F$_1$ mice (0 to 25.0 mg/kg) administered riddelliine by gavage for 5 or 30 days were perfused *in situ*, and a single cell suspension of hepatocytes was obtained using collagenase. Cells were collected by centrifugation, resuspended in cold medium, and seeded into 6-well culture plates containing 25 mm round Thermanox® coverslips (Bio-Labs, Northbrook, IL) in Williams' Medium E supplemented with 2 mM *l*-glutamine, 50 µg/mL gentamycin sulfate (Sigma Chemical Co. St. Louis, MO), and 10% fetal bovine serum. After 1.5 to 2.0 hours of incubation in a humidified, 5% CO_2 atmosphere at 37° C, cultures were washed and then incubated in Williams' Medium E (without serum) containing 10 µCi/mL [^{3}H]-methyl thymidine (specific activity approximately 80 Ci/mmole) for 4 hours at 37° C and 5% CO_2, followed by 14 to 18 hours in Williams' Medium E containing 0.25 mM unlabeled thymidine. Cultures were washed and fixed, and autoradiography was performed as described by Hamilton and Mirsalis (1987). Thirty morphologically unaltered hepatocytes on a randomly selected area of each slide were examined. The highest grain count from two nucleus-sized areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains per nucleus. Three slides were scored for each animal, for a total of 90 cells per animal.

Hepatocytes undergoing DNA replication have jet-black nuclei as a result of the large number of silver grains and are easily distinguished from nonreplicating cells in autoradiographic preparations. Approximately 1,000 cells were counted from a randomly selected area of each slide and classified as "S-phase" (undergoing replicative DNA synthesis) or "non-S-phase." Three slides were scored for each animal, for a total of approximately 3,000 cells per animal. The percentage of cells in S-phase was calculated for each dose group.

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 judgement of the overall evidence for activity of the chemical in an assay.

Results

Riddelliine (100 to 5,000 µg/plate) was mutagenic in *S. typhimurium* strain TA100 in the presence of 30% induced rat or hamster liver S9 (Table E1; Zeiger *et al.*, 1988); it was not mutagenic with 10% S9, and results of mutagenicity testing were negative in strains TA98 and TA1535 with and without S9 activation. The small, dose-related increase in mutant colonies observed with strain TA97 in the presence of 30% rat S9 was judged to be equivocal. Riddelliine induced SCEs in cultured CHO cells with and without S9; the response observed in the presence of S9 was very strong (Table E2; Galloway *et al.*, 1987). In the test conducted with S9, only three cells were counted at the highest scorable dose (30 µg/mL) because of the extremely high number of induced

SCEs. Riddelliine also induced highly significant increases in Abs in cultured CHO cells in the presence of S9 at all three doses scored (Table E3; Galloway *et al.*, 1987). No increase in Abs was observed in the absence of S9.

In single-dose studies in which riddelliine, administered via intraperitoneal injection, was tested for induction of micronucleated erythrocytes in Swiss mice, the increase seen in bone marrow erythrocytes was not dose related and was of insufficient magnitude to be considered positive (Table E4). However, a small but significant dose-related increase in the frequency of micronucleated PCEs was seen in peripheral blood (Table E5). These single-dose studies were not replicated to confirm the positive response; therefore, these results cannot be considered conclusive. In 4- and 13-week gavage studies, riddelliine did not induce micronucleated erythrocytes in peripheral blood of $B6C3F_1$ mice (Tables E6 and E7; Witt *et al.*, 2000). Several protocol differences between the 4- and 13-week studies and the single-dose study may be implicated in the contrasting test results, including the higher doses used in the single-dose study, differences in riddelliine metabolism arising from the different routes of administration, and the different strains of mice that were used.

In tests to measure unscheduled DNA synthesis (UDS), a measure of DNA damage and repair, and S-phase DNA synthesis in cultured hepatocytes from F344/N rats and $B6C3F_1$ mice following 5 or 30 days of treatment by gavage with riddelliine, an increase in UDS was observed at both time points in at least one dose group each in male rats and in male and female mice (Tables E8 and E9). UDS was only observed in female rats following 5 days of treatment. An increase in S-phase DNA synthesis, an indication of DNA replication, was observed at both time points in at least one group in male and female rats. There was no increase in S-phase synthesis in male or female mice following 5 days of treatment; an increase occurred in only a single dose group (3.3 mg/kg) in male mice following 30 days of treatment. In female mice treated for 5 or 30 days, S-phase synthesis appeared inhibited (i.e., less than vehicle control values) at the highest doses (day 5: 25 mg/kg; day 30: 10 and 25 mg/kg). These data suggest that the hepatotoxicity of riddelliine may be due, in part, to an antimitogenic effect at higher doses, which inhibits compensatory cell proliferation that occurs in response to toxicity. However, the high variability in S-phase DNA synthesis in control female mice confounded interpretation of the results.

TABLE E1
Mutagenicity of Riddelliine in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+ hamster S9 10%	+ hamster S9 30%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%	+ rat S9 30%
TA100	0	156 ± 8.4	155 ± 4.4	160 ± 7.4	117 ± 2.1	113 ± 5.2	175 ± 6.8	124 ± 3.8	159 ± 7.1
	100	134 ± 3.2	112 ± 1.5	120 ± 7.0	127 ± 11.8	122 ± 11.2	174 ± 12.5	190 ± 5.8	198 ± 0.6
	333	124 ± 1.2	115 ± 3.6	144 ± 3.5	118 ± 4.5	173 ± 9.7	164 ± 11.1	240 ± 5.6	263 ± 10.5
	1,000	136 ± 4.4	129 ± 4.2	153 ± 4.0	209 ± 13.5	218 ± 11.1	186 ± 7.2	331 ± 15.5	326 ± 12.8
	3,333	147 ± 12.7	176 ± 6.7	166 ± 9.8	291 ± 13.5	315 ± 12.7	186 ± 3.5	355 ± 14.9[c]	370 ± 6.2
	5,000	150 ± 3.0	188 ± 7.8	196 ± 4.6	342 ± 15.1	305 ± 26.4	185 ± 7.4	363 ± 2.1[c]	381 ± 7.3
	Trial summary	Negative	Negative	Negative	Positive	Positive	Negative	Positive	Positive
	Positive control[d]	1,499 ± 63.7	1,484 ± 24.8	2,108 ± 55.9	1,009 ± 33.8	889 ± 9.2	2,373 ± 38.2	863 ± 24.8	738 ± 44.4
TA1535	0	42 ± 1.8	38 ± 3.8	32 ± 4.3	13 ± 2.9		32 ± 2.1	13 ± 3.0	
	100	35 ± 1.2	26 ± 4.6	22 ± 3.2	9 ± 1.5		22 ± 3.9	14 ± 2.7	
	333	34 ± 2.7	32 ± 5.0	21 ± 0.3	12 ± 2.7		23 ± 3.1	13 ± 4.2	
	1,000	34 ± 6.2	29 ± 5.0	20 ± 2.4	13 ± 1.5		20 ± 1.7	8 ± 2.0	
	3,333	30 ± 3.6	27 ± 2.2	19 ± 1.0	7 ± 0.7		16 ± 0.9	6 ± 0.6[c]	
	5,000	32 ± 3.8	23 ± 3.5	23 ± 3.8	5 ± 1.2[c]		17 ± 1.7	8 ± 1.2[c]	
	Trial summary	Negative	Negative	Negative	Negative		Negative	Negative	
	Positive control	1,379 ± 17.9	983 ± 19.6	153 ± 6.1	258 ± 7.9		171 ± 7.5	119 ± 5.0	
TA97	0	107 ± 5.7	78 ± 4.6	150 ± 6.1	161 ± 3.8		140 ± 6.1	171 ± 4.4	152 ± 2.2
	100	107 ± 9.0	73 ± 9.8	140 ± 8.8	150 ± 0.6		143 ± 7.4	205 ± 3.7	177 ± 13.4[c]
	333	106 ± 6.7	85 ± 0.9	139 ± 3.5	160 ± 11.0		133 ± 5.8	184 ± 6.4	184 ± 11.5[c]
	1,000	110 ± 5.6	5 ± 1.5	152 ± 7.2	159 ± 5.0		132 ± 5.8	212 ± 4.9	208 ± 20.8[c]
	3,333	103 ± 2.6	8 ± 0.3	140 ± 1.5	163 ± 2.9		120 ± 7.9	232 ± 13.3[c]	177 ± 17.9[c]
	5,000	97 ± 3.7	10 ± 1.0[c]	141 ± 4.7	168 ± 10.5[c]		147 ± 4.9	248 ± 20.3[c]	209 ± 3.5[c]
	Trial summary	Negative	Negative	Negative	Negative		Negative	Equivocal	Equivocal
	Positive control	1,009 ± 80.5	698 ± 36.0	1,395 ± 54.8	573 ± 19.4		1,636 ± 62.9	429 ± 3.8	732 ± 2.5
TA98	0	13 ± 0.7	20 ± 3.5	24 ± 2.1	29 ± 1.2		29 ± 1.7	28 ± 2.3	
	100	16 ± 2.8	15 ± 1.0	28 ± 0.7	27 ± 2.6		29 ± 2.1	31 ± 2.7	
	333	16 ± 2.0	16 ± 3.5	32 ± 2.5	31 ± 2.3		39 ± 2.3	25 ± 2.0	
	1,000	20 ± 4.3	22 ± 2.3	32 ± 2.1	29 ± 1.3		30 ± 2.1	27 ± 1.9	
	3,333	16 ± 3.1	20 ± 5.5	29 ± 2.7	31 ± 4.3		26 ± 0.9	26 ± 2.8[c]	
	5,000	16 ± 0.7	18 ± 6.1	31 ± 2.0	24 ± 1.8		32 ± 4.7	20 ± 5.2[c]	
	Trial summary	Negative	Negative	Negative	Negative		Negative	Negative	
	Positive control	1,913 ± 86.1	1,392 ± 19.9	1,076 ± 37.1	715 ± 22.8		1,731 ± 26.0	419 ± 5.0	

[a] Study was performed at Microbiological Associates, Inc. The detailed protocol and these data are presented by Zeiger *et al.* (1988). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Riddelliine[a]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Positive								
Dimethylsulfoxide[c]		50	1,031	432	0.41	8.6	25.5	
Riddelliine	30	50	1,028	538	0.52	10.8	25.5	24.90*
	100	50	1,040	702	0.67	14.0	25.5	61.09*
	300	50	1,036	1,224	1.18	24.5	25.5	181.96*
					P<0.001[d]			
Mitomycin-C[e]	0.001	50	1,022	671	0.65	13.4	25.5	56.69
	0.01	5	105	240	2.28	48.0	25.5	445.50
+S9								
Summary: Positive								
Dimethylsulfoxide		50	1,018	432	0.42	8.6	26.0	
Riddelliine	3	50	1,017	1,537	1.51	30.7	26.0	256.14*
	10	20	408	1,539	3.77	77.0	26.0	788.88*
	30	3	63	441	7.00	147.0	26.0	1,549.54*
	100	0					35.0[f]	
					P<0.001			
Cyclophosphamide[e]	0.3	50	1,009	538	0.53	10.8	26.0	25.65
	2	5	101	141	1.39	28.2	26.0	228.97

* Positive response (>20% increase over solvent control)
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol and these data are presented by Galloway *et al*. (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Solvent control
[d] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[e] Positive control
[f] Due to cell cycle delay, harvest time was extended to maximize the number of second-division metaphase cells available for analysis; no suitable cells were found.

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Riddelliine[a]

Compound	Dose (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Harvest time: 10.5 hours					
Summary: Negative					
Dimethylsulfoxide[b]		100	1	0.01	1.0
Riddelliine	402	100	0	0.00	0.0
	498	100	1	0.01	1.0
	600	100	2	0.02	2.0
					P=0.186[c]
Mitomycin-C[d]	1	50	14	0.28	18.0
+S9					
Harvest time: 20.5 hours[e]					
Summary: Positive					
Dimethylsulfoxide		100	3	0.03	3.0
Riddelliine	300	25	73	2.92	92.0*
	400	25	64	2.56	76.0*
	498	25	73	2.92	96.0*
	600[f]	0			
					P<0.001
Cyclophosphamide[d]	15	50	9	0.18	16.0

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol and these data are presented by Galloway *et al.* (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control
[e] Due to cell cycle delay, harvest time was extended to maximize the number of first-division metaphase cells available for analysis.
[f] Not scored due to poor chromosome morphology

TABLE E4
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Swiss Mice Following a Single Intraperitoneal Injection of Riddelliine[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCES/1,000 PCEs[b]	PCEs (%)[b]
Sodium phosphate buffer[c]		8	1.88 ± 0.30	59.50 ± 1.24
Riddelliine	75	8	3.00 ± 0.42	56.63 ± 2.08
	150	8	2.00 ± 0.38	51.38 ± 2.72**
	270	8	3.43 ± 0.57*	48.14 ± 2.43**
	300	1	4.00	61.00
			P=0.071[d]	P=0.006[e]
Urethane[f]	200		16.38 ± 0.60	54.88 ± 1.44

* Significantly different (P≤0.05) from the solvent control by a *t*-test on ranks
** Significantly different (P≤0.01) from the solvent control by Kastenbaum-Bowman's binomial test
[a] Study was performed at Western Regional Research Center, U.S. Department of Agriculture. The detailed protocol is presented by MacGregor *et al*. (1985).
[b] PCE=polychromatic erythrocyte
[c] Mean ± standard error
[d] Solvent control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by a Cochran-Armitage linear regression of proportions
[f] Significance of percent PCEs tested by analysis of variance on ranks
Positive control

TABLE E5
Frequency of Micronuclei in Peripheral Blood Polychromatic Erythrocytes of Male Swiss Mice Following a Single Intraperitoneal Injection of Riddelliine[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCES/1,000 PCEs[b]	PCEs (%)[b]
Sodium phosphate buffer[c]		8	1.65 ± 0.41	3.55 ± 0.30
Riddelliine	75	8	2.40 ± 0.49	3.17 ± 0.34
	150	8	3.62 ± 0.75**	2.38 ± 0.42*
	300	0		
			P=0.005[d]	P=0.105[e]
Urethane[f]	200		14.93 ± 1.53	2.71 ± 0.32

* Significantly different (P≤0.05) from the solvent control by a *t*-test on ranks
** Significantly different (P≤0.01) from the solvent control by Kastenbaum-Bowman's binomial test
[a] Study was performed at Western Regional Research Center, U.S. Department of Agriculture. The detailed protocol is presented by MacGregor *et al.* (1985).
[b] PCE=polychromatic erythrocyte
[c] Mean ± standard error
[d] Solvent control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by a Cochran-Armitage linear regression of proportions
[f] Significance of percent PCEs tested by analysis of variance on ranks
Positive control

Table E6
Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice Following Treatment with Riddelliine by Gavage for 4 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	PCEs (%)[b]
Male				
Sodium phosphate buffer[c]		5	1.44 ± 0.08	1.62 ± 0.06
Riddelliine	3.3	4	1.95 ± 0.28	2.14 ± 0.33
	10.0	4	1.91 ± 0.19	1.71 ± 0.41
	25.0	4	1.45 ± 0.30	2.10 ± 0.14
			P=0.649[d]	P=0.269[e]
Female				
Sodium phosphate buffer		4	1.69 ± 0.36	2.25 ± 0.47
Riddelliine	3.3	5	1.15 ± 0.20	2.36 ± 0.20
	10.0	5	1.01 ± 0.27	2.19 ± 0.22
	25.0	4	1.46 ± 0.36	2.05 ± 0.09
			P=0.543	P=0.711

[a] Study was performed at Western Regional Research Center, U.S. Department of Agriculture. The detailed protocol and these data are presented by Witt *et al*. (2000). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Vehicle control
[d] Significance tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[e] Analysis of variance, significant at P≤0.025

Table E7
Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice Following Treatment with Riddelliine by Gavage for 13 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated Cells/1,000 Cells[b] PCEs	NCEs	PCEs (%)
Male					
Sodium phosphate buffer[c]		10	2.24 ± 0.50	1.33 ± 0.10	2.33 ± 0.13
Riddelliine	10	10	2.42 ± 0.42	1.58 ± 0.13	2.46 ± 0.08
	25	9	1.42 ± 0.15	1.55 ± 0.09	2.13 ± 0.14
			P=0.910[d]	P=0.086[d]	P=0.226[e]
Female					
Sodium phosphate buffer		8	1.09 ± 0.13	1.14 ± 0.08	1.98 ± 0.11
Riddelliine	10	10	1.04 ± 0.10	1.15 ± 0.06	2.14 ± 0.13
	25	10	1.88 ± 0.35	1.28 ± 0.07	2.69 ± 0.55
			P=0.043	P=0.150	P=0.457

[a] Study was performed at Western Regional Research Center, U.S. Department of Agriculture. NCE=normochromatic; PCE=polychromatic erythrocyte. The detailed protocol and these data are presented by Witt *et al.* (2000).
[b] Mean ± standard error
[c] Vehicle control
[d] Significance tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[e] Analysis of variance, significant at P≤0.025

TABLE E8
Induction of Unscheduled DNA Synthesis and S-Phase DNA Synthesis in the Hepatocytes of F344/N Rats Following Treatment with Riddelliine by Gavage for 5 or 30 Days[a]

Dose (mg/kg)	Number of Animals with Hepatocytes Scored	Cells (%) Unscheduled DNA Synthesis	Cells (%) S-Phase Synthesis
5 Days			
Male			
0	5	1.10 ± 0.49	0.99 ± 0.09
0.33	5	2.66 ± 1.35	6.08 ± 0.82**
1.0	5	4.18 ± 1.25*[b]	2.90 ± 0.47
3.3	5	2.20 ± 0.60	2.33 ± 0.29
Female			
0	5	0.66 ± 0.27	0.49 ± 0.04
0.33	5	1.98 ± 0.54	2.25 ± 0.68**
1.0	5	4.22 ± 0.83**	4.77 ± 1.22**
3.3	5	7.56 ± 3.50**	9.06 ± 1.56**
30 Days			
Male			
0	5	0.22 ± 0.22	0.52 ± 0.09
0.33	5	0.66 ± 0.27	1.84 ± 0.21**
1.0	5	0.66 ± 0.44	2.42 ± 0.49**
3.3	4	2.20 ± 0.64*	1.24 ± 0.29*
Female			
0	5	0.22 ± 0.22	0.52 ± 0.12
0.33	5	0.22 ± 0.22	0.82 ± 0.27
1.0	5	0.88 ± 0.41	1.86 ± 0.30**
3.3	5	1.10 ± 0.70	1.15 ± 0.15*

* Significantly different from the control group ($P \leq 0.05$) by Shirley's test
** Significantly different from the control group ($P \leq 0.01$) by Dunn's or Shirley's test
[a] Study was performed at SRI International. Data are presented as mean ± standard error.
[b] n=3

TABLE E9
Induction of Unscheduled DNA Synthesis and S-Phase DNA Synthesis in the Hepatocytes of B6C3F$_1$ Mice Following Treatment with Riddelliine by Gavage for 5 or 30 Days[a]

Dose (mg/kg)	Number of Animals with Hepatocytes Scored	Cells (%)	
		Unscheduled DNA Synthesis	S-Phase Synthesis
5 Days			
Male			
0	5	1.10 ± 0.49	0.26 ± 0.04
3.3	5	0.66 ± 0.44	0.49 ± 0.18
10.0	5	3.34 ± 0.80	0.39 ± 0.06
25.0	5	11.10 ± 3.27**	0.32 ± 0.07
Female			
0	5	0.44 ± 0.27	2.54 ± 1.14
3.3	5	1.54 ± 0.82	4.30 ± 1.36
10.0	4	3.60 ± 1.46*	4.46 ± 1.51
25.0	5	4.88 ± 1.71**	1.35 ± 0.52
30 Days			
Male			
0	5	1.10 ± 0.60	0.87 ± 0.29
3.3	5	0.00 ± 0.00	1.75 ± 0.19*
10.0	5	3.76 ± 1.04	1.26 ± 0.26
25.0	4	21.65 ± 4.89**	1.07 ± 0.16
Female			
0	4	0.55 ± 0.55	5.15 ± 2.05
3.3	5	3.78 ± 1.98	20.99 ± 5.31
10.0	5	2.66 ± 1.30	0.98 ± 0.45
25.0	5	24.90 ± 3.54**	0.66 ± 0.13

* Significantly different from the control group ($P \leq 0.05$) by Dunn's or Shirley's test
** Significantly different from the control group ($P \leq 0.01$) by Shirley's test
[a] Study was performed at SRI International. Data are presented as mean ± standard error.

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Riddelliine

Riddelliine was obtained from the U.S. Food and Drug Administration (Rockville, MD) in one lot (8194-110-01). Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC), and the study laboratory. Reports on analyses performed in support of the riddelliine studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white, crystalline solid, was identified as riddelliine by infrared, ultraviolet/visible, and proton nuclear magnetic resonance spectroscopy and by low-resolution mass spectrometry (MS). All spectra were consistent with the literature spectra (MRI, 1983) or with the structure of riddelliine. The infrared and nuclear magnetic resonance spectra are presented in Figures F1 and F2. The melting point of 189° to 193° C was in agreement with literature values (MRI, 1982).

The purity of lot 8194-110-01 was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC) by systems A and B (Table E1) and gas chromatographic water analysis. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). The analytical chemistry laboratory performed additional analyses using HPLC/MS by system C to identify and quantify impurities detected by systems A and B. Gas chromatographic water analysis was performed with a Varian 3700 gas chromatograph (Varian, Inc., Palo Alto, CA) and an 80/100 Poropak QS column (6 ft × 0.4 mm). The temperature program was 40° C for 1 minute and then 40° to 120° C at 15° C/minute; the carrier gas was helium at 100 mL/minute. The study laboratory confirmed the purity using HPLC by system D.

Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were in agreement with the theoretical values for riddelliine. Gas chromatography indicated less than 0.1% water. HPLC by system A indicated one major peak and five minor peaks accounting for 6.3% of the total integrated area; HPLC by system B indicated one major peak and two minor peaks accounting for 8.0% of the total integrated area. By both systems, only one impurity peak was present at greater than 1% of the total peak area. This component was identified as retrorsine by HPLC/MS; the identity of an early eluting impurity with a molecular weight of 367 was not determined. The overall purity of lot 8194-110-01 was determined to be approximately 92%. HPLC by system D indicated one major peak and two impurities with a combined area of 6.4% relative to the major peak area. One impurity peak was negligible; the other was identified as retrorsine by comparison to the spectrum of a retrorsine sample obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). The study laboratory confirmed a purity of 92.6% for lot 8194-110-01.

The stability of of riddelliine (lot U101981, not used in the current studies) was tested by Midwest Research Institute (Kansas City, MO) using HPLC by system E. The results indicated that riddelliine is stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at approximately 5° C, protected from light. Stability was monitored during the 2-year studies with HPLC by system D. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 4 weeks by mixing riddelliine with 0.1 M sodium phosphate buffer (pH 6.0) (EM Science, Gibbstown, NJ; Fischer Scientific, Fairlawn, NJ) (Table F2). The dose formulations were stored at 5° C in amber glass bottles for up to 35 days.

Stability studies of a 0.00204 mg/mL formulation were performed by the analytical chemistry laboratory using HPLC by system A with β-hydroxyethyltheophylline added as an internal standard. Homogeneity studies of the

0.0020 and 0.30 mg/mL dose formulations were performed by the study laboratory with HPLC by system D. Homogeneity was confirmed, and stability was confirmed by the analytical chemistry laboratory for at least 35 days for dose formulations stored in sealed glass containers refrigerated at 1° to 3° C or at room temperature.

Periodic analyses of the dose formulations of riddelliine were conducted by the study laboratory using HPLC by system D. The dose formulations were analyzed every 8 to 12 weeks (Table F3). Of the dose formulations analyzed for rats, 51 of 54 were within 10% of the target concentrations; 18 of 20 animal room samples were within 10% of the target concentrations. The three dose formulations that were not within specifications were remixed and reanalyzed; the remixes were found to be within 10% of the target concentrations. Of the dose formulations analyzed for mice, all 44 were within 10% of the target concentrations, with no value greater than 109% of the target concentration; all 16 animal room samples also were within 10% of the target concentrations.

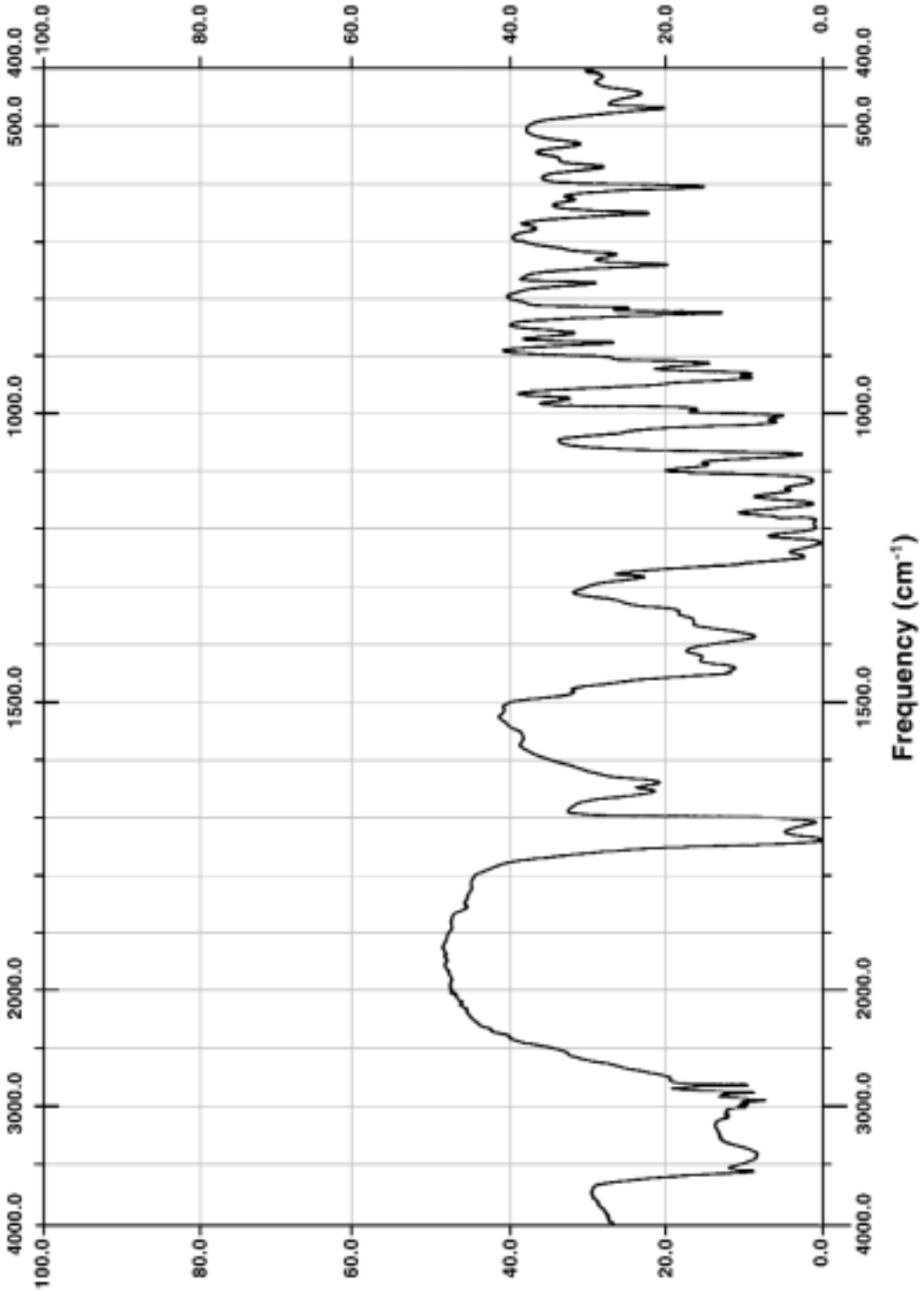

Figure F1
Infrared Absorption Spectrum of Riddelliine

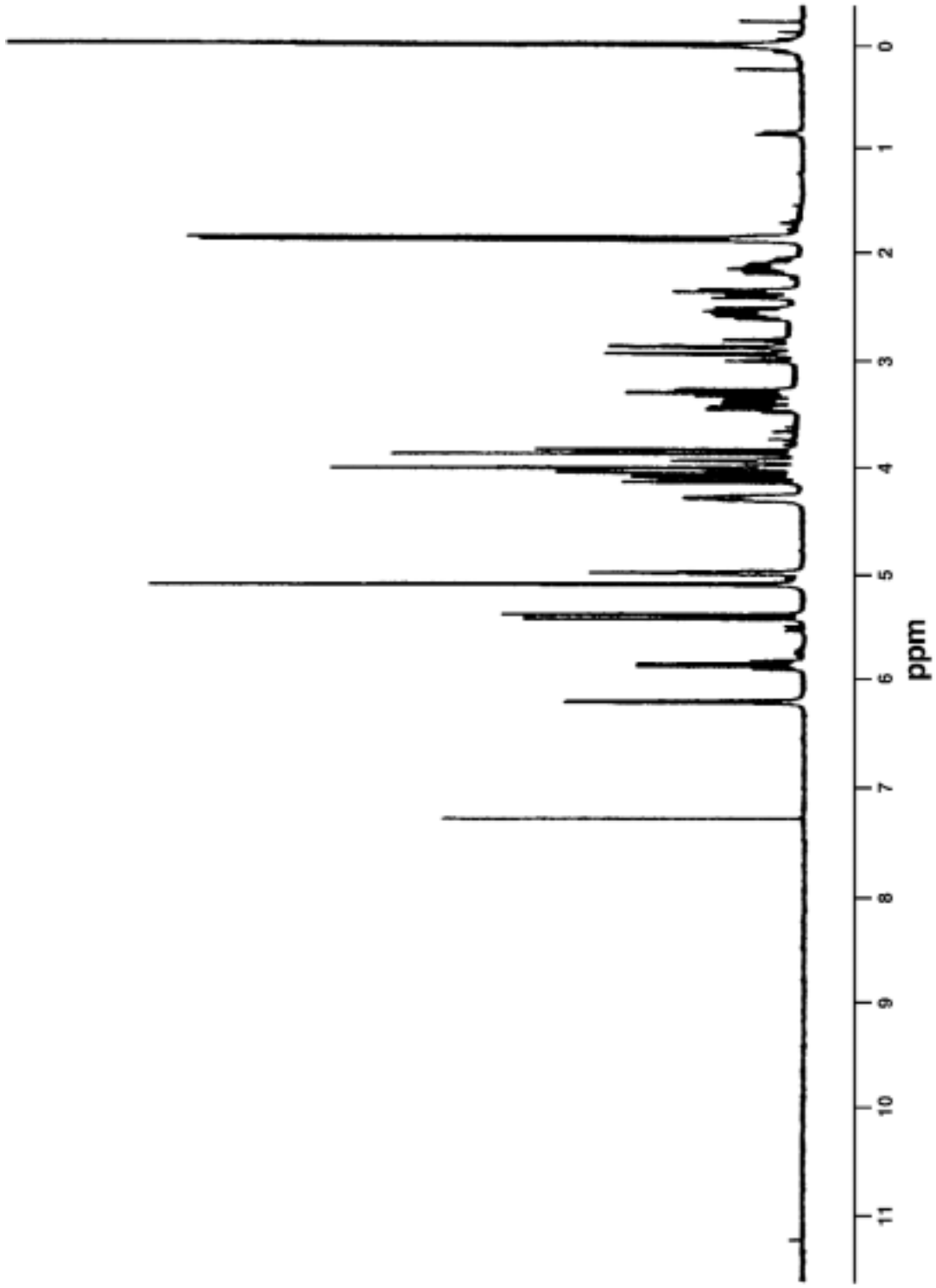

Figure F2
Nuclear Magnetic Resonance Spectrum of Riddelliine

Table F1
High-Performance Liquid Chromatography Systems Used in the 2-Year Gavage Studies of Riddelliine[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (215 nm) light	Phenomenex Primesphere 5 C18-HC 15 cm × 3.2 mm, (Phenomenex, Torrance, CA)	A) Acetonitrile and B) water containing 0.1 M phosphate buffer (pH 6); 10% A:90% B, isocratic, or 10% A:90% B for 5 minutes, then 10% A:90% B to 50% A:50% B in 10 minutes, held 20 minutes; flow rate 0.5 mL/minute
System B		
Ultraviolet (215 nm) light	Hamilton PRP-1, 15 cm × 4.1 mm	A) Acetonitrile and B) water containing 0.049 M ammonium hydroxide (pH 10); 26% A:74% B, isocratic, or 10% A:90% B to 90% A:10% B in 15 minutes, held 30 minutes; flow rate 1.0 mL/minute
System C		
Mass spectrometry with electrospray interface	Hamilton PRP-1, 15 cm × 4.1 mm	A) Acetonitrile and B) water containing 0.005 M ammonium hydroxide (26% A:74% B), flow rate 1.0 mL/minute, split 10:1 prior to mass spectrometry; propionic acid:isopropyl alcohol (75:25), flow rate 100 µL/minute, added post column
System D		
Ultraviolet light (200 to 350 nm or 215 nm)	Phenomenex Primesphere 5 C18-HC, 15 cm × 3.2 mm	0.1 M phosphate buffer (pH 6):acetonitrile (90:10), isocratic; flow rate 0.5 mL/minute
System E		
Ultraviolet (215 nm) light	Altex Ultrasphere Octyl (C_8), 250 mm × 4.6 mm, (Beckman Coulter, Inc., Fullerton, CA)	Aqueous 0.067 M phosphate buffer (pH 6.4): methanol (57:43); flow rate 1.0 mL/minute; xylene sulfonic acid as an internal standard

[a] High-performance liquid chromatographs were manufactured by Waters Corp. (Milford, MA) (systems A, B, C, and E) and Hewlett-Packard (Palo Alto, CA) (system D).

Table F2
Preparation and Storage of Dose Formulations in the 2-Year Gavage Studies of Riddelliine

Preparation
The required amount of riddelliine was added to 0.1 M sodium phosphate buffer (pH 6.0) and stirred with a magnetic stirrer until a homogeneous preparation was obtained. The doses were prepared every 4 weeks.

Chemical Lot Number
8194-110-01

Maximum Storage Time
35 days

Storage Conditions
Stored in sealed amber glass at 5° C

Study Laboratory
Southern Research Institute (Birmingham, AL)

Table F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Riddelliine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
February 29-March 1, 1996	March 1-2, 1996	0.00200	0.00199	0
		0.00660	0.00647	–2
		0.0200	0.0192	–4
		0.0660	0.0994[b]	+51
		0.200	0.191	–4
March 4, 1996	March 4, 1996	0.0660	0.0630[c]	–5
	April 3-4, 1996[d]	0.00200	0.00182	–9
		0.00660	0.00613	–7
		0.0200	0.0191	–4
		0.0660	0.0640	–3
		0.200	0.191	–4
May 22, 1996	May 23-24, 1996	0.00200	0.00191	–4
		0.00660	0.00628	–5
		0.0200	0.0193	–3
		0.0660	0.0634	–4
		0.200	0.193	–3
August 15, 1996	August 15-16, 1996	0.00200	0.00171[b]	–14
		0.00660	0.00617	–7
		0.0200	0.0192	–4
		0.0660	0.0641	–3
		0.200	0.199	0
August 20, 1996	August 20, 1996	0.00200	0.00195[c]	–2
November 7, 1996	November 8-9, 1996	0.00200	0.00197	–1
		0.00660	0.00634	–4
		0.0200	0.02000	0
		0.0660	0.0679	+3
		0.200	0.202	+1
	December 11-12, 1996[d]	0.00200	0.00211	+6
		0.00660	0.00658	0
		0.0200	0.0188	–6
		0.0660	0.0646	–2
		0.200	0.197	–1
January 2, 1997	January 3-4, 1997	0.00200	0.00184	–8
		0.00660	0.00650	–2
		0.0200	0.0196	–2
		0.0660	0.0648	–2
		0.200	0.197	–1

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Riddelliine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
March 27, 1997	March 27-28, 1997	0.00200	0.00207	+4
		0.00660	0.00646	–2
		0.0200	0.0197	–1
		0.0660	0.0648	–2
		0.200	0.196	–2
May 22, 1997	May 22-23, 1997	0.00200	0.00198	–1
		0.00660	0.0064	–3
		0.0200	0.0202	+1
		0.0660	0.0665	+1
		0.200	0.198	–1
	June 25-26, 1997[d]	0.00200	0.00177	–11
		0.00660	0.00626	–5
		0.0200	0.0198	–1
		0.0660	0.0666	+1
		0.200	0.203	+2
August 14, 1997	August 14-16, 1997	0.00200	0.00180	–10
		0.00660	0.00631	–4
		0.0200	0.0184	–8
		0.0660	0.0662	0
		0.200	0.194	–3
October 9, 1997	October 10-11, 1997	0.00200	0.00194	–3
		0.00660	0.00652	–1
		0.0200	0.0194	–3
		0.0660	0.0667	+1
		0.200	0.205	+3
December 4, 1997	December 4-6, 1997	0.00200	0.00184	–8
		0.00660	0.00668	+1
		0.0200	0.0204	+2
		0.0660	0.0677	+3
		0.200	0.213	+7
	January 7-9, 1998[d]	0.00200	0.00173	–13
		0.00660	0.00598	–9
		0.0200	0.0192	–4
		0.0660	0.0639	–3
		0.200	0.210	+5
February 26, 1998	February 27-28, 1998	0.00200	0.00280[b]	+40
		0.00660	0.00602	–9
		0.0200	0.0196	–2
		0.0660	0.0663	0
March 3, 1998	March 3, 1998	0.00200	0.00238[c,b]	+19
		0.00200	0.00210[c]	+5

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Riddelliine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
February 29-March 1, 1996	March 1-2, 1996	0.0100	0.0101	+1
		0.0300	0.0300	0
		0.100	0.0974	–3
		0.300	0.285	–5
	April 3-4, 1996[d]	0.0100	0.00969[e]	–3
		0.0300	0.0288	–4
		0.100	0.0949	–5
		0.300	0.282	–6
May 22, 1996	May 23-24, 1996	0.0100	0.0100	0
		0.0300	0.0289	–4
		0.100	0.0961	–4
		0.300	0.289	–4
August 15, 1996	August 15-16, 1996	0.0100	0.00925	–7
		0.0300	0.0289	–4
		0.100	0.0980	–2
		0.300	0.298	–1
November 7, 1996	November 8-9, 1996	0.0100	0.00980	–2
		0.0300	0.0307	+2
		0.100	0.0973	–3
		0.300	0.307	+2
	December 11-12, 1996[d]	0.0100	0.00969	–3
		0.0300	0.0301	0
		0.100	0.0998	0
		0.300	0.293	–2
January 2, 1997	January 3-4, 1997	0.0100	0.00991	–1
		0.0300	0.0294	–2
		0.100	0.0973	–3
		0.300	0.294	–2
March 27, 1997	March 27-28, 1997	0.0100	0.00946	–5
		0.0300	0.0291	–3
		0.100	0.0987	–1
		0.300	0.294	–2
May 22, 1997	May 22-23, 1997	0.0100	0.0102	+2
		0.0300	0.0299	0
		0.100	0.0990	–1
		0.300	0.298	–1
	June 25-26, 1997[d]	0.0100	0.00986	–1
		0.0300	0.0320	+7
		0.100	0.0967	–3
		0.300	0.310	+3

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Riddelliine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
August 14, 1997	August 14-16, 1997	0.0100	0.0101	+1
		0.0300	0.0303	+1
		0.100	0.0962	–4
		0.300	0.297	–1
October 9, 1997	October 10-11, 1997	0.0100	0.0105	+5
		0.0300	0.0297	–1
		0.100	0.0974	–3
		0.300	0.302	+1
December 4, 1997	December 4-6, 1997	0.0100	0.0102	+2
		0.0300	0.0327	+9
		0.100	0.101	+1
		0.300	0.301	0
	January 7-9, 1998[d]	0.0100	0.0108[e]	+8
		0.0300	0.0312	+4
		0.100	0.101	+1
		0.300	0.292	–3
February 26, 1998	February 27-28, 1998	0.0100	0.00973	–3
		0.0300	0.0288	–4
		0.100	0.0994	–1
		0.300	0.311	+4

[a] Results of duplicate analyses. For rats, 0.00200 mg/mL=0.01 mg/kg; 0.00660 mg/mL=0.033 mg/kg; 0.0200 mg/mL=0.1 mg/kg; 0.0660 mg/mL=0.33 mg/kg; 0.200 mg/mL=1 mg/kg. For mice, 0.0100 mg/mL=0.1 mg/kg; 0.0300 mg/mL=0.3 mg/kg; 0.100 mg/mL=1 mg/kg; 0.300 mg/mL=3 mg/kg.
[b] Remixed; not used in study
[c] Results of remix
[d] Animal room samples
[e] Result of single analysis due to insufficient volume of sample

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

TABLE G1 Ingredients of NTP-2000 Rat and Mouse Ration 238
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TABLE G1
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 G2
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 G3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.5 ± 0.53	12.5 – 14.7	24
Crude fat (% by weight)	8.1 ± 0.30	7.5 – 8.7	24
Crude fiber (% by weight)	9.6 ± 0.45	8.5 – 10.3	24
Ash (% by weight)	5.0 ± 0.16	4.8 – 5.4	24
Amino Acids (% of total diet)			
Arginine	0.731 ± 0.050	0.670 – 0.800	8
Cystine	0.224 ± 0.012	0.210 – 0.240	8
Glycine	0.684 ± 0.041	0.620 – 0.740	8
Histidine	0.333 ± 0.018	0.310 – 0.350	8
Isoleucine	0.524 ± 0.046	0.430 – 0.590	8
Leucine	1.061 ± 0.061	0.960 – 1.130	8
Lysine	0.708 ± 0.056	0.620 – 0.790	8
Methionine	0.401 ± 0.035	0.350 – 0.460	8
Phenylalanine	0.598 ± 0.036	0.540 – 0.640	8
Threonine	0.501 ± 0.051	0.430 – 0.590	8
Tryptophan	0.126 ± 0.014	0.110 – 0.150	8
Tyrosine	0.390 ± 0.056	0.280 – 0.460	8
Valine	0.640 ± 0.049	0.550 – 0.690	8
Essential Fatty Acids (% of total diet)			
Linoleic	3.97 ± 0.284	3.59 – 4.54	8
Linolenic	0.30 ± 0.042	0.21 – 0.35	8
Vitamins			
Vitamin A (IU/kg)	4,570 ± 1,223	2,780 – 8,140	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)[b]	8.0 ± 1.09	6.0 – 10.3	24
Riboflavin (ppm)	5.6 ± 1.12	4.20 – 7.70	8
Niacin (ppm)	74.3 ± 5.94	66.4 – 85.8	8
Pantothenic acid (ppm)	22.5 ± 3.96	17.4 – 29.1	8
Pyridoxine (ppm)[b]	9.04 ± 2.37	6.4 – 12.4	8
Folic acid (ppm)	1.64 ± 0.38	1.26 – 2.32	8
Biotin (ppm)	0.333 ± 0.15	0.225 – 0.704	8
Vitamin B_{12} (ppb)	68.7 ± 63.0	18.3 – 174.0	8
Choline (ppm)[b]	3,155 ± 325	2,700 – 3,790	8
Minerals			
Calcium (%)	0.963 ± 0.047	0.867 – 1.050	24
Phosphorus (%)	0.567 ± 0.025	0.496 – 0.620	24
Potassium (%)	0.659 ± 0.022	0.627 – 0.691	8
Chloride (%)	0.357 ± 0.027	0.300 – 0.392	8
Sodium (%)	0.189 ± 0.019	0.160 – 0.212	8
Magnesium (%)	0.199 ± 0.009	0.185 – 0.213	8
Sulfur (%)	0.178 ± 0.021	0.153 – 0.209	8
Iron (ppm)	160 ± 14.7	135 – 177	8
Manganese (ppm)	50.3 ± 4.82	42.1 – 56.0	8
Zinc (ppm)	50.7 ± 6.59	43.3 – 61.1	8
Copper (ppm)	6.29 ± 0.828	5.08 – 7.59	8
Iodine (ppm)	0.461 ± 0.187	0.233 – 0.843	8
Chromium (ppm)	0.542 ± 0.128	0.330 – 0.707	7
Cobalt (ppm)	0.23 ± 0.049	0.20 – 0.30	7

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

Table G4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.126	0.10 – 0.50	24
Cadmium (ppm)	0.05 ± 0.014	0.04 – 0.10	24
Lead (ppm)	0.09 ± 0.028	0.06 – 0.17	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.16 ± 0.032	0.11 – 0.26	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	16.6 ± 7.55	9.04 – 39.6	24
Nitrite nitrogen (ppm)[c]	0.73 ± 0.39	0.61 – 2.00	24
BHA (ppm)[d]	1.1 ± 0.37	1.0 – 2.5	24
BHT (ppm)[d]	1.1 ± 0.21	1.0 – 1.8	24
Aerobic plate count (CFU/g)[e]	283,250 ± 477,839	41,000 – 1,000,000	4
Coliform (MPN/g)	1.2 ± 2.7	0 – 9	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[f]	5.3 ± 3.75	2.1 – 20.9	24
N-Nitrosodimethylamine (ppb)[f]	2.3 ± 1.69	1.0 – 6.4	24
N-Nitrosopyrrolidine (ppb)[f]	3.0 ± 2.8	1.0 – 14.5	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.079 ± 0.061	0.020 – 0.217	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.263 ± 0.570	0.020 – 2.810	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[a] 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] Nonirradiated samples; microbial counts for 20 irradiated samples were below the detection limit.
[f] All values were corrected for percent recovery.

APPENDIX H
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from randomly selected rats and mice during the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed. At 18 months, live mice were shipped to Microbiological Associates, Inc. for evaluation of viral serology and bacterial profile, including *Helicobacter hepaticus*.

Method and Test	Time of Analysis
RATS	
ELISA	
Mycoplasma arthritidis	6 months and study termination
Mycoplasma pulmonis	6 months and study termination
PVM (pneumonia virus of mice)	6, 12, and 18 months, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
M. arthritidis	Study termination
Parvovirus	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	6, 12, and 18 months
KRV (Kilham rat virus)	6, 12, and 18 months

Method and Test	Time of Analysis
MICE	
Bacterial Assays	
Oral	18 months
Fecal	18 months
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM (epizootic diarrhea of infant mice)	6, 12, and 18 months, study termination
GDVII (mouse encephalomyelitis virus)	6, 12, and 18 months, study termination
LCM (lymphocytic choriomeningitis virus)	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV (mouse hepatitis virus)	6, 12, and 18 months, study termination
M. arthritidis	12 and 18 months, study termination
M. pulmonis	12 and 18 months, study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Ectromelia virus	12 months
GDVII	12 and 18 months
Helicobacter spp.	18 months
Mouse adenoma virus-FL	Study termination
MCMV (mouse cytomegalovirus)	12 and 18 months, study termination
Parvovirus	Study termination
Hemagglutination Inhibition	
K (papovavirus)	6, 12, and 18 months
MVM (minute virus of mice)	6, 12, and 18 months
Polyoma virus	6, 12, and 18 months

RESULTS

For the 2-year study in rats, all serology tests were negative. Bacterial profiles of sentinel mice at 18 months indicated *Enterococcus faecalis* in five males. These had no impact on the study results. Two mice had positive titers for *M. arthritidis* at 12 months. Further evaluation of the samples positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may have been due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only sporadic samples were positive and there were no clinical findings or histopathologic changes of *M. arthritidis* infection in animals with positive titers. Accordingly, *M. arthritidis*-positive titers were considered false positives.

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DNA ADDUCT CHARACTERIZATION STUDIES

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DNA ADDUCT CHARACTERIZATION STUDIES

Introduction

The mechanistic studies reported here include metabolic activation of riddelliine and identification of activated metabolites, development and validation of a ^{32}P-postlabeling/HPLC method for identification of riddelliine-derived DNA adducts, detection and quantitation of these adducts in livers of female F344/N rats orally gavaged with riddelliine for 3 or 6 months, and determination of metabolic activation of riddelliine mediated by human liver microsomes and riddelliine-DNA adduct formation *in vitro* to determine the relevance of the interspecies mechanistic data.

Materials and Methods

Riddelliine was obtained from the NTP (Research Triangle Park, NC). Phenobarbital (sodium salt), calf thymus DNA (sodium salt, type I), 2′-deoxyguanosine-3′-monophosphate (sodium salt) (3′-dGMP), 2′-deoxyguanosine-5′-monophosphate (free acid) (5′-dGMP), adenosine 5′-triphosphate (disodium salt) (ATP), glucose-6-phosphate, glucose-6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate ($NADP^+$), nuclease P1, micrococcal nuclease (MN), spleen phosphodiesterase (SPD), bicine, spermidine, and dithiothreitol were purchased from Sigma Chemical Co. (St. Louis, MO). Monocrotaline, *o*-bromanil, and barium hydroxide octahydrate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Cloned T4 polynucleotide kinase (PNK) was obtained from U.S. Biochemical Corp. (Cleveland, OH). [γ-^{32}P]-Adenosine-5′-triphosphate ([γ-^{32}P]-ATP; specific activity of at least 7,000 Ci/mmol) was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA). All other reagents were obtained through commercial sources and were the highest grade available. All solvents used were high-performance liquid chromatography (HPLC) grade.

Retronecine was prepared from barium hydroxide-catalyzed hydrolysis of monocrotaline by the procedure of Hoskins and Crout (1977). A solution of monocrotaline (1 gram, 3.1 mmol) and barium hydroxide octahydrate (2 grams, 6.3 mmol) in water (10 mL) was heated at reflux for 2 hours. The solution was cooled, treated with carbon dioxide (dry ice), filtered, acidified with 1 N hydrochloric acid to adjust the pH to 3 to 4, and then extracted repeatedly with ethyl ether. The aqueous phase was collected and concentrated under reduced pressure. The residue was then passed through a column of AG 1-X8 ion-exchange resin (20 grams, hydroxide form, 200-400 mesh; Bio-Rad Laboratories, Hercules, CA) and eluted with water until the eluate was neutral. The combined eluates were evaporated and extracted three times with hot acetone. After filtration to remove the precipitate, the filtrate was collected and the solvent was removed under reduced pressure. The resulting residue was crystallized from acetone to give 392 mg retronecine in 81% yield.

As reported by Christie *et al.* (1949) and Mattocks *et al*. (1989), DHR can be prepared either by dehydrogenation of retronecine or dehydration of retronecine-*N*-oxide. Dehydrogenation of retronecine was chosen for the DHR preparation. Briefly, to a solution of retronecine (100 mg, 650 µmol) in chloroform ($CHCl_3$) (30 mL) in an ice bath was added *o*-bromanil (300 mg, 708 µmol) in $CHCl_3$ (6 mL) dropwise with stirring, in 2 minutes. The hydroquinone by-product was removed by extraction with anion exchange AG 1-X8 resin (200-400 mesh, hydroxide form). The organic phase was separated and concentrated under reduced pressure, providing practically pure DHR, which was recrystallized from light petroleum ether giving pure DHR as white prisms in 40% yield.

Riddelliine-*N*-oxide was synthesized by oxidation of riddelliine (350 mg, 1 mmol) in hot alcohol (5 mL) with 30% hydrogen peroxide (0.1 mL, 1 mmol) at 60° C for 4 hours (Christie *et al.*, 1949). Upon removal of solvent under reduced pressure, the residue was partitioned between water and $CHCl_3$. The aqueous phase was collected and concentrated under reduced pressure, providing crude riddelliine-*N*-oxide in 85% yield. Pure riddelliine-*N*-oxide was obtained by recrystallization from ethanol; mass *m/z* 365 (M^+).

Metabolism of Riddelliine Leading to Activated Metabolites

For the studies of microsomal metabolism of riddelliine, female F344/N rats were obtained from the breeding colony of the National Center for Toxicological Research (NCTR) as weanlings. Liver microsomes of female F344/N rats treated with phenobarbital (PB-microsomes) were prepared according to published procedures (Chou *et al.*, 1987). The rats were intraperitoneally injected once daily with 75 mg phenobarbital/kg body weight in 0.5 mL water for three consecutive days. Twenty-four hours after the final injection, rats were sacrificed by carbon dioxide asphyxiation. The livers were perfused with cold 1.15% potassium chloride via the portal vein and immediately stored at -78° C. The liver microsomes were prepared from the thawed livers by differential centrifugation methods (Chou *et al.*, 1987) and stored at -78° C prior to use. Liver microsomes of untreated female F344/N rats (control microsomes) were prepared similarly. Protein concentrations were determined using a protein assay based on the Bradford method using a Bio-Rad protein detection kit (Bio-Rad Laboratories, Hercules, CA).

Metabolism of riddelliine by PB-microsomes was performed in a 1.0 mL incubation volume containing 100 mM sodium phosphate buffer (pH 7.6), 5 mM magnesium chloride, 1 mM $NADP^+$, 8 mM glucose 6-phosphate, 2 units glucose-6-phosphate dehydrogenase, 4 mg PB-microsomes, and riddelliine (2 µmol in 50 µL DMSO) at 37° C for 30 minutes. The incubation mixture was centrifuged at 105,000 g for 30 minutes at 4° C to remove microsomal proteins. The supernatant was collected and the metabolite mixture was separated by reversed-phase HPLC employing a Prodigy 5 µm ODS column (4.6 mm x 250 mm, Phenomenex, Torrance, CA) eluted isocratically with 20 mM ammonium acetate (NH_4OAc) buffer (pH 7) at 1 mL/minute for 10 minutes followed by a 40-minute linear gradient from 20 mM NH_4OAc buffer (pH 7) to 50% methanol in the buffer. The metabolite mixture was subsequently analyzed by liquid chromatography/mass spectrometry (LC/MS).

Metabolism of riddelliine by control-microsomes was similarly conducted and the metabolites were analyzed by HPLC and LC/MS.

Development of a ^{32}P-Postlabeling/HPLC Method for Detection of DHR-Derived DNA Adducts *In vivo* and *In vitro*:

Synthesis of 3′-monophosphate-7-(deoxyguanosin-N^2-yl)dehydrosupinidine (DHR-3′-dGMP) adducts was performed by purging a solution of 3′-dGMP (20 mg, 60 µmol) in 4 mL of 20 mM K_2CO_3 buffer (pH 8.0) with argon for 5 minutes. DHR (18 mg, 120 µmol) was added, and the resulting solution was stirred anaerobically at 60° C for 6 hours. The reaction mixture was then filtered through a 0.22 µm Millipore filter. The filtrate was concentrated to half of the volume under reduced pressure and the adducts were purified by HPLC on a Whatman Partisil ODS-3 (4.6 mm × 250 mm; Whatman, Maidstone, Kent, England) column eluted with 20 mM K_2CO_3 buffer (pH 8.0) isocratically at a flow rate of 1 mL/minute, and monitored at 254 and 220 nm. The DHR-3′-dGMP adducts were further purified on an analytical Prodigy 5 µm ODS column (4.6 mm × 250 mm; Phenomenex) eluted isocratically with 10% methanol in 20 mM NH_4OAc buffer (pH 7.0) at flow rate of 1 mL/minute. The synthesis was repeated on a larger scale so that a greater quantity of DHR-3′-dGMP adducts was obtained for structural determination and for use as external standards for ^{32}P-postlabeling/HPLC.

Synthesis of DHR-2′-deoxyguanosine-3′,5′-bisphosphate (DHR-dG 3′,5′-bisphosphate) adducts was performed by incubating a solution of 10 nmol DHR-3′-dGMP in 50 µL water with a 50 µL reaction mix containing 0.4 µmol ATP, 150 units PNK, 40 mM bicine-NaOH (pH 9.5), 20 mM magnesium chloride, 2 mM spermidine, and 20 mM dithiothreitol (DTT) at 37° C for 40 minutes. The resulting products were separated by HPLC using a Prodigy 5 µm ODS column (4.6 mm × 250 mm; Phenomenex), eluted isocratically with 10% methanol in 20 mM NH_4OAc buffer at 1 mL/minute, and monitored at 254 nm with a Waters 996 photodiode array detector (Waters, Milford, MA).

Synthesis of DHR-2′-deoxyguanosine-5′-monophosphate (DHR-5′-dGMP) adducts was performed following the procedure of Wickramanayake *et al*. (1985). DHR-5′-dGMP was synthesized by reaction of DHR (18 mg, 120 µmol) with 5′-dGMP (64 mg, 180 µmol) in aqueous K_2CO_3 buffer at pH 7.4 at 60° C for 6 hours. The

precipitate was removed by filtration and the products in the filtrate were separated by semipreparative HPLC. The crude DHR-5′-dGMP adducts were isolated using a Prodigy 5 µ ODS column (10 mm × 250 mm; Phenomenex) equilibrated with 20 mM NH_4OAc buffer (pH 7.0). After applying the sample, the column was eluted with a 40-minute linear gradient from 20 mM NH_4OAc buffer to 50% methanol in 20 mM NH_4OAc buffer with a flow rate of 2 mL/minute. The adducts were further purified on an analytical Prodigy 5 µ ODS column (4.6 mm × 250 mm; Phenomenex) eluted isocratically with 20% methanol in 8 mM NH_4OAc buffer (pH 7.0) at flow rate of 1 mL/minute. The collected fractions were lyophilized and stored at -70° C until use.

Synthesis of 3′-monophosphate-7-(deoxyadenosin-N^6-yl)dehydrosupinidine (DHR-3′-dAMP) adducts was performed in a manner similar to the synthesis of DHR-3′-dGMP. DHR-3′-dAMP was prepared by reaction of 3′-dAMP (43.2 mg, 130 µmol) in 8 mL of 20 mM K_2CO_3 buffer (pH 8.0) with DHR (40 mg, 261 µmol) at 60° C for 40 hours. The resulting products were separated by HPLC using a Prodigy 5 µ ODS column (4.6 mm × 250 mm; Phenomenex) eluted at 1.0 mL/minute by a 40-minute linear gradient from 20 mM NH_4OAc to 50% methanol in 20 mM NH_4OAc buffer. The collected adducts were further purified by reversed-phase HPLC using the same conditions for the purification of DHR-3′-dGMP.

To achieve chemical reaction of DHR with calf thymus DNA, purified calf thymus DNA (2.5 mg, 7.5 µmol) in 2.5 mL 20 mM K_2CO_3 buffer (pH 7.5) was reacted with 64 nmol of DHR at 37° C for 40 minutes. After incubation, the reaction mixture was extracted twice with 2.5 mL of chloroform/isoamyl alcohol (24/1). The DNA in the aqueous phase was precipitated by adding 250 µL of 3 M sodium acetate followed by an equal volume of cold isopropanol and washed with 70% ethanol. After redissolving in 20 mM K_2CO_3 buffer (pH 7.5), the DNA concentration and purity were analyzed spectrophotometrically. The DNA was stored at -78° C prior to ^{32}P-postlabeling/HPLC analysis.

Initially, conventional ^{32}P-postlabeling enzymatic digestion procedures were employed for analysis of DHR-3′-dGMP and DHR-3′-dAMP adducts (Reddy and Randerath, 1986). Briefly, 10 µg of DNA (in 10 µL distilled water) from reaction of DHR and calf thymus DNA was enzymatically hydrolyzed to the corresponding 2′-deoxyribonucleoside-3′-monophosphates at 37° C for 4 hours by 1.25 units of MN and 62 milliunits of SPD contained in a 20 µL solution of 20 mM sodium succinate and 10 mM of calcium chloride (pH 6.0). Two enrichment methods, nuclease P1 treatment and *n*-butanol extraction, were employed. For the nuclease P1 method, the MN/SPD digested DNA solutions were incubated at 37° C for 40 minutes with nuclease P1 (8 µg, in 4 µL of buffer containing 0.24 M sodium acetate and 2 mM zinc chloride at pH 5.0) to remove normal 3′-monophosphate-2′-deoxyribonucleosides. The resulting incubation mixture was then evaporated to dryness under reduced pressure and redissolved in 10 µL of distilled water for ^{32}P-postlabeling. For enrichment by *n*-butanol extraction, the incubation mixture (30 µL) was extracted with water-saturated *n*-butanol (2 × 100 µL) in the presence of the phase-transfer agent, tetrabutylammonium chloride. A buffer solution (4 µL) containing 50 mM bicine (pH 9.0) and 0.1 M DTT (2:1) was added, and the *n*-butanol was removed under reduced pressure. The samples were redissolved in 25 µL of water for ^{32}P-postlabeling.

To determine optimal conditions for DNA digestion and adduct enrichment, enzymatic digestion of DNA from reaction of DHR and calf thymus DNA was also conducted with different quantities of digestion enzymes, including using 1/4, 1/8, 1/16, and 1/32 of the MN and SPD quantities employed above. To determine whether or not MN and SPD enzymes would affect the yield of DHR-derived DNA adducts, incubation of the synthetically prepared DHR-3′-dGMP and DHR-3′-dAMP adducts (30-60 fmol) with MN/SPD was compared.

Each MN/SPD-digested DNA sample was dissolved in 10 µL of distilled water and ^{32}P-phosphorylated by incubating with 10 µL of PNK mix containing 100 µCi of [γ-^{32}P]-ATP (specific activity of at least 7,000 Ci/mmol), 12 units of PNK, and 2 µL of 10X PNK buffer (200 mM bicine-NaOH, pH 9.6, 100 mM DTT, 100 mM $MgCl_2$, and 10 mM spermidine) at 37° C for 40 minutes. The labeled mixture was injected onto a Prodigy 5 µ ODS column (4.6 mm × 250 mm; Phenomenex) and eluted isocratically with 20 mM NaH_2PO_4 buffer (pH 4.5) for 10 minutes, followed by a 60-minute linear gradient of 20 mM NaH_2PO_4 (pH 4.5) to 15% methanol in 20 mM NaH_2PO_4 buffer. The HPLC flow rate was 1.0 mL/minute, and the scintillation fluid flow rate was 3.0 mL/minute.

To avoid interference by the high radioactivity of the free ^{32}P and the unreacted [γ-^{32}P]-ATP, the on-line FLO-ONE radioactivity detector (Radiomatic Instruments, Tampa, FL) was equipped with a diverter, and the eluent from the first 40 minutes was diverted away from the radioactivity detector.

Besides using 10 µg of DNA, ^{32}P-postlabeling/HPLC analysis of DHR-3′-dGMP was also conducted with 5, 3, 1, and 0.5 µg of DNA under conditions similar to those described above.

An aliquot containing 10 µg DNA from reaction of DHR and calf thymus DNA was digested by MN/SPD, enriched by nuclease P1, and ^{32}P-postlabeled as described above. As a control experiment, 8.3 fmol of the synthetic DHR-3′-dGMP was also ^{32}P-postlabeled in parallel under the same conditions. To analyze the [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts, the labeled mixture was applied onto 10 cm × 10 cm polyethyleneimine (PEI) cellulose plates (Macherey-Nagel, Düren, Germany) and a two-dimensional development as previously described (Reddy and Randerath, 1986; Gupta, 1993) was carried out. Autoradiography was performed on DuPont Cronex (DuPont Corp., Wilmington, DE) films and the radioactivity on the thin-layer chromatography (TLC) spots was quantitated by Cerenkov counting.

The labeled mixture containing [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts obtained above was adjusted to pH 5.0 with 4 µL of 0.4 M acetic acid and then 3′-dephosphorylated with 17.5 µg of nuclease P1 (5 µg/µL in 0.42 M sodium acetate and 2 mM $ZnCl_2$) at 37° C for 5 hours. The dephosphorylated mixture was then subjected to NaH_2PO_4/methanol elution HPLC analysis under conditions described above.

^{32}P-Postlabeling/HPLC Analysis of DHR-Derived DNA Adducts in NTP Female F344/N Rat Liver Samples

A DNA sample that served as a negative control was prepared by reaction of purified calf thymus DNA (2.5 mg, 7.5 µmol, dissolved in 2.5 mL distilled water) with riddelliine (64 nmol) at 37° C for 40 minutes. After incubation, the reaction mixture was extracted twice with 10 mL of $CHCl_3$ (24:1). The DNA in the aqueous phase was precipitated by adding 250 µL of 3 M sodium acetate followed by an equal volume of cold isopropanol and washed with 70% ethanol. After being dissolved in 20 mM K_2CO_3 buffer (pH 7.5), the DNA concentration and purity were analyzed spectrophotometrically. The DNA was stored at -78° C prior to ^{32}P-postlabeling/HPLC analysis.

In the 2-year NTP gavage study, groups of 6 female F344/N rats were administered riddelliine (5 days/week) in 0.1 M sodium phosphate buffer at doses of 0, 0.01, 0.033, 0.1, 0.33, or 1.0 mg/kg per day until terminal sacrifice at 3 or 6 months. After sacrifice, liver tissue was collected, stored at -78° C, and shipped to NCTR for DNA adduct analysis. Following extraction using RecoverEase DNA Isolation Kit (Stratagene, Cedar Creek, TX), DHR-derived DNA adducts contained in the liver samples were analyzed according to the developed ^{32}P-postlabeling/HPLC methodology described above.

To identify the DHR-derived DNA adducts in the NTP liver samples, DNA from reaction of DHR and calf thymus DNA was ^{32}P-postlabeled and analyzed by HPLC in parallel. For quantitation of each sample, the two epimeric DHR-3′-dGMP synthetic standards, in amounts that closely matched the range of modification in the liver DNA samples, were also analyzed in parallel. To provide a negative control, DNA from the chemical reaction of riddelliine with calf thymus DNA was also ^{32}P-postlabeled and subjected to HPLC analysis.

The levels of adducts *in vivo* were compared by a two-way analysis of variance (ANOVA) combined with Dunnett's test with dose and time as factors and total and individual adducts as the dependent variable (Sigmastat, version 2.0, SPSS, Inc., Chicago, IL). Separate analyses to compare individual adducts were performed using Student's *t*-test.

Human Liver Microsome-Mediated DHR-Derived DNA Adduct Formation

Human livers were obtained from organ donor samples through a gift from Dr. Fred F. Kadlubar. Four male (#4886, 80-year-old; #9504, 62-year-old; #9603, 57-year-old; and #9310, 50-year-old) and four female (#5807, 69-year-old; #C055G, 52-year-old; #9603, 50-year-old, and #9502, 30-year-old) liver samples were used for study. Donor #9502 was African; all other donors were Caucasian.

Human liver microsomes were prepared and stored as described by Culp *et al.* (1997). Protein concentrations were determined using the Bio-Rad protein detection kit described earlier (Bio-Rad Laboratories). For comparison, liver microsomes of female F344/N rats obtained from the NCTR breeding colony were similarly prepared.

The metabolism of riddelliine by human liver microsomes was first conducted in a 2.0 mL incubation volume containing 100 mM sodium phosphate buffer (pH 7.6), 5 mM $MgCl_2$, 2 mM $NADP^+$, 8 mM glucose-6-phosphate, 4 units glucose-6-phosphate dehydrogenase, riddelliine (3.0 mM in 40 µL DMSO), and 7.0 mg microsomal protein at 37° C for 30 minutes. After incubation, the incubation mixture was centrifuged at 105,000 × g for 30 minutes at 4° C to remove microsomal proteins. The supernatant was collected and the metabolite mixture was separated by reversed-phase HPLC employing a Prodigy 5 µ ODS column (10 mm × 250 mm; Phenomenex) eluted isocratically with 20 mM NH_4OAc buffer (pH 7) at 5 mL/minute for 10 minutes followed by a 30-minute linear gradient from 20 mM NH_4OAc buffer (pH 7.0) to 50% methanol in the buffer.

The metabolism of riddelliine by human liver microsomes in the presence of calf thymus DNA was conducted in a 0.5 mL incubation volume containing 100 mM sodium phosphate buffer (pH 7.6), 5 mM magnesium chloride, 1 mM NADPH, 0.1 mg microsomal protein, and calf thymus DNA (1 mg/mL) at 37° C for 5 minutes. After riddelliine (0.01, 0.025, 0.05, 0.1, or 0.25 µM) was added, incubation was continued for 30 minutes. After incubation, the reaction was terminated by cooling with ice water, and sequentially extracted with 0.5 mL phenol, twice with 0.5 mL phenol:chloroform:isoamyl alcohol (25:24:1), and 0.5 mL chloroform:isoamyl alcohol (24:1). The DNA in the aqueous phase was precipitated by adding 0.05 mL 5 M sodium chloride followed by an equal volume of cold ethanol and three washes with 70% ethanol. After being dissolved in 300 µL distilled water, the DNA concentration and purity were analyzed spectrophotometrically. The DNA was stored at -78° C prior to ^{32}P-postlabeling/HPLC analysis.

A Waters HPLC system (Waters, Inc.), consisting of a Model 600 controller, a Model 996 photodiode array detector, and a pump, was used for the separation and purification of DHR-derived DNA adducts. Electrospray (ES) mass spectrometry was performed using a Platform II single quadrupole instrument (Micromass, Inc., Altrincham, England). ES tandem mass spectrometry was performed using a Quattro LC (Micromass, Inc.). Separate MS functions were used to acquire full-scan data at a low and a high cone voltage in a single chromatographic run (e.g., 20 and 40 V, respectively, for *m/z* 100-600). ES tandem mass spectrometry was performed using the negative and/or positive ion mode with a source temperature of 80° C for infusion with a syringe pump. Product ion scans were obtained from collisional-induced dissociation of selected ions using a cone voltage between 37 and 40, and collision energies between 24 and 31 eV. The collision gas was argon at pressures between 2×10^{-3} and 4×10^{-3} mbar. LC/MS samples (5 µL injection volume) were introduced into the ES probe following separation with a Prodigy 5 µ ODS column (4.6 mm × 250 mm; Phenomenex), eluting with the conditions previously described, and split to approximately 0.2 mL/minute entering the probe. The ^1H nuclear magnetic resonance (NMR) experiments were carried out on a Bruker AM 500 MHz spectrometer (Bruker Instruments, Billerica, MA) at 301 K. Samples were dissolved in 0.6 mL deuterated water (D_2O). The D_2O peak was assigned a resonance of 4.7 ppm. Typical NMR spectral acquisition parameters were as follows: data size, 32K; flip angle, 90 degrees; sweep width, 6,000 Hz; and relaxation delay, 1 second. Nuclear Overhauser enhancement (NOE) difference and homonuclear coupling NMR experiments were conducted to assist in proton resonance assignment. Circular dichroism (CD) spectra of DHR-3′-dGMP adducts were determined with a quartz cell of 1 cm path length at ambient temperature on a Jasco 500A spectropolarimeter (Jasco, Inc., Easton, MD). CD spectra are expressed by ellipticity (in millidegrees) for 20 mM NH_4OAc solutions that read 1.0 absorbance in an ultraviolet (UV)-visible spectrophotometer at the wavelength of maximum absorption in a quartz cell of 1-cm path length.

RESULTS

Metabolism of Riddelliine Leading to Activated Metabolites

Riddelliine was incubated aerobically with PB-microsomes for 30 minutes. Upon removal of microsomal protein by ultracentrifugation, the metabolism products contained in the supernatant were separated and analyzed by reversed-phase HPLC (Figure I1). The chromatographic peak eluting at 43 to 48 minutes contained the recovered substrate, riddelliine. By comparison of the HPLC retention times, UV-visible absorption, and mass spectral data (not shown) with those of the synthetically prepared standards, the metabolites contained in chromatographic peaks eluting at 28.3 and 35.2 minutes were identified as DHR and riddelliine-*N*-oxide. Because the chromatographic peaks eluted prior to 26 minutes were also detected from a control incubation to which riddelliine was not added, they were not metabolites. Mass spectral analysis of the materials contained in these peaks (data not shown) confirmed that no metabolites were present.

Metabolism of riddelliine by liver microsomes of untreated rats (control-microsomes) was conducted under similar conditions. Based on comparison of HPLC peak area and UV-visible absorption intensity of the riddelliine-*N*-oxide and DHR metabolites, the rate of metabolism of riddelliine by PB-microsomes was 3.5-fold greater than that by control-microsomes (Table I1).

Development of a ^{32}P-Postlabeling/HPLC Method for Detection of DHR-derived DNA Adducts *In vivo* and *In vitro*:

Retronecine was prepared from barium hydroxide-catalyzed hydrolysis of monocrotaline by the procedure of Hoskins and Crout (1977). DHR was synthesized by dehydrogenation of retronecine with *o*-bromanil in $CHCl_3$ (Mattocks *et al.*, 1989). Its structure was confirmed by ^{1}H NMR spectral analysis: (DMSO-d_6): δ 6.53 (d, $J_{2,3}$ = 2.5 Hz, 1, H_3), 6.01 (d, $J_{2,3}$ = 2.5 Hz, 1, H_2), 4.99 (m, 1, H_7), 4.90 (d, 1, OH_7), 4.36 (m, 1, H_9), 4.32 (m, 1, H_9), 3.97 (m, 1, H_5), 3.77 (m, 1, H_5), 2.61 (m, 1, H_6), 2.17 (m, 1, H_6). The NMR chemical shift assignment of DHR was determined by NOE difference experiments, homonuclear coupling experiments, peak splitting, and peak integration.

DHR-3′-dGMP adducts were synthesized by reaction of DHR with 3′-dGMP. The resulting reaction products were purified by HPLC, first onto a Whatman ODS-3 column to remove most of the excess 3′-dGMP (data not shown) and further purified with a Prodigy 5 μ ODS column (Figure I2). The materials contained in the chromatographic peaks eluted at 21.9 and 25.5 minutes showed a baseline separation and exhibited identical UV-visible absorption spectra (Figure I3a) that were identical to that of DHR-5′-dGMP reported by Wickramanayake *et al*. (1985). These products were also characterized from the ES/MS negative product ion spectra, which were identical (Figure I4). The spectrum included the deprotonated molecule $(M-H)^-$ *m/z* 481, and product ions corresponding to the deprotonated base (*m/z* 285), deoxyribose-monophosphate (*m/z* 195), deoxyribose-monophosphate-H_2O (*m/z* 177), and ions derived from phosphate ion (*m/z* 97 and 79). The positive ion product spectrum (not shown) contained the protonated molecule $(M+H)^+$ of *m/z* 483, loss of H_2O (*m/z* 465), the protonated DHR-guanine (*m/z* 269), dGMP (*m/z* 348), guanine (*m/z* 152), and DHR-H_2O (*m/z* 136). These data suggested that the materials contained in the chromatographic peaks at 21.9 and 25.5 minutes shown in Figure I2 were epimeric DHR-3′-dGMP adducts.

The CD spectra of both adducts were determined (Figure I3b). The CD Cotton effects of the first adduct were a mirror image of those of the second adduct, indicating that these two adducts were a pair of epimers. These CD spectra also had Cotton effects similar to those of 7-(deoxyguanosin-N^2-yl)dehydrosupinidine previously reported by Wickramanayake *et al.* (1985). The ^{1}H NMR spectrum of the adduct mixture was determined by NMR decoupling and NOE techniques as well as by comparison of the NMR data of DHR and 3′-dGMP measured under identical conditions (Figure I5 and Table I2). Consequently, based on mass, CD, and NMR spectral analysis, the structures of these two products were characterized as a pair of epimeric DHR-3′-dGMP adducts.

Based on the analysis by proton NMR using an internal standard (1,4-dioxane), the quantity of these adducts obtained from organic reaction followed by repeated purification was 0.91 mg. Using the UV-visible absorbance measurement in water, the molar extinction coefficient of DHR-3′-dGMP adducts at 254 nm was determined to be 4.7×10^4 $M^{-1}cm^{-1}$.

Reactions of DHR and 3′-dGMP were carried out three times, with DHR:3′-dGMP in 1:1, 2:1, and 3:1 molar ratio. The reaction with a ratio of 2:1 provided the highest yield (2.5 %).

Similar to the synthesis and HPLC purification of DHR-3′-dGMP, DHR-5′-dGMP was prepared by reaction of DHR with 5′-dGMP followed by two HPLC separations. The HPLC profile of the second HPLC purification provided a baseline separation of the DHR-5′-dGMP adducts (Figure I6). Negative ion ES mass spectral analysis showed that both of these adducts had product ion spectra that were identical to the DHR-3′-dGMP adducts (Figure I7). The positive ion product spectrum (not shown) contained the protonated molecule $(M+H)^+$ of *m/z* 483, loss of H_2O (*m/z* 465), the protonated DHR-guanine (*m/z* 269), dGMP (*m/z* 348), guanine (*m/z* 152), and DHR-H_2O (*m/z* 136).

The ^1H NMR spectrum was assigned by NOE difference experiments, homonuclear coupling experiments, peak splitting, and peak integration. H1′ (6.35 ppm) to H5′ (4.04 ppm) and H8 (8.77 ppm) were assigned by NOE techniques. The chemical shift and coupling constant assignments are shown in Table I2.

There was a very strong similarity between the chemical shifts of 3′-dGMP and DHR-3′-dGMP, and between 5′-dGMP and DHR-5′-dGMP. The biggest difference between the 3′-dGMP and 5′-dGMP chemical shifts was seen along the backbone H3′, H5′, and H5′′ NMR chemical shifts. There was a very strong similarity between the DHR NMR chemical shifts of DHR-3′-dGMP and the DHR-5′-dGMP.

These adducts had the UV-visible absorption spectra identical to those of the DHR-5′-dG reported by Wickramanayake *et al*. (1985). Thus, based on UV-visible absorption, mass, and NMR spectral analysis, the compounds contained in chromatographic peaks eluted at 25.1 and 27.7 minutes in Figure I6 were identified as DHR-5′-dGMP adducts, respectively. The reaction yield was 0.9%.

DHR-3′,5′-dG-bisphosphate was synthesized by 5′-phosphorylation of the synthetically prepared epimeric DHR-3′-dGMP adducts with cold (nonradioactive) ATP catalyzed by PNK. The resulting reaction mixture was purified by reversed-phase HPLC (Figure I8). The chromatographic peaks eluted at 19.2 and 23.8 minutes contained the two recovered substrates, epimeric DHR-3′-dGMP adduct I and adduct II, respectively. The materials contained in chromatographic peaks eluted at 6.2 and at 7.3 minutes, respectively had UV-visible absorption spectra similar to that of DHR-3′-dGMP (Figure I3a). These materials were characterized by analysis of their identical negative ion ES mass spectra (Figure I9). The deprotonated molecule, $(M-H)^-$, was *m/z* 561 and product ions corresponding to loss of H_2O (*m/z* 543), loss of one H_3PO_4 (*m/z* 463), loss of the DHR moiety (*m/z* 426), loss of two H_3PO_4 (*m/z* 365), the ribose-3′,5′-bisphosphate ion (*m/z* 275), loss of H_2O from the ribose-3′,5′-bisphosphate ion (*m/z* 257), ribose-monophosphate ion (*m/z* 195), $H_3P_2O_7^-$ (*m/z* 177), $HP_2O_6^-$ (*m/z* 159), $H_2PO_4^-$ (*m/z* 97), and PO_3^- (*m/z* 79). Based on UV-visible absorption and mass spectral analysis, the structures of these two reaction products were identified as DHR-3′,5′-dG-bisphosphate adduct I and DHR-3′,5′-dG-bisphosphate adduct II, respectively.

Similar to the synthesis of DHR-3′-dGMP, DHR-3′-dAMP was prepared by reaction of DHR with 3′-dAMP followed HPLC separations. The profile of HPLC purification is shown in Figure I10. The chromatographic peak eluted at 18.2 minutes contained the recovered substrate, DHR. The materials contained in the chromatographic peaks eluted at 13.2, 15, 20.5, and 23.5 minutes were analyzed using LC-ES/MS. As shown in Figures 11A through 11D, these four products had identical mass spectra with $(M-H)^-$ ion (*m/z* 465), M-deoxyribose-PO_4^- (*m/z* 269), deoxyribose-PO_4^- (*m/z* 195), and $H_2PO_4^-$ (*m/z* 97). Thus, based on mass spectral analysis, they were all DHR-3′-dAMP adducts.

^{32}P-Postlabeling methodology was selected to detect and quantify DHR-modified DNA adducts *in vitro* and *in vivo*. The synthesized epimeric DHR-3′-dGMP adducts with a specific quantity ranging from 1 to 60 fmol were first employed to develop optimal conditions for the entire process, including DNA enzyme digestion, adduct enrichment, ^{32}P-postlabeling, and HPLC separation. To confirm the ^{32}P-postlabeling products, [^{32}P]-DHR-3′,5′-dG-bisphosphate, and the cold synthetically prepared DHR-3′,5′-dG-bisphosphate adducts I and II were co-chromatographed by HPLC with the ^{32}P-postlabeling reaction product mixture.

To examine the effect of MN/SPD on ^{32}P-postlabeling of DHR-3′-dGMP and DHR-3′-dAMP, 10 µg of DNA from reaction of DHR and calf thymus DNA was digested with 1.25 units of MN and 62 mU of SPD, quantities commonly reported for enzyme digestion (Reddy and Randerath, 1986; Gupta, 1993; Nath and Chung, 1994; Chung *et al.*, 1996). No DHR-3′,5′-dG-bisphosphate adducts were detected by HPLC (Figure I12a). With a concern that the digestion enzymes may interact with DHR-3′-dGMP and/or [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts, optimal quantities of MN and SPD and digestion time were then pursued. Digestion of DNA was repeated by using 312 mU MN and 16 mU SPD; 156 mU MN and 8 mU SPD;, 78 mU MN and 4 mU SPD; and 39 mU MN and 2 mU SPD, respectively (Figures I12b through I12e). As shown in Figures I12c and I12d, the eight chromatographic peaks eluted at 47.6, 48.3, 51.4, 53.9, 55.3, 60.1, 61.0, and 62.6 minutes are designated as P1 through P8, respectively. These chromatographic peaks were not detected from ^{32}P-postlabeling/HPLC analysis of the untreated calf thymus DNA (Figure I12f) or from incubation of riddelliine with calf thymus DNA in the absence of rat liver microsomes (data not shown). Thus, the eight chromatographic peaks P1 through P8 are all DHR-derived DNA adducts. As compared with the HPLC profile from ^{32}P-postlabeling/HPLC analysis of the synthetically prepared DHR-3′-dGMP adducts (Figure I13a), the DNA adducts designated as P4 and P6 are [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts derived from DHR-3′-dGMP adduct I and adduct II, respectively (each indicated with an arrow in Figures I12a through I12e). Thus, the results shown in Figure I12 clearly indicate that the use of 78 mU MN and 4 mU SPD provided the highest yield of DHR-3′,5′-dG-bisphosphate adducts (Figure I12d).

The conditions for analysis of DHR-3′-dAMP adducts by ^{32}P-postlabeling were then pursued using different amounts of MN and SPD and different incubation times. As shown in Figures I13a through I13e, similar to DHR-3′-dGMP adducts, the optimal conditions for analysis of DHR-3′-dAMP adducts were also the use of 78 mU MN and 4 mU SPD. These were also the optimal conditions for ^{32}P-postlabeling a mixture of DHR-3′-dGMP and DHR-3′-dAMP adducts (Figures I14a through I14e).

To measure the enrichment of DHR-3′-dGMP adducts by nuclease P1 incubation, DNA was extracted from the livers of F344/N female rats treated with riddelliine, and 10 µg aliquots were enzymatically digested under optimal conditions (same conditions described in Figure I12d); and the resulting DHR-3′-monophosphate deoxyribonucleosides were enriched by various amounts of nuclease P1 using incubation times ranging from 20 to 40 minutes. The use of 8 µg of nuclease P1 and 20 minutes of incubation at 37° C provided the best enrichment.

Enrichment by *n*-butanol was similarly studied. Following the conventional procedure (Gupta, 1993), the DHR-3′-monophosphate deoxyribonucleosides formed from enzymatic digestion of DNA were fortified with the phase-transfer agent tetrabutylammonium chloride and then extracted four times with *n*-butanol. The adducts collected from the pooled *n*-butanol fractions were ^{32}P-postlabeled followed by HPLC analysis (data not shown). Based on three trials, the yields of [^{32}P]-DHR-3′,5′-deoxyribonucleoside-bisphosphate were erratic and much lower than those by nuclease P1 enrichment. Therefore, this approach was found to be less satisfactory than nuclease P1 enrichment.

After the synthetically prepared DHR-3′-dGMP adducts, combined synthetically prepared DHR-3′-dGMP and DHR-3′-dAMP adducts, and DNA from reaction of DHR and calf thymus DNA were ^{32}P-postlabeled under the optimal conditions described above, the resulting [^{32}P]-DHR-3′,5′-deoxyribonucleoside-bisphosphate adducts were separated by HPLC. After a number of trials using different HPLC columns, solvent systems, and elution profiles (data not shown), optimal conditions for separation of the DHR-3′,5′-bisphosphate adducts were developed. The developed HPLC profile for separation of the two epimeric DHR-3′,5′-dG-bisphosphate adducts is shown in

Figure I13a. The optimal HPLC conditions for separation of the mixture of DHR-3′,5′-dG-bisphosphate and DHR-3′,5′-dA-bisphosphate adducts are those shown in Figure I13d. Similarly, the optimal HPLC conditions for separation of the DNA adducts from reaction of DHR and calf thymus DNA are those shown in Figure I12d.

Based on experience in employing or developing ^{32}P-postlabeling/HPLC methodologies for detection of carcinogen-modified DNA adducts (Gupta, 1993), attempts were made to establish reliable conditions for validating interexperimental reproducibility. However, the DNA enzyme digestion products (e.g., DHR-3′-dGMP) and/or ^{32}P-postlabeling products (e.g., DHR-3′,5′-dG-bisphosphate) are highly unstable to the DNA digestion enzymes, incubation media, and experimental conditions. As shown in Figure I12, the yield of ^{32}P-postlabeling is dependent on the amount of the MN and SPD enzymes employed. It was also found that the storage of DNA, the use of [γ-^{32}P]-ATP from different batches or the same batch but on a different day, and repreparation of the buffer media can all result in poor interexperimental reproducibility (data not shown). Consequently, it was decided to develop a ^{32}P-postlabeling/HPLC methodology that allowed the detection and quantitation of up to 30 samples of DNA adducts on the same day.

Thus, intra-experimental reproducibility was determined using the synthetic DHR-3′-dGMP adduct standards and DNA sample from reaction of DHR with calf thymus DNA for ^{32}P-postlabeling/HPLC analysis. Triplicate DNA samples were concurrently enzymatically digested, adduct enriched (by nuclease P1), and ^{32}P-postlabeled. The levels of modification of the DNA from reaction of DHR with calf thymus DNA were 615.6, 577.4, and 578.0 adducts/10^8 nucleotides (mean ± standard deviation = 590.4 ± 21.6; 4% relative standard deviation). Among these adducts, the amounts of the two enantiomeric DHR-3′,5′-dG-bisphosphate adducts I (P4) and II (P6) were 33.7 ± 2.1 and 23.9 ± 1.2 adducts/10^8 nucleotides, respectively.

Attempts were made to enzymatically 3′-dephosphorylate [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts to the corresponding [^{32}P]-DHR-5′-dGMP adducts followed by HPLC analysis. However, based on comparison of the HPLC retention time of the synthetic DHR-5′-dGMP standard, no DHR-5′-dGMP was detected (data not shown).

^{32}P-Postlabeling/TLC analysis of DHR-3′-dGMP adducts was performed. After ^{32}P-postlabeling of the DHR-3′-dGMP adducts as described above, the resulting [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts were separated using TLC on PEI cellulose plates (Reddy and Randerath, 1986; Gupta, 1993). Comparison of the autoradiography results with those from controls indicated no adducts were detected (data not shown).

^{32}P-Postlabeling/HPLC Analysis of DHR-Derived DNA Adducts in NTP Female F344/N Rat Liver Samples

Two control ^{32}P-Postlabeling/HPLC profiles of DHR-derived DNA adduct standards were developed to analyze DHR-derived DNA adducts in the liver of rats chronically exposed to riddelliine. The first profile was obtained from analysis of a mixture of synthetically prepared DHR-3′-dGMP and DHR-3′-dAMP adducts, and the second profile was modified DNA from reaction of DHR with calf thymus DNA. A negative control was obtained from the ^{32}P-postlabeling/HPLC of DNA obtained from incubation of riddelliine with calf thymus DNA. These HPLC profiles are shown in Figures I14a, I14b, and I14c, respectively. As shown in Figure I14a, the HPLC retention times of DHR-3′,5′-dG-bisphosphate adducts I and II are 53.9 and 60.1 minutes. Identical to previously presented data, ^{32}P-postlabeling of adducts from reaction of DHR and calf thymus DNA resulted in eight DHR-derived DNA adducts (Figure I14b), which were not formed in the control experiment using DNA obtained from incubation of riddelliine with calf thymus DNA (Figure I14c).

The eight DHR-derived DNA adducts contained in the chromatographic peaks eluted at 47.6, 48.3, 51.4, 53.9, 55.3, 60.1, 61.0, and 62.6 minutes are designated as P1 through P8 (Figure I14). The DNA adducts designated as P4 and P6 are DHR-3′-dGMP adduct I and adduct II. Due to lack of synthetic standards, the structures of the other six DHR-derived adducts (P1, P2, P3, P5, P7 and P8) were not characterized.

The levels of riddelliine-modified DNA adducts contained in the DNA of the female rat liver samples were analyzed by ^{32}P-postlabeling/HPLC. It has been previously established that the ^{32}P-postlabeling methodology has a high intra-experimental reproducibility, but the interexperimental reproducibility was much less satisfactory. Thus,

to conduct the experiment on the same day, four liver DNA samples from each of six groups were analyzed simultaneously. For identification and quantification of the modified 3′,5′-bisphosphate adduct, external standards were also analyzed in parallel with the biological samples. The standards are DHR-3′-dGMP containing a known level of adducts (8.3 fmol, equal to 9.6 adducts/10^8 nucleotides) that corresponded closely to the modification level expected in each DNA sample. In order to verify the two chromatographic peaks of [^{32}P]-DHR-3′,5′-dG-bisphosphate, an unlabeled synthetic standard of DHR-3′,5′-dG-bisphosphate was co-chromatographed as a UV marker with the first two DNA samples from the liver samples. Verification of the chromatographic peaks of the other six DHR-derived DNA adducts was based on comparison of their HPLC retention times with those from DHR-modified calf thymus DNA (Figure I14b). The lack of [^{32}P]-DHR 3′,5′-dA-bisphosphate adducts was based on comparison of HPLC retention times with those of the synthetic DHR-3′,5′-dA-bisphosphate samples (Figure I14a).

The DNA samples from liver of untreated rats and rats administered riddelliine at concentrations of 0.01, 0.033, 0.1, 0.33, or 1.0 mg/kg per day, 5 days per week beginning at 5 to 6 weeks of age and continuing until sacrifice at 3 months were ^{32}P-postlabeled and analyzed by HPLC. The HPLC profiles of these samples (Figures I14d through I14i) are identical to that from reaction of DHR and calf thymus DNA (Figure I14b), albeit with different amounts of adducts). This comparison confirms that in the livers of rats chronically exposed to riddelliine, all eight DHR-derived DNA adducts (P1 through P8) were formed; among these eight DNA adducts, two were the epimeric [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts I and II; and that no DHR-3′,5′-dADP adducts were detected. As shown in Figure I14d, these eight DHR-derived DNA adducts were not detected in the livers of untreated rats.

Because the DHR-3′-dGMP standard containing 8.3 fmol (equal to 9.6 adducts/10^8 nucleotides) was ^{32}P-postlabeled in parallel, the quantities of the two [^{32}P]-DHR-3′,5′-dG-bisphosphate adducts I and II (P4 and P6) in each of the liver samples were determined based on comparison of the radioactivity contained in the chromatographic peaks and in the peaks from the synthetic DHR-3′-dGMP standards. However, since the structures of the other six DNA adducts (P1, P2, P3, P5, P7, and P8) are not known and therefore no synthetic standards of known level of modification can be used for quantitation, the quantities of these six DNA adducts were estimated by comparison of their radioactivity contained in the chromatographic peak and the DHR-3′-dGMP adduct synthetic standard of known quantity (8.3 fmol). The total quantity of DHR-derived DNA adduct formation that includes these six adducts and the two DHR-3′-dGMP adducts I and II (which are also designated as P4 and P6, respectively) are listed in Table I3.

The DNA samples from liver of untreated rats and rats exposed to riddelliine and sacrificed at 6 months were similarly ^{32}P-postlabeled and analyzed by HPLC. The HPLC profile (figure not shown) was closely similar to that of rats sacrificed at 3 months, and the quantities of each adduct peak are tabulated in Table I4.

The data presented in Tables I3 and I4 indicated a positive dose-response trend for DHR-3′-dGMP adducts I and II in the liver of rats exposed to riddelliine for 3 or 6 months.

Statistical analysis of the data shown in Tables I3 and I4 by two-way ANOVA combined with Dunnett's test indicate that compared with the animals exposed to the same dose of riddelliine, the levels of DNA adducts formed in the liver of rats exposed for 6 months are higher than those exposed for 3 months ($P<0.05$), and also that in all the liver DNA from the riddelliine-treated rats, the yield of adduct I was higher than that of adduct II ($P<0.05$). The data shown in Figure I16 indicate a positive dose-response trend for the total DHR-derived DNA adducts from the liver of rats exposed to riddelliine for 3 or 6 months. The total DNA adduct formation of the 6-month group was greater than that of the 3-month group, and total adduct formation in all exposed groups except the 0.01 and 0.033 mg/kg groups of rats exposed for 3 months was significantly greater than that of the control groups ($P<0.05$).

To verify that optimal conditions were used for ^{32}P-postlabeling, analyses were repeated using different amounts of the digestion enzymes MN and SPD. Results clearly confirmed that the use of 78 mU MN and 4 mU SPD provided the highest yield of DHR-3′,5′-dG-bisphosphate adducts (data not shown).

Human Liver Microsome-Mediated DHR-Derived DNA Adduct Formation

Riddelliine was incubated aerobically with liver microsomes from a 62-year-old male human for 30 minutes. Upon removal of microsomal protein by ultracentrifugation, the metabolism products contained in the supernatant were separated and analyzed by reversed-phase HPLC (Figure I17). The chromatographic peak eluting at 35.7 to 39.2 minutes contained the recovered substrate, riddelliine. By comparison of HPLC retention times and the UV-visible absorption spectrum (not shown) with those of synthetically prepared standards, the metabolites contained in chromatographic peaks eluting at 25.4 and 30.1 minutes were identified as DHR and riddelliine-*N*-oxide, respectively (Figure I17).

To determine the relevance of the mechanistic study in rodents to humans, DHR-derived DNA adduct formation mediated by human liver microsomes was studied and the results compared with those obtained from female F344/N rat liver microsomal metabolism of riddelliine in the presence of calf thymus DNA. The substrate (riddelliine) concentration-dependent formation of the total set of eight DHR-derived DNA adducts from female rat microsomal incubation was first determined (Table I5 and Figure I18a). Metabolism of riddelliine by liver microsomes from two female humans and one male human in the presence of calf thymus DNA was subsequently studied. Comparison of the DNA adduct formation profile with that from rat liver microsomal incubation (Figure I19) showed that the same set of eight DHR-derived DNA adducts were formed from all the human liver microsomal incubations. The substrate concentration-dependent formation of DHR-derived DNA adducts from these three human liver microsomal incubations was subsequently determined (Table I5 and Figures I18b through I18d). These results indicate that metabolism of riddelliine by human liver microsomes in the presence of calf thymus DNA produces the same set of eight DHR-derived DNA adducts formed from female F344/N rat microsomal metabolism, and that the levels of DHR-derived DNA adducts formed from human liver microsomal metabolism were at least five times lower than those from rat liver microsomal metabolism.

Discussion

As reported earlier in this Technical Report, the 2-year carcinogenicity study of riddelliine found that riddelliine induced hemangiosarcoma in the liver of male and female rats and male mice and hepatocellular adenoma in male and female rats. This study describes the mechanisms by which riddelliine induces tumors in rats and mice. A study of the metabolic activation of riddelliine by female F344/N rat liver microsomes determined that DHR is one of the major metabolites. DHR was found to be able to bind with DNA, 3′-dGMP, and 5′-dAMP, which indicates that it is a potential activated metabolite. A ^{32}P-postlabeling/HPLC method for detection and quantitation of DHR-derived DNA adducts *in vivo* and *in vitro* was developed, and liver samples from female F344/N rats exposed to riddelliine at five different doses for 3 or 6 months were analyzed for DNA content and identity. A dose-response relationship was obtained between the dose and level of total DNA adducts, which are all derived from DHR (Tables I3 and I4). The DNA from reaction of riddelliine and calf thymus DNA was similarly analyzed by ^{32}P-postlabeling/HPLC. In this case, no DHR-derived DNA adducts were formed (Figure I14c), indicating that riddelliine cannot bind with DNA in the absence of metabolism. Thus, these combined results suggest that riddelliine induces liver tumors in rats through a genotoxic mechanism. Eight DHR-derived DNA adducts were detected in all the liver samples of rats treated with riddelliine, of which two adducts had their structures fully characterized as epimeric DHR-3′-dGMP (Figure I16). Because a dose relationship exists, these eight DHR-derived DNA adducts may be responsible in part for development of liver tumors in the female rats exposed to riddelliine. In the current study, microsomal metabolism of riddelliine generated riddelliine-*N*-oxide and DHR. The formation of these metabolites is also in agreement with the *in vitro* metabolism pattern reported for other pyrrolizidine alkaloids (Mattocks, 1986).

The CYP3A1 and CYP2C11 isozymes are the metabolizing enzymes responsible for pyrrolizidine alkaloid metabolism (Buhler and Kedzierski, 1986; Williams *et al*., 1989; Chu *et al*., 1993; Chung *et al*., 1995; Kasahara *et al.*, 1997; Reid *et al*., 1998). In the current study, the rate of riddelliine metabolism was higher with PB-microsomes than with control microsomes. Because phenobarbital induces both CYP2B1 and CYP3A1 isozymes (Omiecinski *et al.*, 1999), these results suggest that CYP2B1 and/or CPY3A1 isozymes are the major metabolizing enzymes responsible for riddelliine metabolism and that these results are consistent with those reported in the literature.

The same set of eight DHR-derived DNA adducts found in rat livers was found from metabolism of riddelliine by human liver microsomes in the presence of calf thymus DNA. These results strongly suggest that the use of rodents for studying the mechanisms of liver tumor induction by riddelliine is highly relevant to humans.

Overall, these results suggest that riddelliine induces liver tumors in rats gavaged with riddelliine through a genotoxic mechanism, and the eight DHR-derived adducts are responsible in part for the liver tumor development. It is known that hydroxylation of pyrrolizidine alkaloids at the necine base, particularly at the C-8 and C-3 positions, to form 8- and 3-hydroxynecine derivatives, followed by dehydration to form the corresponding dehydropyrrolizidine (pyrrolic) derivatives, is a general metabolic pathway for these alkaloids (Bull *et al*., 1968; Mattocks, 1986). Therefore, metabolism of riddelliine catalyzed by CYP isozymes may first provide 8-hydroxyriddelliine and/or 3-hydroxyriddelliine as the primary metabolites, which upon enzymatic dehydration produce dehydroriddelliine. Two possible pathways may lead to DHR-derived DNA adducts. The first pathway is that dehydroriddelliine, a potent electrophile, covalently binds to cellular DNA to form dehydroriddelliine-derived DNA adducts (Figure 6), which are subsequently hydrolyzed to DHR-derived DNA adducts. The second pathway is that dehydroriddelliine, like the other dehydropyrrolizidine alkaloids, is unstable and easily hydrolyzed by esterases and/or other hepatic enzymes to form DHR (Mattocks, 1968, 1986; Kim *et al*., 1999), which subsequently binds to DNA. At present, the pathway from which these eight DHR-derived DNA adducts are formed is unknown. Dehydropyrrolizidine alkaloids (pyrroles) are highly unstable and DHR is the most stable pyrrolic compound (Galloway *et al*., 1987; Huxtable *et al*., 1996); therefore more binding should occur through DHR than through dehydroriddelliine.

Although pyrrolizidine alkaloids are a class of naturally occurring phytochemicals and a number of pyrrolizidine alkaloids have been found to induce tumors in experimental animals (Schoental *et al*., 1954; Schoental and Head, 1957; Mattocks, 1968; Svoboda and Reddy, 1972; Newbern and Rogers, 1973; NCI, 1978; Juhara *et al*., 1980; NTP, 1993; Chan *et al*., 1994), the mechanisms that lead to tumorigenicity are not clear. This study is the first to establish a mechanism by which a pyrrolizidine alkaloid (riddelliine) induces liver tumors; it is a genotoxic mechanism mediated by a set of eight DHR-derived DNA adducts. This set of DHR-derived DNA adducts is expected to be formed *in vivo* and *in vitro* when exposure occurs to other genotoxic pyrrolizidine alkaloids. ^{32}P-Postlabeling methodology is a highly sensitive method for detection and quantitation of carcinogen-modified DNA adducts *in vivo* and *in vitro*. The ^{32}P-postlabeling/HPLC methodology developed for these studies can serve as a sensitive and reliable technique to identify and quantify the DHR-derived DNA adducts formed *in vivo* and *in vitro* after exposure to genotoxic pyrrolizidine alkaloids.

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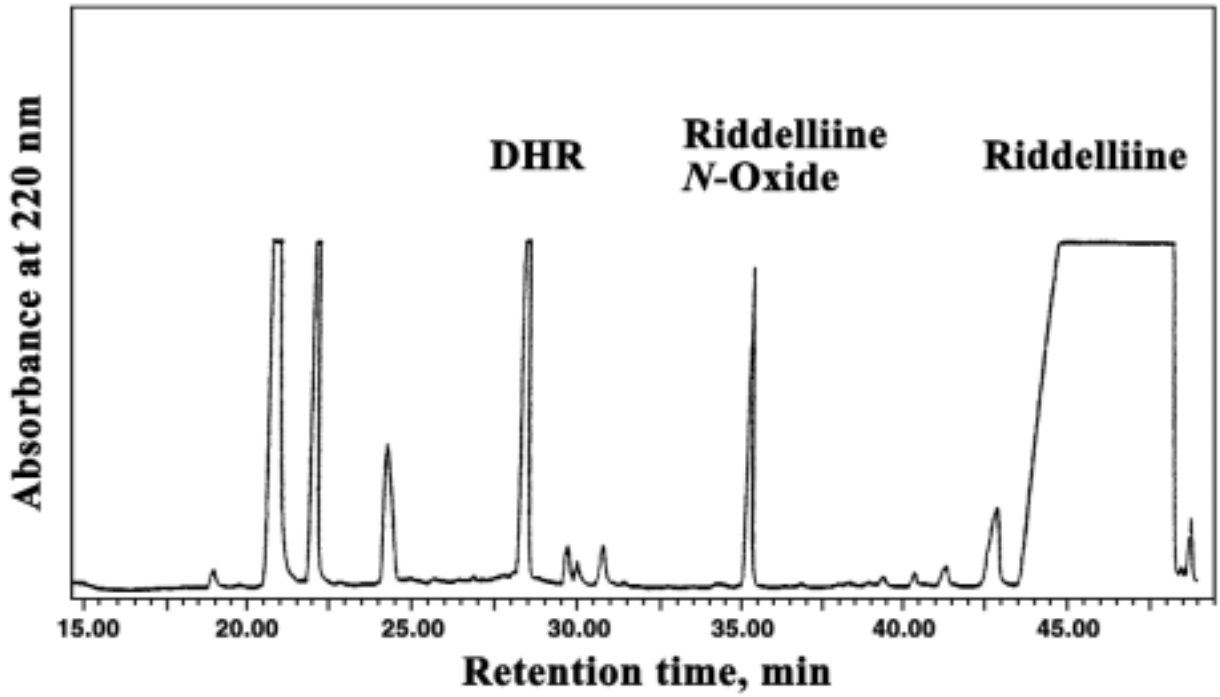

FIGURE I1
Reversed-Phase HPLC Analysis of Riddelliine Metabolites Formed from Metabolism of Riddelliine by PB-Microsomes

TABLE I1
***In vitro* Metabolism of Riddelliine by Liver Microsomes from Female F344/N Rats[a]**

Microsomes	Metabolites[b]	
	Dehydroretronecine	Riddelliine-*N*-oxide
Control	8.3 ± 0.7	14.7 ± 1.1
Phenobarbital-induced	31.1 ± 1.5	50.0 ± 1.7
Phenobarbital-induced/control ratio	3.8	3.4

[a] Data presented as mean ± standard deviation (n=3)
[b] nmol metabolite formed/mg microsomal protein/30 minutes

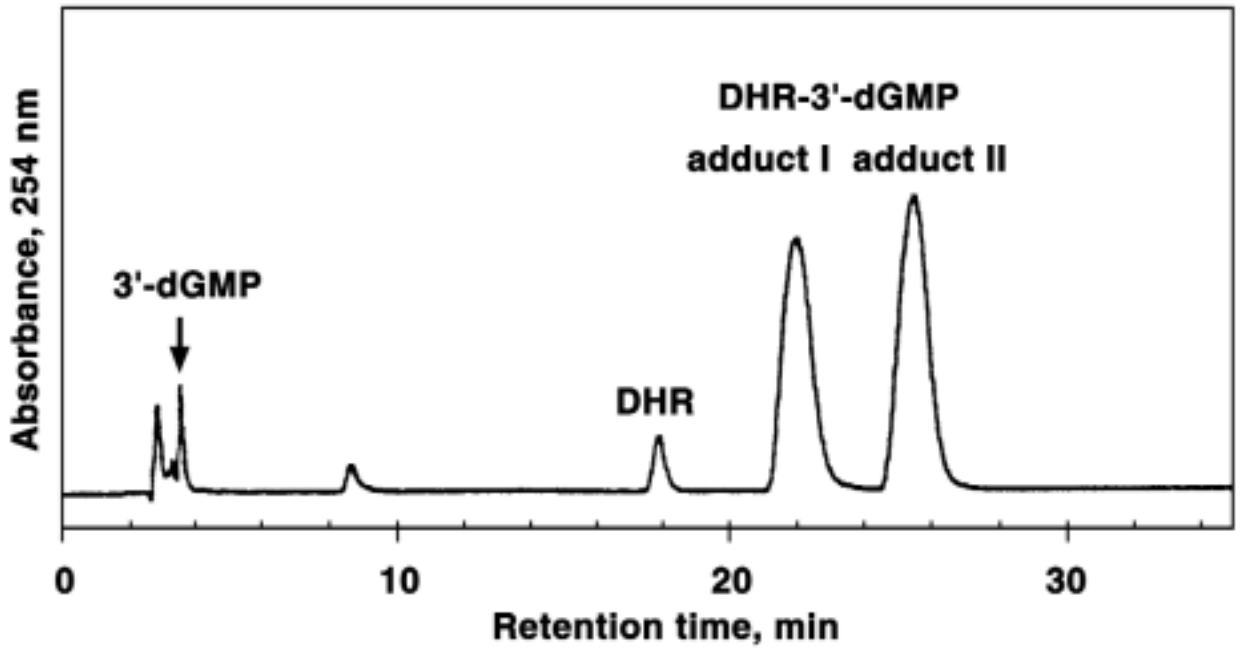

FIGURE I2
Reversed-Phase HPLC Purification of the Synthetically Prepared DHR-3′-dGMP Adducts I and II

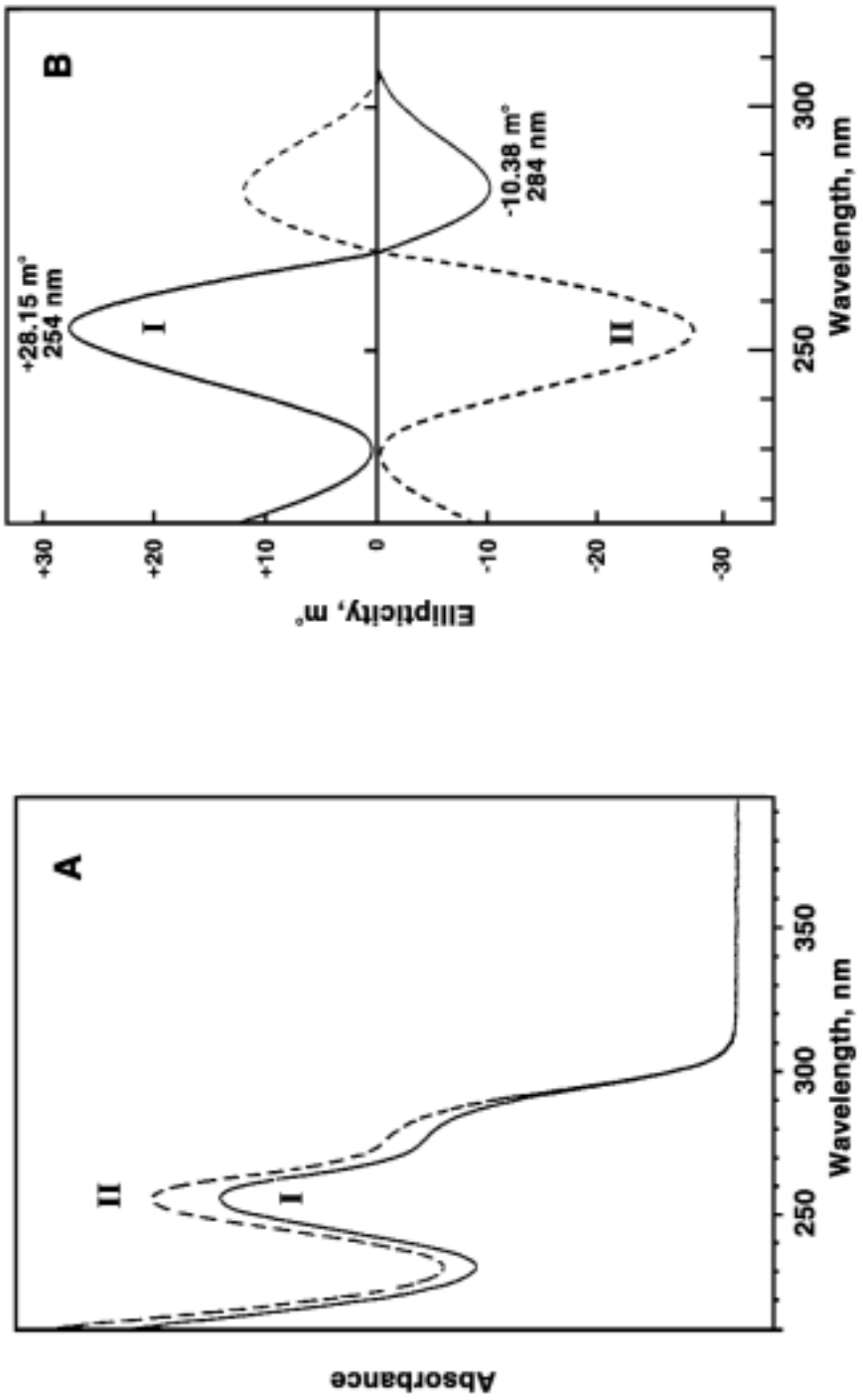

FIGURE I3
(A) Ultraviolet-Visible Absorption Spectra and (B) CD Spectra of the Synthetically Prepared Epimeric DHR-3′-dGMP Adducts I and II Obtained from HPLC Purification

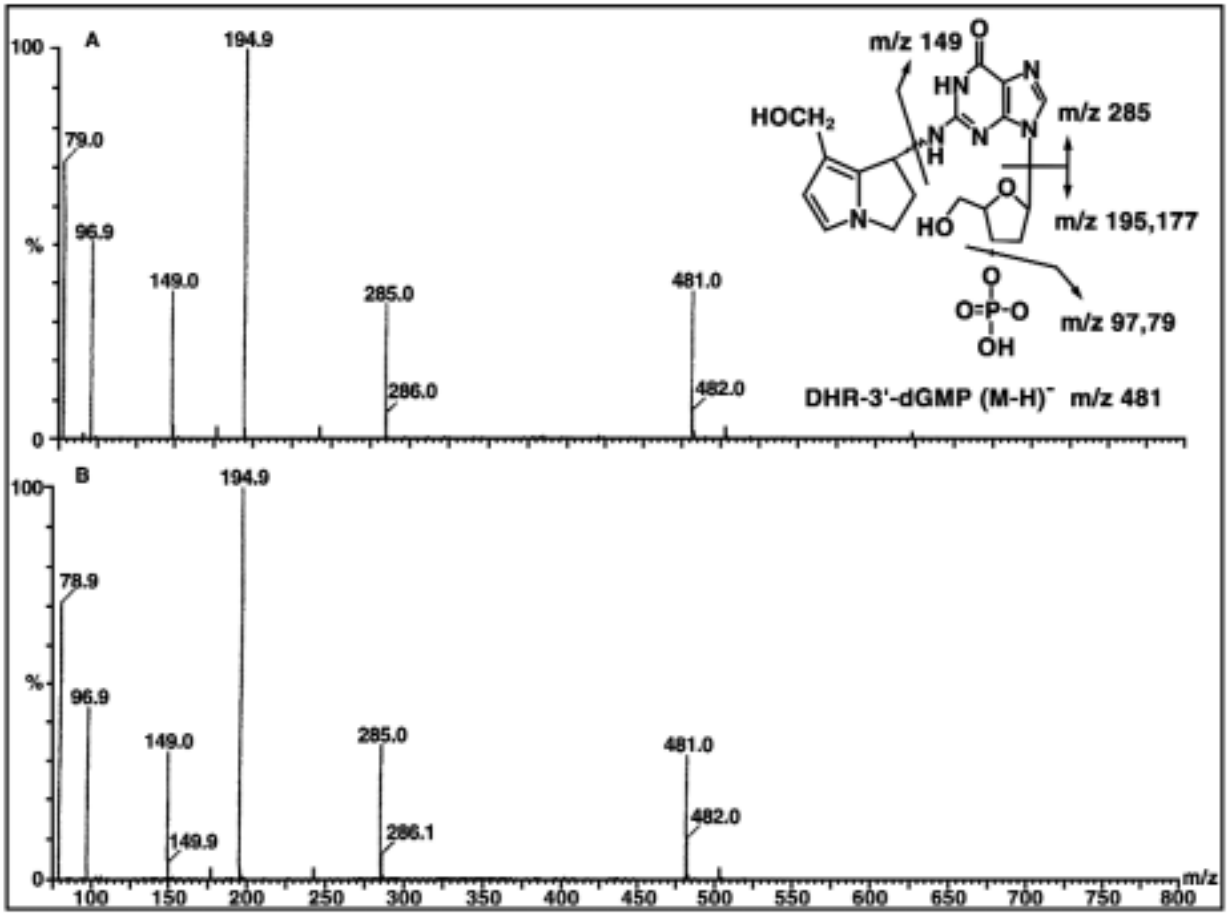

FIGURE I4
Negative Ion Electrospray Mass Spectra
of Synthetically Prepared DHR-3′-dGMP Adducts I (A) and II (B)

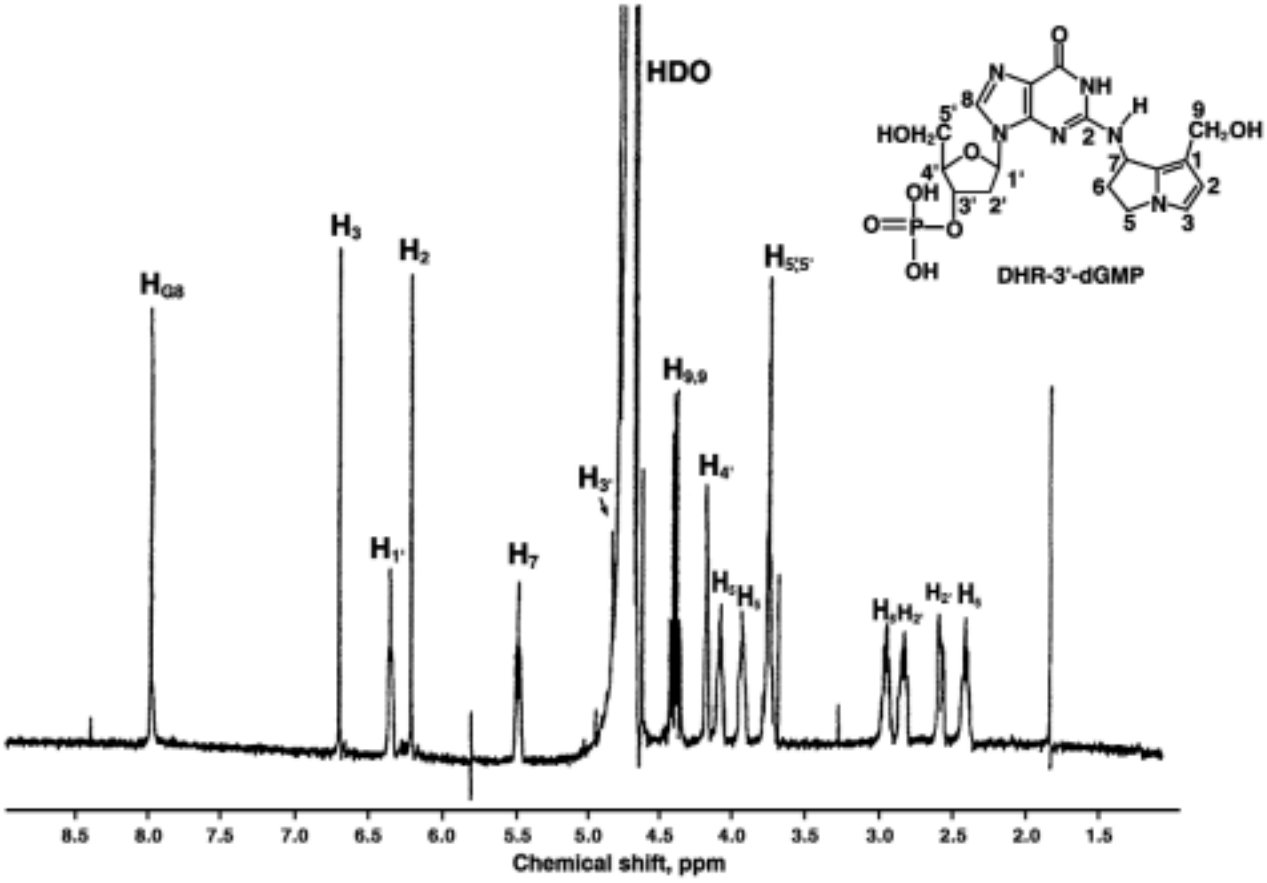

FIGURE I5
Proton NMR Spectrum (500 MHz) of the Synthetically Prepared DNA Adduct Standards Identified as the Mixture of DHR-3′-dGMP Adducts I and II

Table I2
500 MHz ^{1}H NMR Spectral Data of DHR, 3′-dGMP, DHR-3′-dGMP, 5′-dGMP, and DHR-5′-dGMP

	Chemical Shift (ppm)				
	DHR	3′-dGMP	DHR-3′-dGMP	5′-dGMP	DHR-5′-dGMP
H2	6.01		6.29		6.31
H3	6.53		6.76		6.73
H5	3.97		4.13		4.17
H5	3.77		3.99		4.15
H6	2.61		2.96		3.02
H6	2.17		2.51		2.59
H7	4.99		5.56		5.57
H9	4.36		4.41		4.71
H9	4.32		4.36		4.24
OH7	4.90				
H8		7.97	8.01	8.77	8.14
H1′		6.27	6.41	6.35	6.46
H2′		2.62	2.88	2.72	2.87
H2′		2.74	2.59	2.60	2.59
H3′		4.82	4.75	4.63	4.77
H4′		4.21	4.25	4.25	4.24
H5′		3.76	3.83	4.04	4.49
H5′		3.77	3.83	4.09	4.49
J	**Coupling Constant (Hz)**				
1′,2′		7.0		7.0	
2,3	2.5		2.5		2.5
9,9			12.1		

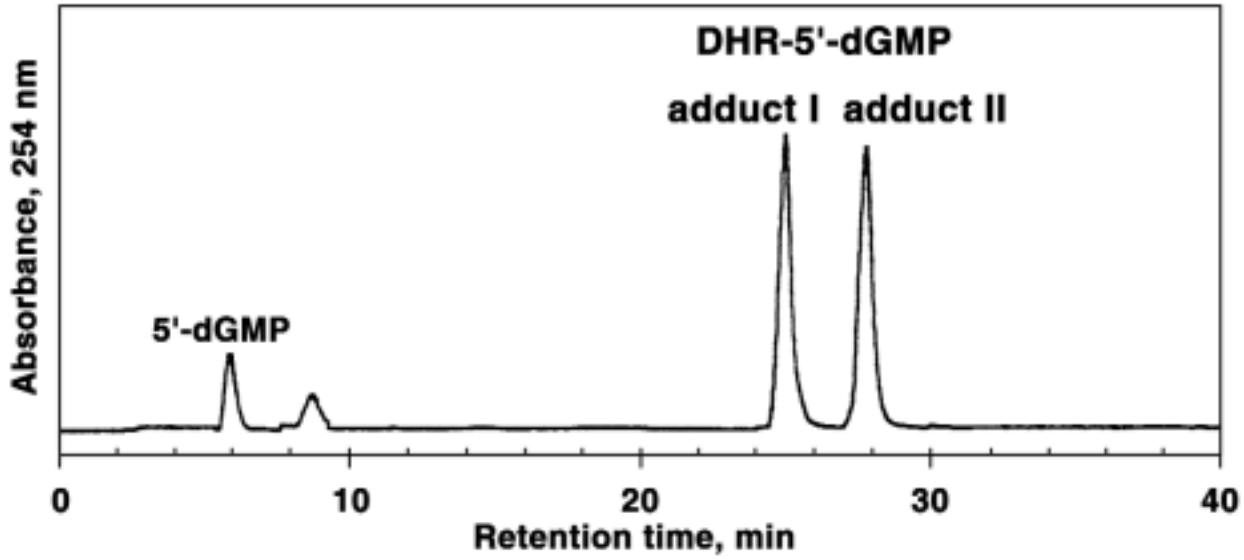

FIGURE I6
HPLC Purification of DHR-5′-dGMP

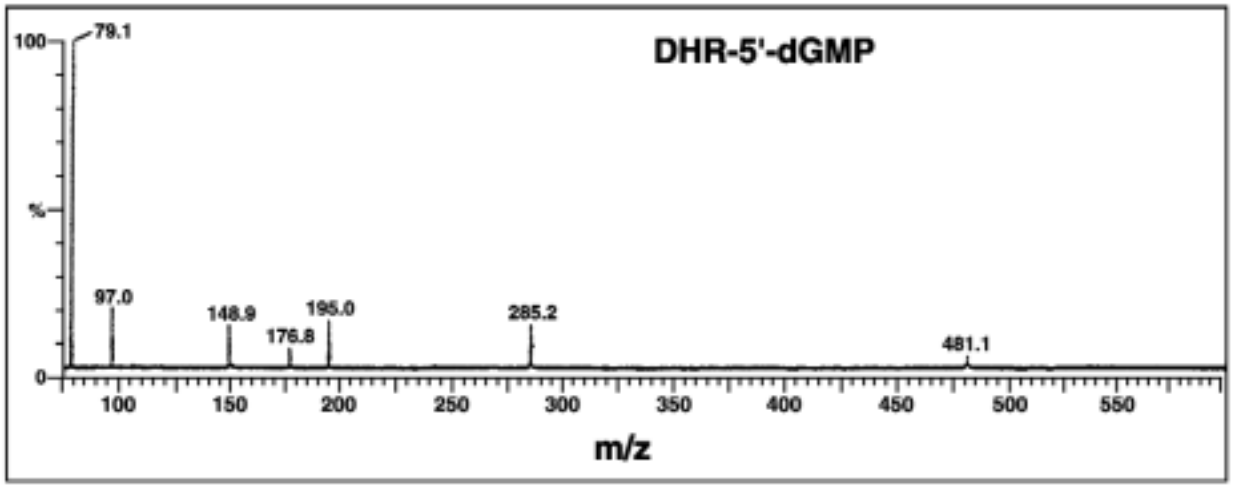

FIGURE I7
Negative Ion Electrospray Mass Spectrum of DHR-5′-dGMP

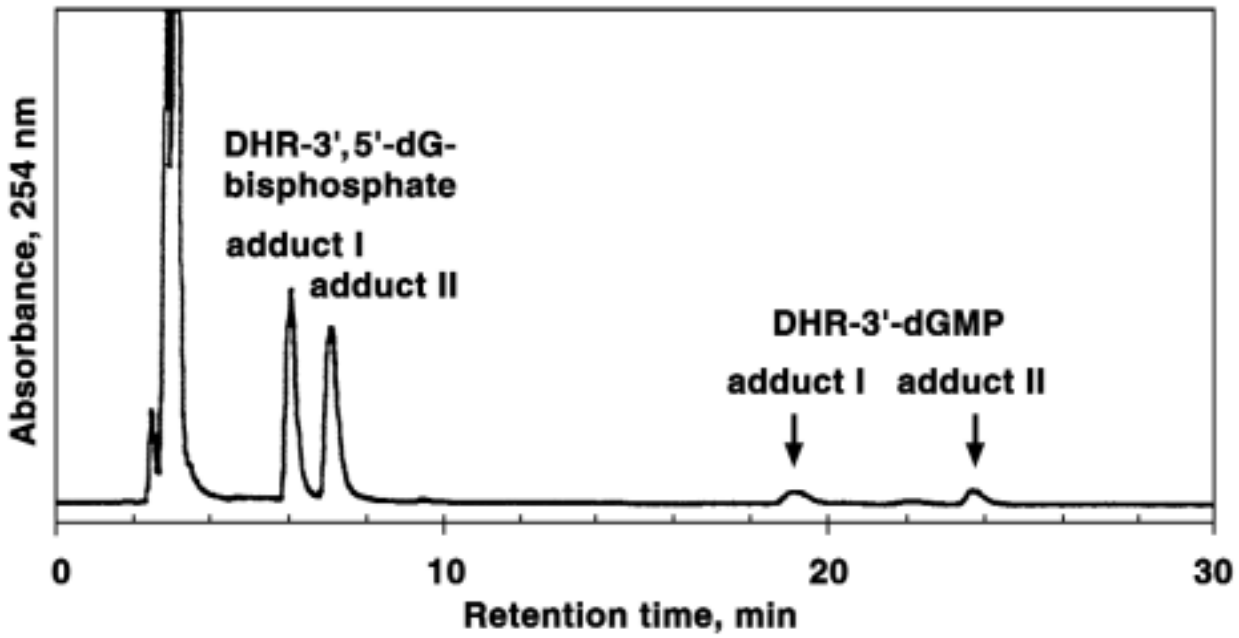

FIGURE I8
HPLC Separation of DHR-3′,5′-dG-bisphosphate Adducts Formed from the Reaction of DHR-3′-dGMP with Cold ATP Catalyzed by Cloned T4 PNK Following HPLC

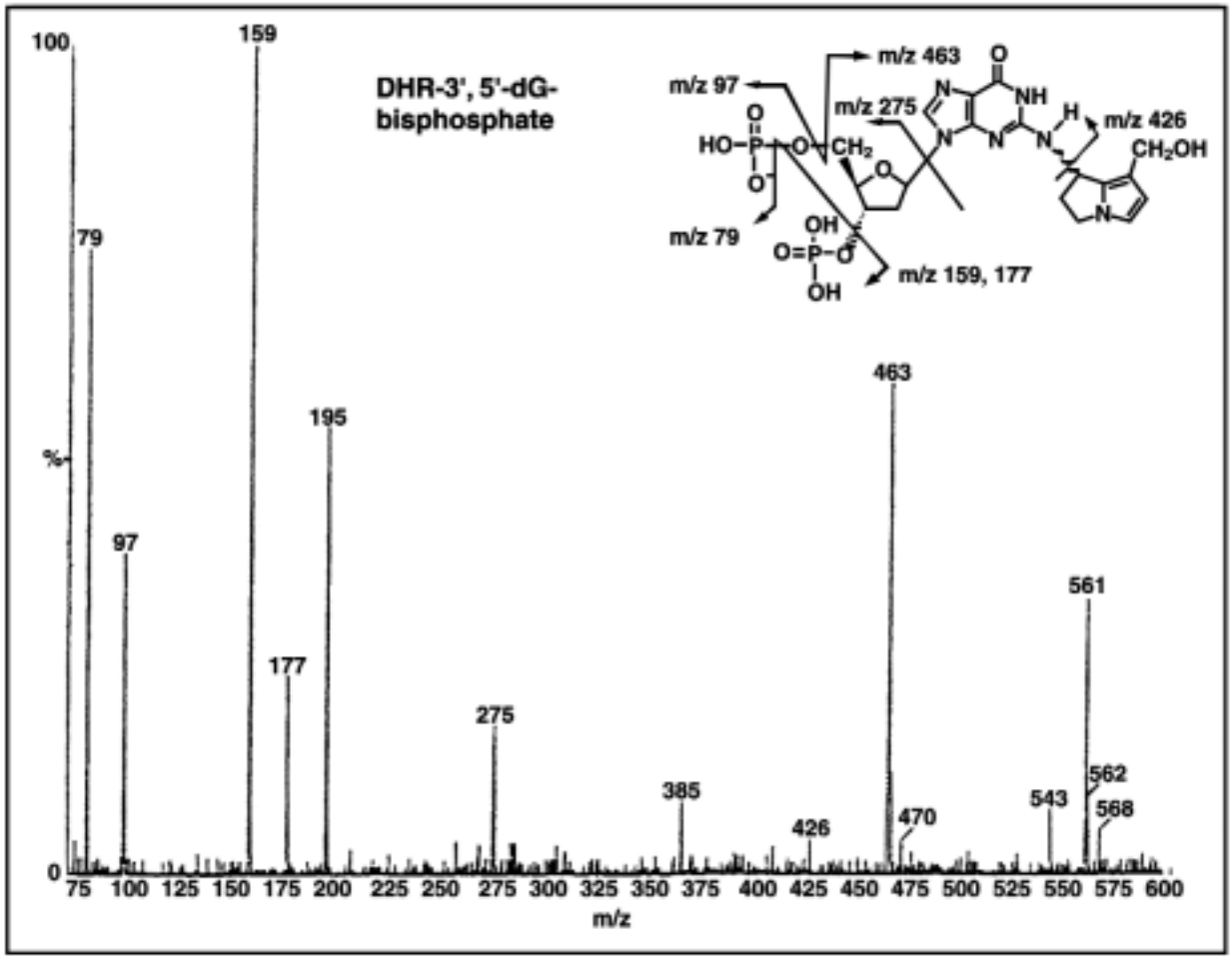

FIGURE I9
Negative Ion Electrospray Mass Spectrum of DHR-3′,5′-dG-bisphospate

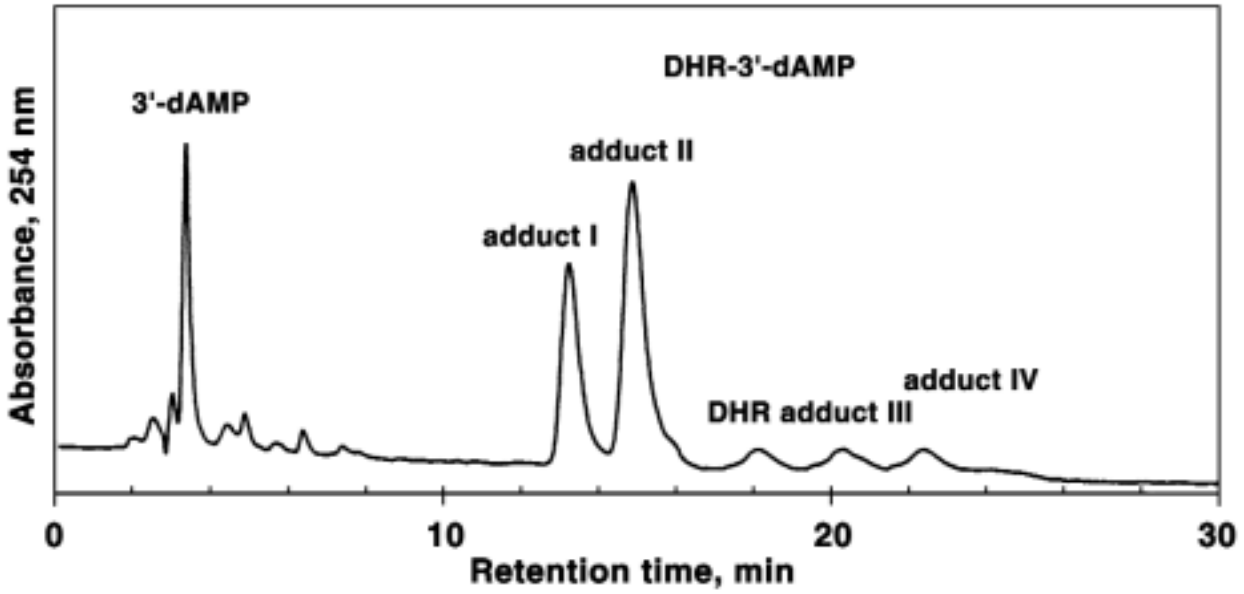

Figure I10
HPLC Separation of DHR-3′-dAMP

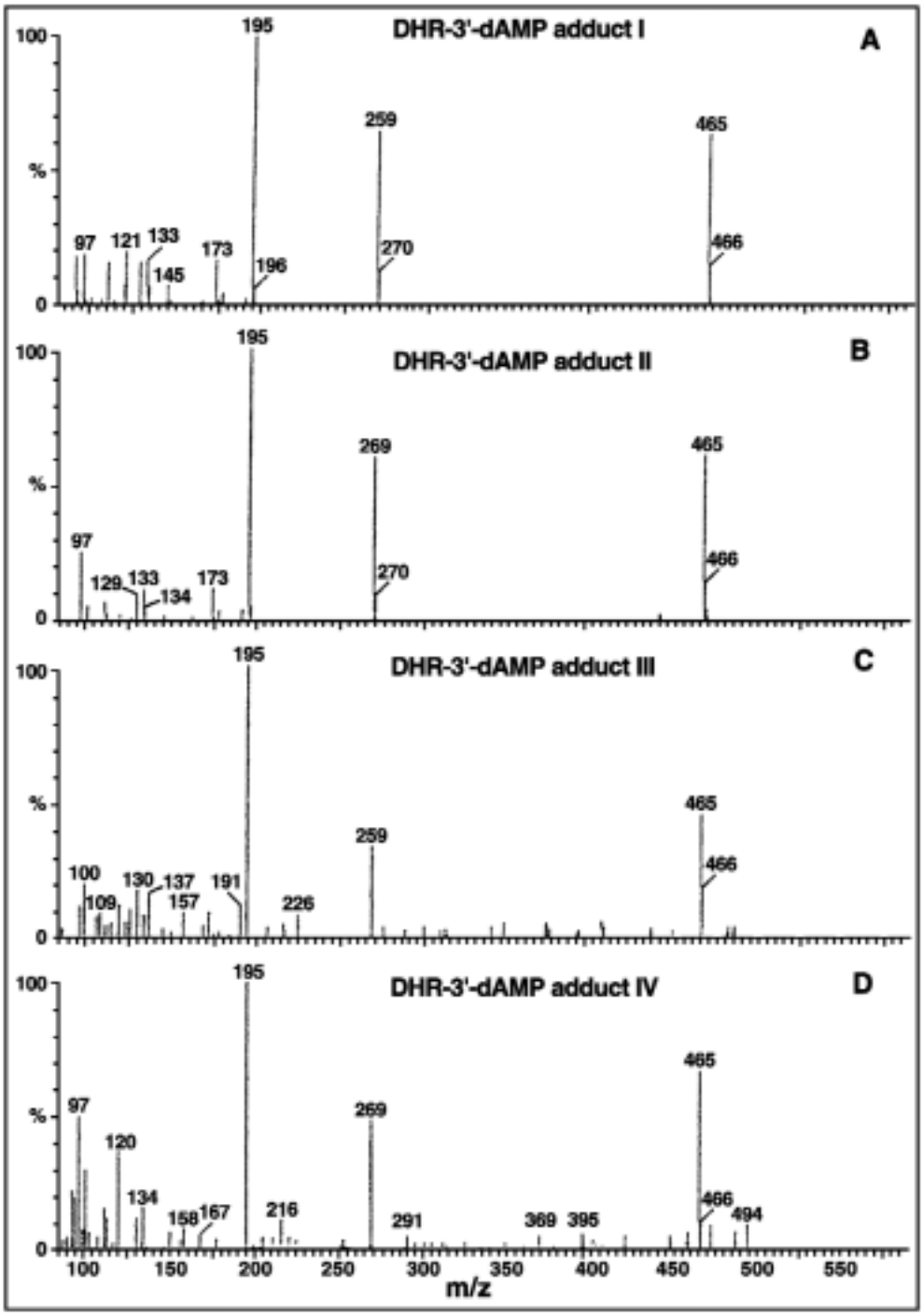

FIGURE I11
Negative Ion Electrospray Mass Spectra of Four Isomeric DHR-3′-dAMP Adducts Prepared from Reaction of DHR and 3′-dAMP Followed by HPLC Purification

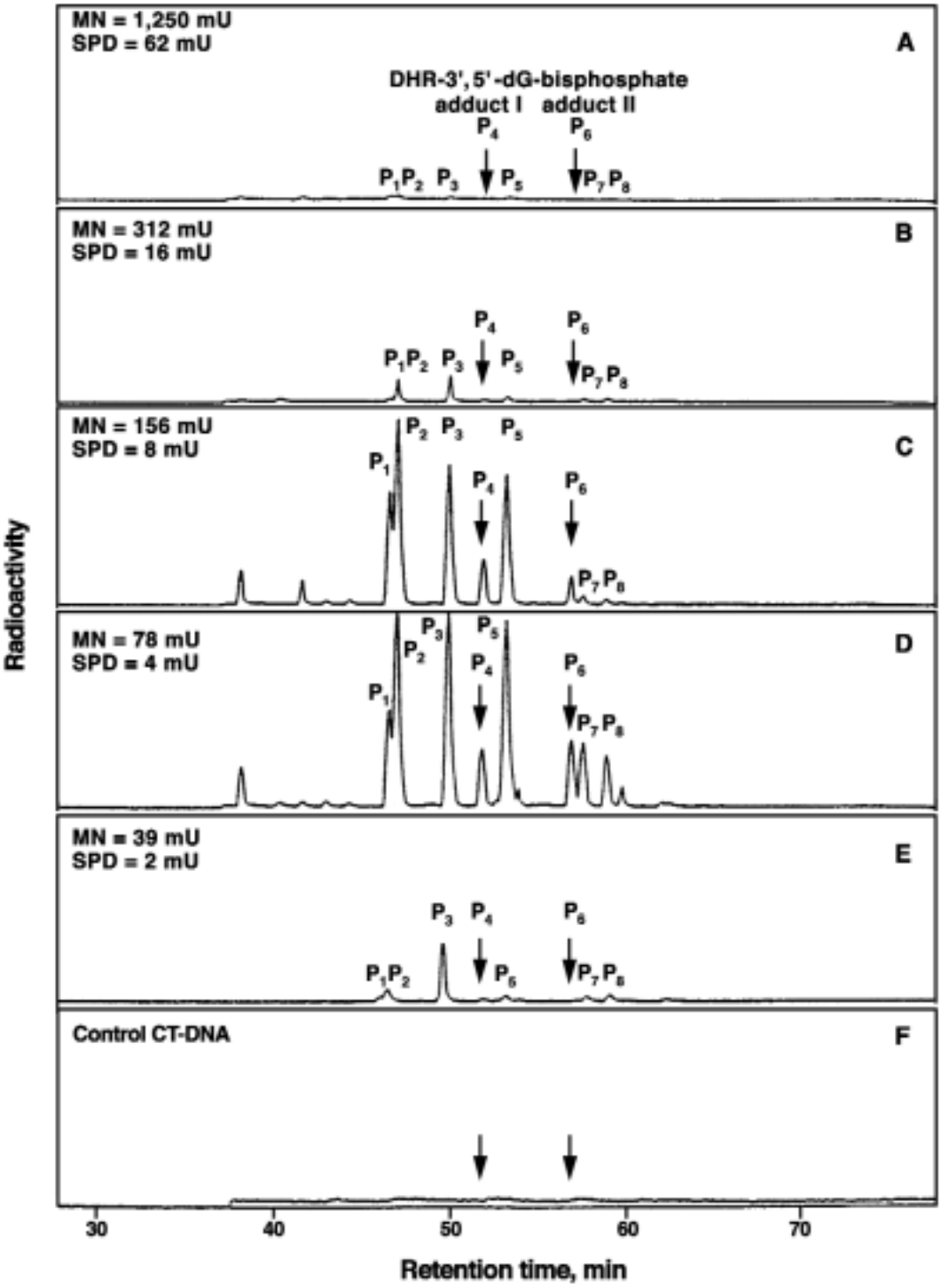

FIGURE I12
Effect of Micrococcal Nuclease (MN) and Spleen Phosphodiesterase (SPD) Concentration on ^{32}P-Postlabeling/HPLC Analysis of DHR-Modified DNA Adducts Contained in DNA from Reaction of DHR and Calf Thymus DNA

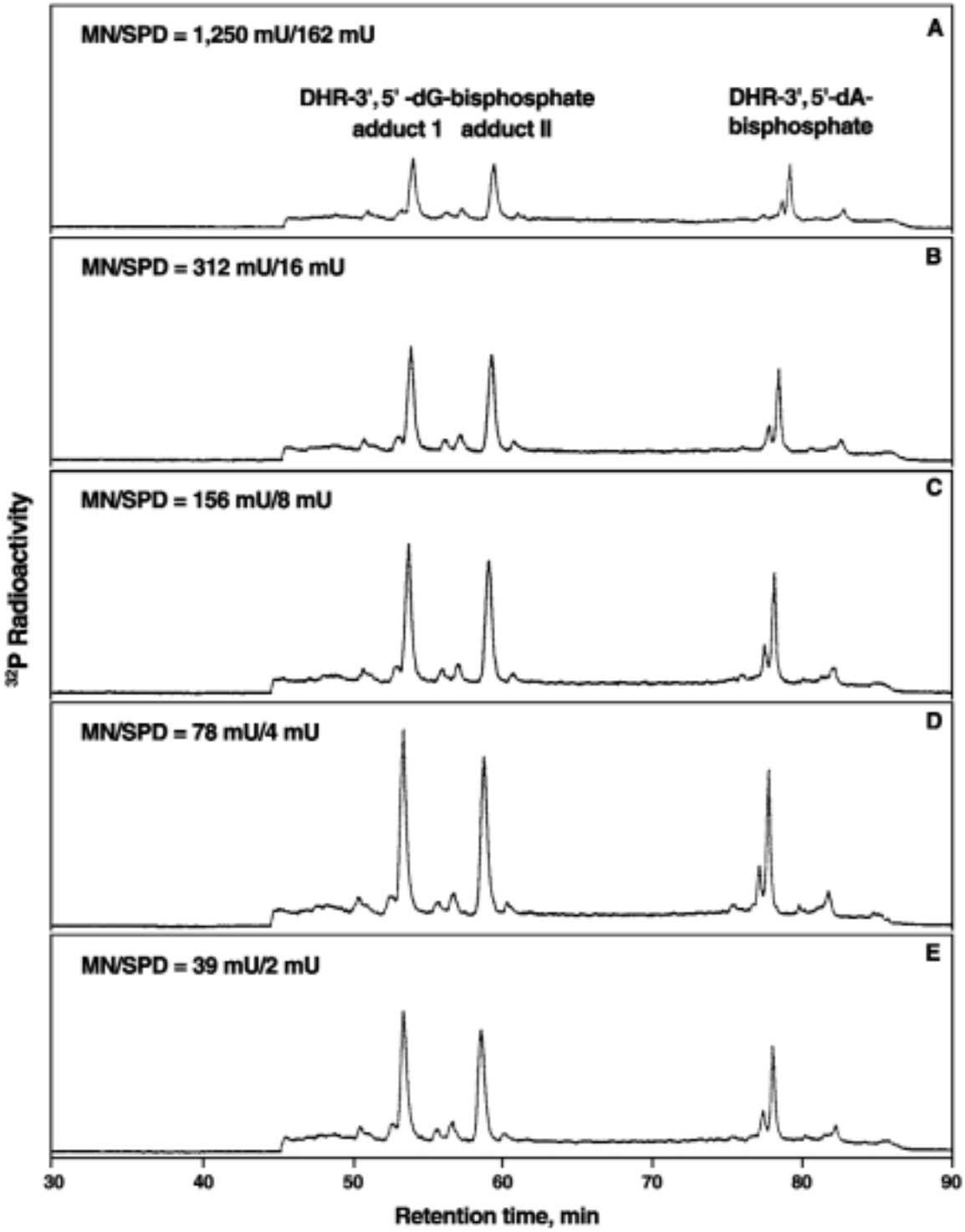

FIGURE I13
Effect of Micrococcal Nuclease (MN) and Spleen Phosphodiesterase (SPD) Concentration on ^{32}P-Postlabeling/HPLC Analysis of a Mixture of 8.3 fmol of DHR-3′-dGMP and 20 fmol of DHR-3′-dAMP Adducts

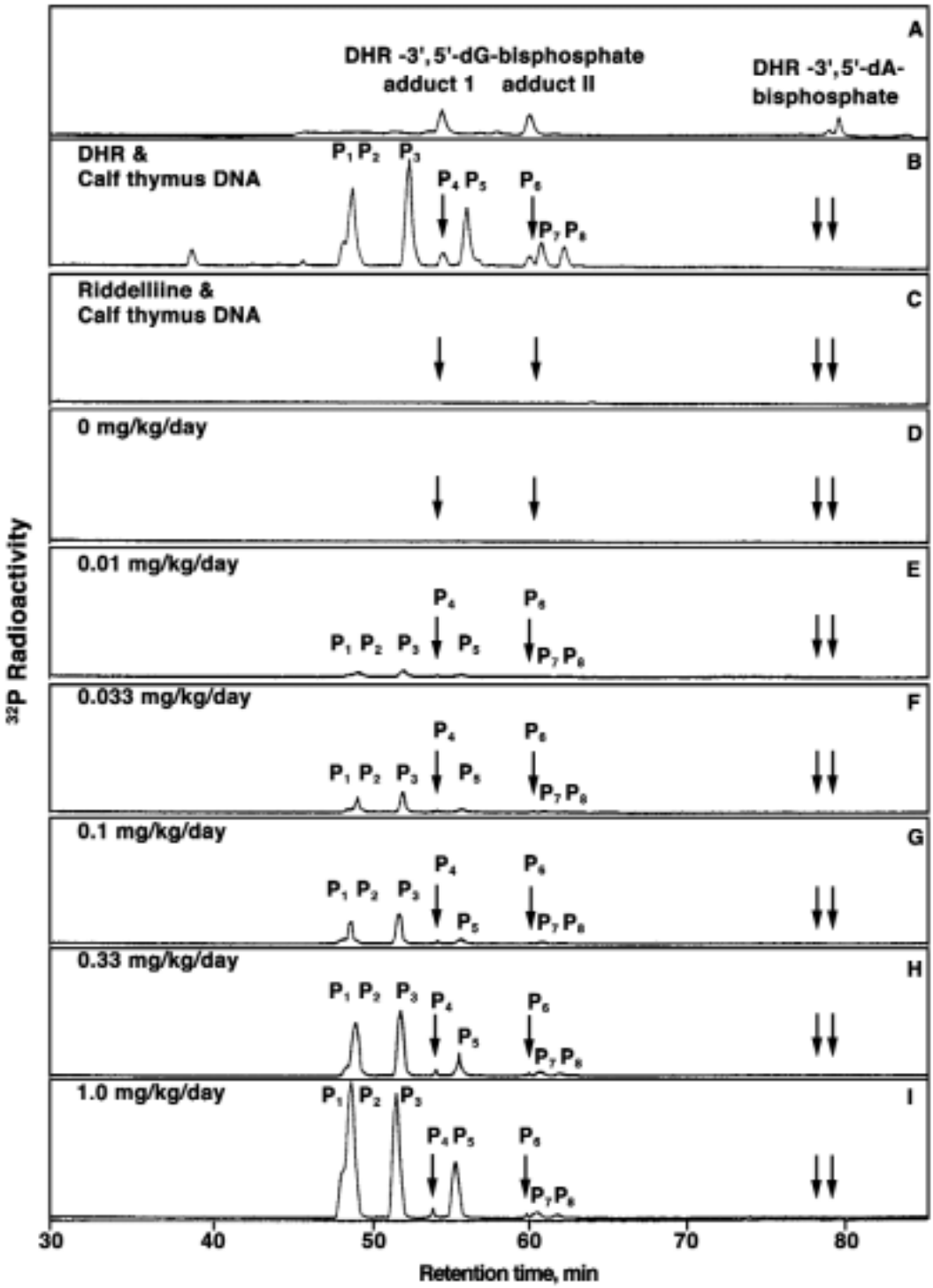

FIGURE I14
^{32}P-Postlabeling/HPLC Analysis of DHR-Derived DNA Adducts
The adducts are contained in (A) a mixture of synthetically prepared DHR-3′-dGMP and DHR-3′-dAMP adducts; (B) modified DNA from reaction of DHR with calf thymus DNA; (C) DNA obtained from chemical reaction of riddelliine with calf thymus DNA; and (D through I) livers of rats treated by gavage with one of six doses of riddelliine 5 days per week for 3 months.

TABLE I3
^{32}P-Postlabeling/HPLC Quantitation of Riddelliine-Derived DNA Adducts in Liver DNA of Female F344/N Rats Administered Riddelliine by Gavage for 3 Months[a]

Dose (mg/kg)	Adducts/10^7 Nucleotides							
	P1 + P2	P3	P4 (Adduct I)	P5	P6 (Adduct II)	P7	P8	Total
Vehicle Control	0	0	0	0	0	0	0	0
0.01	2.0 ± 0.8	2.4 ± 1.5	0.3 ± 0.1	0.7 ± 0.3	0.1 ± 0.03	0.3 ± 0.1	0.2 ± 0.7	6.0 ± 2.9
0.033	2.3 ± 0.5	3.4 ± 0.3	0.3 ± 0.01	0.8 ± 1.0	0.1 ± 0.2	0.36 ± 0.01	0.2 ± 0.01	7.4 ± 0.9
0.1	4.8 ± 0.4	5.7 ± 0.3	0.4 ± 0.03	1.1 ± 1.0	0.14 ± 0.01	0.51 ± 0.02	0.28 ± 0.02	12.0 ± 0.9
0.33	13.4 ± 2.8	12.8 ± 2.6	0.6 ± 0.02	4.2 ± 1.9	0.23 ± 0.04	0.86 ± 0.1	0.42 ± 0.05	32.6 ± 7.4
1.0[b]	20.0 ± 3.1	18.7 ± 1.5	0.8 ± 0.1	6.4 ± 0.8	0.32 ± 0.03	1.07 ± 0.23	0.6 ± 0.1	47.8 ± 4.9

[a] Data presented as mean ± standard deviation (n=4). P1 to P8 represent the chromatographic fractions from ^{32}P-postlabeling/HPLC assays.
[b] n=3

TABLE I4
^{32}P-Postlabeling/HPLC Quantitation of Riddelliine-Derived DNA Adducts in Liver DNA of Female F344/N Rats Administered Riddelliine by Gavage for 6 Months[a]

Dose (mg/kg)	Adducts/10^7 Nucleotides							
	P1 + P2	P3	P4 (Adduct I)	P5	P6 (Adduct II)	P7	P8	Total
Vehicle Control	0	0	0	0	0	0	0	0
0.01	8.9 ± 3.7	15.6 ± 4.0	0.55 ± 0.1	5.65 ± 2.1	0.2 ± 0.04	0.9 ± 0.2	0.65 ± 0.06	32.4 ± 10.2
0.033	15.8 ± 6.3	24.6 ± 5.3	0.74 ± 0.1	9.9 ± 3.5	0.35 ± 0.1	2.2 ± 0.6	1.03 ± 0.1	54.7 ± 15.9
0.1	23.8 ± 5.9	35.4 ± 4.9	1.3 ± 0.7	14.1 ± 3.2	0.4 ± 0.02	3.7 ± 0.5	2.4 ± 0.4	81.0 ± 15.5
0.33	41.1 ± 8.9	51.9 ± 4.2	2.2 ± 0.24	22.7 ± 2.8	0.7 ± 0.07	5.5 ± 0.3	3.8 ± 0.3	129.0 ± 15.8
1.0[b]	51.0 ± 4.3	72.1 ± 1.0	3.8 ± 0.23	30.1 ± 0.5	0.98 ± 0.1	8.5 ± 0.6	6.0 ± 0.7	172.4 ± 3.7

[a] Data presented as mean ± standard deviation (n=4). P1 to P8 represent the chromatographic fractions from ^{32}P-postlabeling/HPLC assays.
[b] n=3

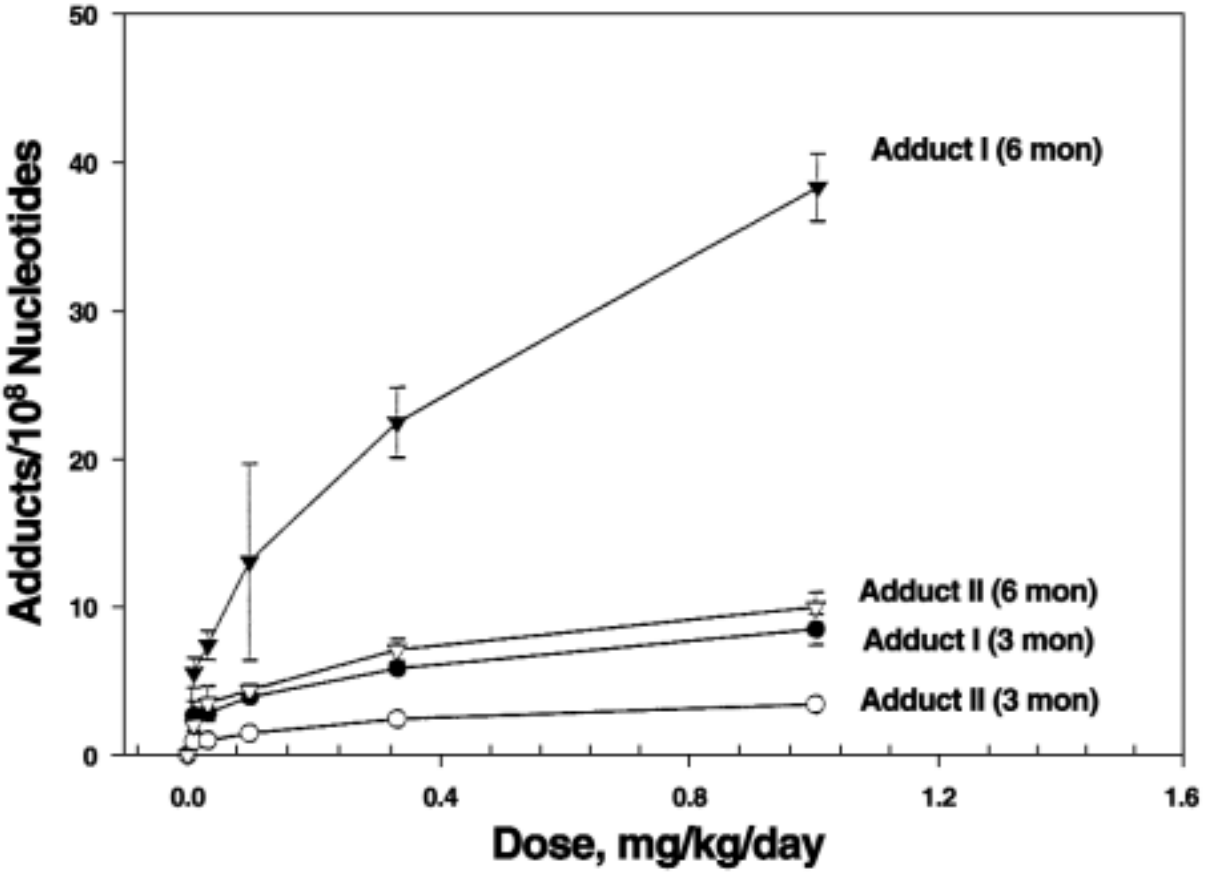

FIGURE I15
Formation of DHR-3′-dGMP Adducts I and II Contained in Liver DNA of Female F344/N Rats Administered Riddelliine by Gavage for 3 or 6 Months

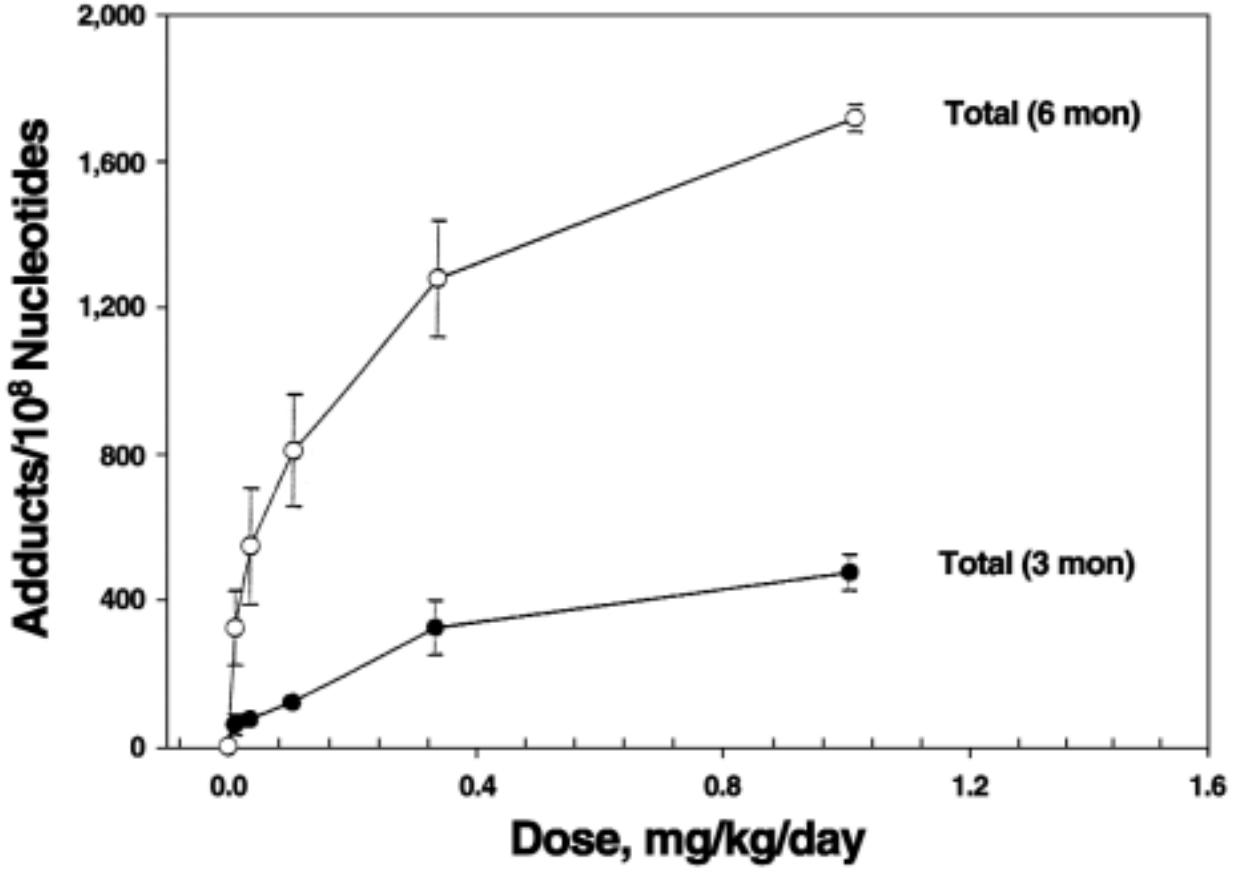

FIGURE I16
Total Riddelliine-Derived DNA Adduct Formation in Liver DNA of Female F344/N Rats Administered Riddelliine by Gavage for 3 or 6 Months

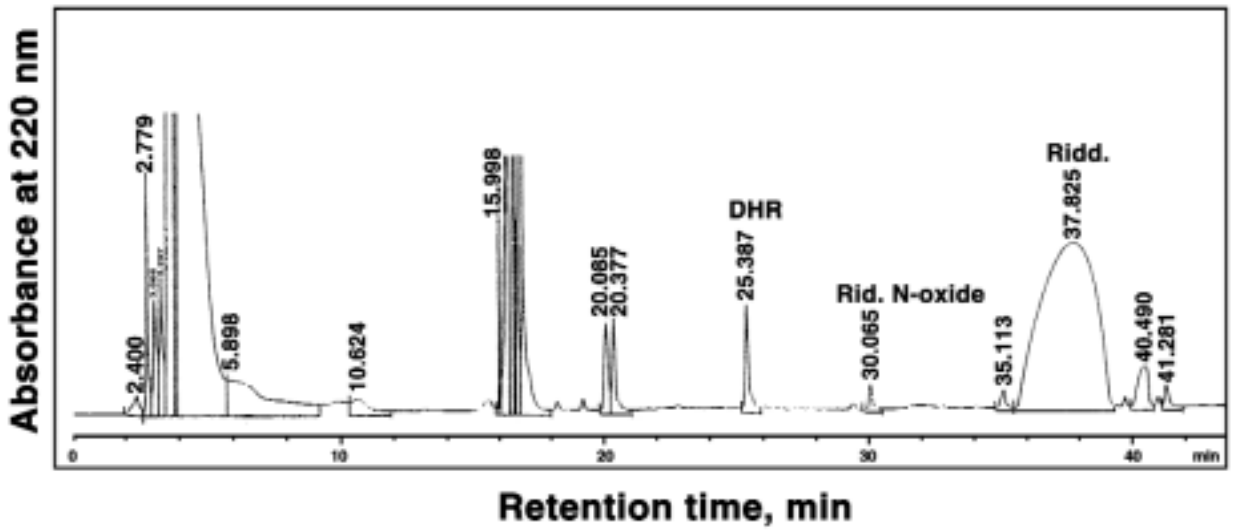

FIGURE I17
Reversed-Phase HPLC Analysis of Riddelliine Metabolites Formed from Metabolism of Riddelliine by Human Liver Microsomes

TABLE I5
^{32}P-Postlabeling/HPLC Quantitation of Total DHR-Derived DNA Adducts Formed from Rat and Human Liver Microsomal Metabolism of Riddelliine in the Presence of Calf Thymus DNA

Riddelliine (mM)	Total DNA Adducts[a]			
	Female F344/N Rat[b]	Human Female[b] (52-year-old white)	Human Male[c] (80-year-old white)	Human Female[c] (69-year-old white)
0.0025	105 ± 5			
0.004	181 ± 28.5			
0.0065	288 ± 12			
0.01	505 ± 32.3	59 ± 2		115 ± 2.1
0.02				258 ± 4.1
0.025	719 ± 85.1	98 ± 1	138 ± 14	
0.05		202 ± 2	156 ± 20.3	
0.08				720 ± 12.3
0.1		389 ± 2	407 ± 46.3	
0.16				1,188 ± 17.2
0.25		530 ± 2	536 ± 113.5	

[a] Data presented as adducts/10^8 nucleotides (mean ± standard deviation)
[b] Results of duplicate analyses
[c] Results of triplicate analyses

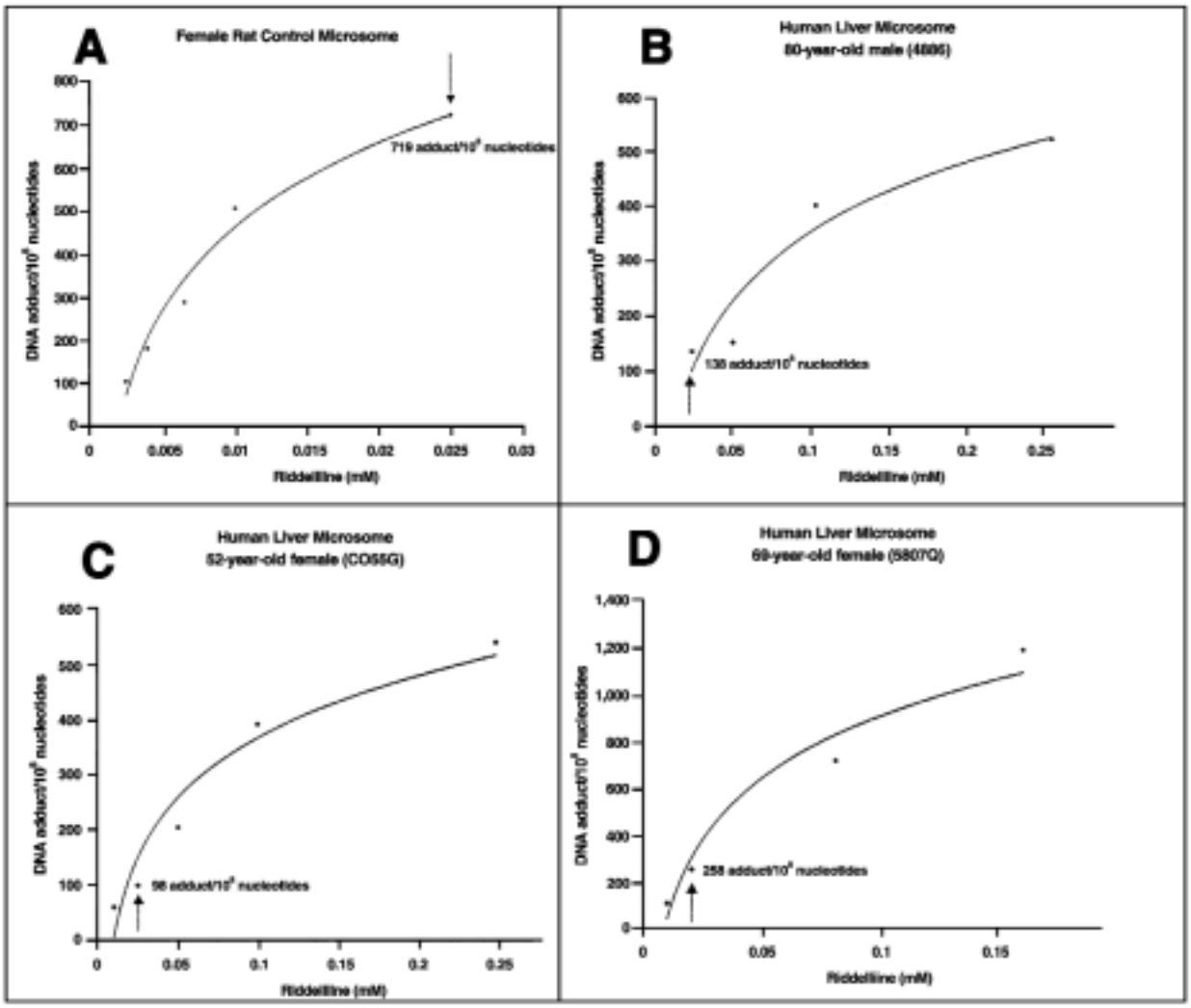

FIGURE I18
Total DHR-Derived DNA Adducts Formed from Metabolism of Riddelliine by Rat or Human Liver Microsomes in the Presence of Calf Thymus DNA
The microsomes were from (A) female F344/N rats or (B through D) male or female humans

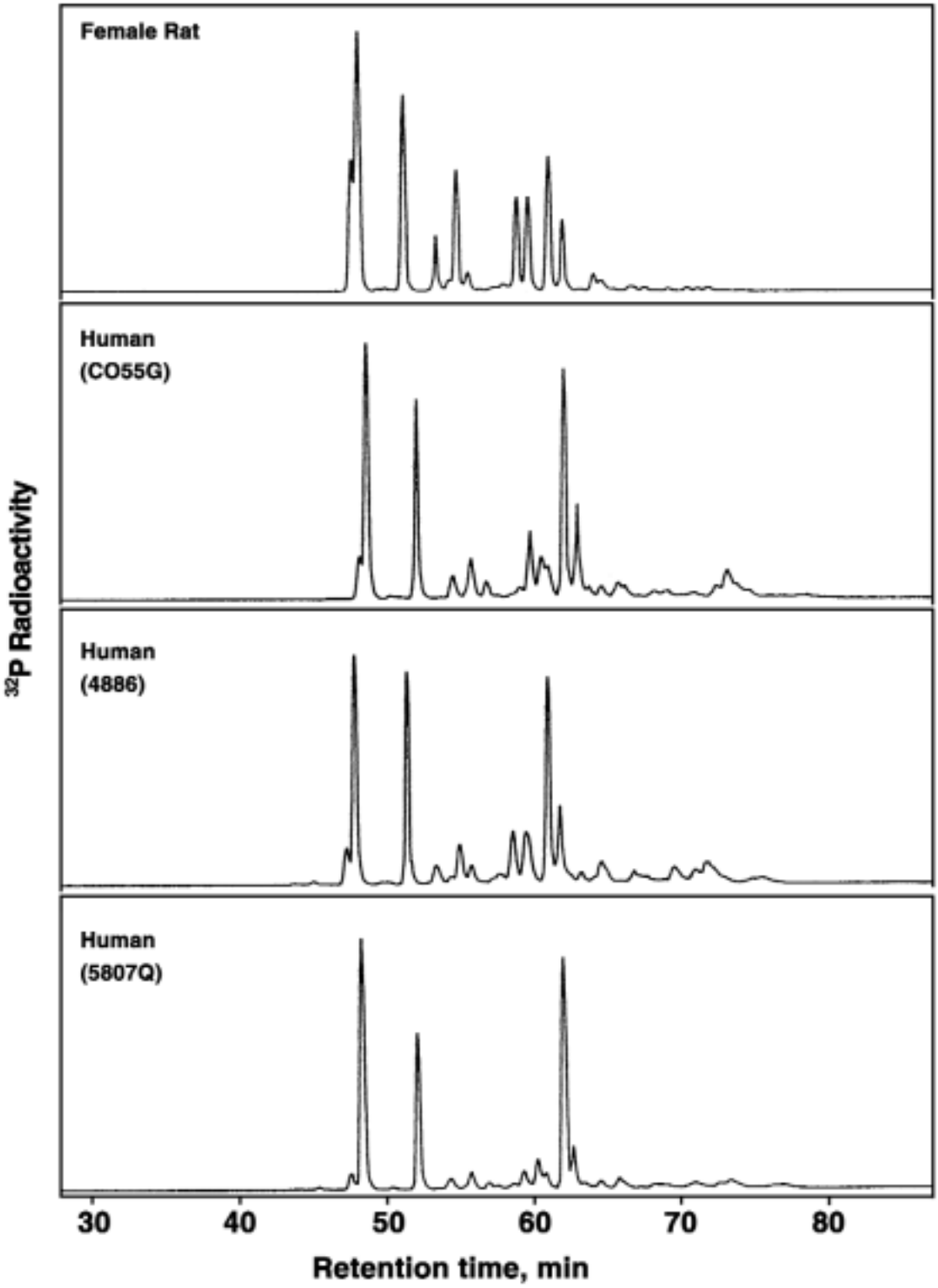

Figure I19
^{32}P-Postlabeling/HPLC Analysis of DHR-Derived Adducts Formed from Metabolism of Riddelliine by Liver Microsomes
The liver microsomes were from female F344/N rats or male or female humans.

Chemical	TR No.
Ethylbenzene	466
Ethylene Glycol	413
Ethylene Glycol Monobutyl Ether	484
Ethylene Oxide	326
Ethylene Thiourea	388
Eugenol	223
FD&C Yellow No. 6	208
Fumonisin B_1	496
Furan	402
Furfural	382
Furfuryl Alcohol	482
Furosemide	356
Gallium Arsenide	492
Geranyl Acetate	252
Glutaraldehyde	490
Glycidol	374
Guar Gum	229
Gum Arabic	227
HC Blue 1	271
HC Blue 2	293
HC Red 3	281
HC Yellow 4	419
Hexachlorocyclopentadiene	437
Hexachloroethane	361
4-Hexylresorcinol	330
Hydrochlorothiazide	357
Hydroquinone	366
8-Hydroxyquinoline	276
Indium Phosphide	499
Iodinated Glycerol	340
Isobutene	487
Isobutyl Nitrite	448
Isobutyraldehyde	472
Isophorone	291
Isoprene	486
Lauric Acid Diethanolamine Condensate	480
d-Limonene	347
Locust Bean Gum	221
60-Hz Magnetic Fields	488
Magnetic Field Promotion	489
Malonaldehyde, Sodium Salt	331
Manganese Sulfate Monohydrate	428
D-Mannitol	236
Marine Diesel Fuel and JP-5 Navy Fuel	310
Melamine	245
2-Mercaptobenzothiazole	332
Mercuric Chloride	408
Methacrylonitrile	497
8-Methoxypsoralen	359
α-Methylbenzyl Alcohol	369
Methyl Bromide	385
Methyl Carbamate	328
Methyldopa Sesquihydrate	348
Methylene Chloride	306
4,4′-Methylenedianiline Dihydrochloride	248
Methyleugenol	491
Methyl Methacrylate	314
N-Methylolacrylamide	352
Methylphenidate Hydrochloride	439
Mirex	313
Molybdenum Trioxide	462
Monochloroacetic Acid	396
Monuron	266
Nalidixic Acid	368
Naphthalene (Mice)	410
Naphthalene (Rats)	500
Nickel (II) Oxide	451
Nickel Sulfate Hexahydrate	454
Nickel Subsulfide	453
p-Nitroaniline	418
o-Nitroanisole	416

Chemical	TR No.
p-Nitrobenzoic Acid	442
Nitrofurantoin	341
Nitrofurazone	337
Nitromethane	461
p-Nitrophenol	417
o-Nitrotoluene	504
p-Nitrotoluene	498
Ochratoxin A	358
Oleic Acid Diethanolamine Condensate	481
Oxazepam (Mice)	443
Oxazepam (Rats)	468
Oxymetholone	485
Oxytetracycline Hydrochloride	315
Ozone and Ozone/NNK	440
Penicillin VK	336
Pentachloroanisole	414
Pentachloroethane	232
Pentachloronitrobenzene	325
Pentachlorophenol, Purified	483
Pentachlorophenol, Technical Grade	349
Pentaerythritol Tetranitrate	365
Phenolphthalein	465
Phenylbutazone	367
Phenylephrine Hydrochloride	322
N-Phenyl-2-Naphthylamine	333
o-Phenylphenol	301
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Gavage)	244
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Feed)	398
Polysorbate 80 (Glycol)	415
Polyvinyl Alcohol	474
Primidone	476
Probenecid	395
Promethazine Hydrochloride	425
Propylene	272
1,2-Propylene Oxide	267
Propyl Gallate	240
Pyridine	470
Quercetin	409
Riddelliine	508
Resorcinol	403
Rhodamine 6G	364
Rotenone	320
Roxarsone	345
Salicylazosulfapyridine	457
Scopolamine Hydrobromide Trihydrate	445
Sodium Azide	389
Sodium Fluoride	393
Sodium Nitrite	495
Sodium Xylenesulfonate	464
Stannous Chloride	231
Succinic Anhydride	373
Talc	421
Tara Gum	224
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Dermal)	201
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Gavage)	209
1,1,1,2-Tetrachloroethane	237
Tetrachloroethylene	311
Tetracycline Hydrochloride	344
Tetrafluoroethylene	450
1-Trans-Delta^9-Tetrahydrocannabinol	446
Tetrahydrofuran	475
Tetrakis(Hydroxymethyl)Phosphonium Sulfate	296
Tetrakis(Hydroxymethyl)Phosphonium Chloride	296
Tetranitromethane	386
Theophylline	473
4,4-Thiobis(6-*t*-Butyl-*m*-Cresol)	435
Titanocene Dichloride	399
Toluene	371
2,4- & 2,6-Toluene Diisocyanate	251
Triamterene	420
Tribromomethane	350

Chemical	TR No.
Trichloroethylene	243
Trichloroethylene	273
1,2,3-Trichloropropane	384
Tricresyl Phosphate	433
Triethanolamine	449
Tris(2-Chloroethyl) Phosphate	391
Tris(2-Ethylhexyl) Phosphate	274
Turmeric Oleoresin (Curcumin)	427
Vanadium Pentoxide	507
4-Vinylcyclohexene	303
4-Vinyl-1-Cyclohexene Diepoxide	362
Vinylidene Chloride	228
Vinyl Toluene	375
Xylenes (Mixed)	327
2,6-Xylidine	278
Zearalenone	235
Ziram	238