National Toxicology Program
Toxicity Report Series
Number 27

NTP Technical Report
on Toxicity Studies of

Riddelliine

(CAS No. 23246-96-0)

Administered by Gavage
to F344/N Rats and B6C3F$_1$ Mice

Po C. Chan, PhD, Study Scientist
National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 94-3350
December 1993

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

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This NTP report on the toxicity studies of riddelliine was based primarily on 2-week studies that took place in November 1986 and 13-week studies that began in March 1987 and ended in September 1987 at SRI International, Menlo Park, CA.

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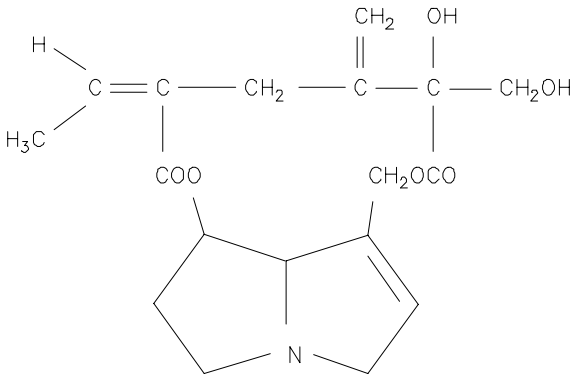
Riddelliine



Molecular Formula $C_{18}H_{23}NO_6$
CAS Number 23246-96-0
Molecular Weight 349.4
Synonyms 13,19-didehydro-12,18-dihydroxy senecionan-11,16-dione;
trans-15-ethylidine-12b-hydroxy-12a-hydroxymethyl-13-methylenesenec-1-enine;
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

ABSTRACT

Riddelliine is a naturally occurring pyrrolizidine alkaloid, a class of compounds occurring in rangeland plants of the genera *Crotalaria*, *Amsinckia*, and *Senecio*. Two-week and 13-week rodent toxicity studies of riddelliine were conducted because riddelliine can be a contaminant of foodstuffs, such as meat, grains, seeds, milk, herbal tea, and honey. In addition to histopathology, evaluations included clinical pathology and reproductive toxicity. *In vitro* genetic toxicity studies included assessments of mutagenicity in *Salmonella typhimurium* and of the induction of chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells. Riddelliine was also evaluated *in vivo* for the induction of micronuclei in mouse bone marrow and in peripheral blood and for the induction of S-phase synthesis and unscheduled DNA synthesis in the liver of rats and mice.

In the 2-week studies, groups of five male and five female F344/N rats and B6C3F$_1$ mice were administered riddelliine in 0.1 M phosphate buffer by gavage at dose levels of 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg body weight five times per week, for a total of 12 doses. Four of five male rats in the 25 mg/kg group 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. No deaths or body weight effects were observed in female rats. Male rats had dose-related hemorrhagic centrilobular hepatic necrosis, hepatocytic karyomegaly and cytologic alterations, pulmonary hemorrhage and/or edema, splenic extramedullary hematopoiesis, and pancreatic edema. Female rats exhibited fewer and less severe lesions than identically treated male rats. Heart weights of treated male and female rats were lower than those of the controls.

No deaths or effects on body weight were observed in treated mice. Dose-related increases in absolute and relative liver weights and increased incidences of hepatic cytomegaly were the only treatment-related findings in male and female mice administered riddelliine.

In the 13-week studies, groups of 20 male and 20 female F344/N rats and B6C3F$_1$ mice were administered riddelliine in 0.1 M phosphate buffer by gavage five times per week for 13 weeks. Rats received 0, 0.1, 0.33, 1.0, 3.3, or 10 mg/kg and mice received 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg. Ten animals from each dose group were killed after 13 weeks of treatment. The remaining 10 animals in each dose group were observed without further treatment for up to 14 weeks; five animals from each dose group were killed after 7 weeks of recovery, and the remaining five animals per dose group were killed at the end of the 14-week recovery period.

During the 13-week treatment period, 19 of 20 male rats in the high-dose group died; all others survived. Body weight gains were decreased with increasing dose at Week 13. During the 14-week recovery period, all male rats survived, but five high-dose females died. Mean body weight gains of dosed and control male rats were similar throughout the 14-week recovery period; the final mean body weights of the treated males approached the final mean body weight of the controls. Similarly, mean body weight gains among the treated female rats were similar to the control value at the end of the 14-week recovery period. However, the final mean body weight of female rats given 1.0 or 3.3 mg/kg remained lower than that of controls at the end of the 14-week recovery period.

In the 13-week study, the most significant treatment-related histopathologic lesions in rats occurred in the liver and included hepatocyte cytomegaly and karyomegaly, cytoplasmic vacuolization, centrilobular necrosis, mixed inflammatory cell infiltration, and bile duct hyperplasia. Vascular lesions in the kidneys and lungs were observed in most high-dose rats after 13 weeks of riddelliine administration. Additional lesions were found in the heart, spleen, kidneys, and pancreas at 13 weeks. At the end of the 14-week recovery period, hepatocyte karyomegaly, cytomegaly, and cytoplasmic vacuolization persisted. In addition, the incidence of bile duct hyperplasia was markedly increased in dosed female rats, and foci of cytologic alteration or hyperplastic hepatocytes were observed in dosed rats that were allowed to recover for up to 14 weeks.

Adenomas of the liver occurred in 2 of 10 females in the 10 mg/kg group at 13 weeks and in one of five females in this group after the 14-week recovery period; no adenomas were found in the livers of control females.

Serum activities of alkaline phosphatase in male rats and sorbitol dehydrogenase in female rats increased with increasing dose. Reticulocyte counts consistently increased and platelet counts consistently decreased with increasing dose in treated male and female rats. The clinical pathology findings were indicative of liver damage and erythrocyte and platelet sequestration.

In mice in the 13-week study, no deaths related to riddelliine treatment occurred. Body weight gains were depressed at the two highest dose levels (10 and 25 mg/kg); the depression in body weight persisted throughout the 14-week recovery period. Dose-related increases in erythrocyte counts in male mice and in reticulocyte counts in female mice were observed. Dose-related decreases in platelet counts were also observed in both males and females. Centrilobular cytomegaly in the liver was noted at 13 weeks in males and females administered 25 mg/kg riddelliine; this lesion persisted through the recovery period in females. At the end of the 14-week recovery period, bile duct hyperplasia was seen in the liver in high-dose female mice. Epithelial hyperplasia of the forestomach was noted in male and female mice in the 10 and 25 mg/kg groups after 13 weeks of treatment, but this lesion became less severe during the recovery period.

In male rats administered up to 3.3 mg/kg and in male mice administered up to 25 mg/kg for 13 weeks, riddelliine did not adversely affect any of the reproductive end points evaluated. In female rats given 10 mg/kg and in female mice given 25 mg/kg, the length of the estrous cycle was increased. However, no unequivocal adverse effects were noted on fertility, pup growth and survival, or weight gain of dams during pregnancy during the mating trial in rats, although mean body weights of dams given 0.1 or 1.0 mg/kg were significantly lower than the mean body weight of the controls throughout gestation and lactation. In contrast, riddelliine administered at a dose of 25 mg/kg was toxic to the dams in the mouse mating trial, resulting in lower body weights at the beginning of gestation and throughout lactation. Administration of 25 mg/kg riddelliine to mouse dams also affected fetal growth and survival; the average live litter size was significantly reduced, the number of pups born dead was increased, and the average pup weight was reduced throughout the 21-day postpartum period.

Riddelliine was mutagenic in *Salmonella typhimurium* strain TA100 with, but not without, S9 activation; results of mutagenicity testing were negative in strains TA97, TA98, and TA1535. Riddelliine induced sister chromatid exchanges in Chinese hamster ovary (CHO) cells with and without S9. Chromosomal aberrations were induced in CHO cells only in the presence of S9. The frequency of micronucleated erythrocytes in mouse peripheral blood samples was not elevated after 4 or 13 weeks of daily gavage treatments; however, a weakly positive response was noted in the peripheral blood and bone marrow of male mice administered a single, high dose of riddelliine by gavage.

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 increase in S-phase DNA synthesis was observed in cultured hepatocytes of male and female rats treated for either time period.

In summary, the administration of riddelliine to rodents by gavage for up to 13 weeks resulted in a spectrum of neoplastic and nonneoplastic effects similar to those previously described for other pyrrolizidine alkaloids. Rats were found to be somewhat more sensitive than mice, and males more sensitive than females, to the toxic effects of riddelliine. The no-observed-adverse-effect level (NOAEL) for histopathologic changes in the 13-week studies was 3.3 mg/kg body weight for mice and 0.1 mg/kg body weight for rats. The liver was the primary target of riddelliine-induced injury that resulted in lesions characterized

by cytomegaly and cytologic alteration in rats and mice and also by marked necrotic and proliferative changes in rats. Riddelliine is carcinogenic to female F344/N rats, based on the occurrence of hepatocellular adenomas.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of riddelliine on June 24, 1992, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On June 24, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of riddelliine.

Dr. Po Chan, NIEHS, introduced the short-term toxicity studies of riddelliine by reviewing the occurrence of the chemical, the rationale for the studies, the experimental design, and the results.

Dr. Goodman, a principal reviewer, said that the report was thoroughly prepared and to the point. However, given what is known about the toxicity and carcinogenicity of the pyrrolizidine alkaloids, he wondered if there was adequate rationale to perform the studies. He requested that more detail be added to the report concerning the interest of the United States Food and Drug Administration (USFDA) in this particular alkaloid. Dr. Chan replied that the USFDA was concerned about the contamination of meat, milk, honey, and herbal teas, and that in some Western grasses, riddelliine is the predominant pyrrolizidine alkaloid found. Dr. Goodman stated that longer exposures to lower levels of riddelliine might cause lung toxicity, as is seen with other pyrrolizidine alkaloids, rather than liver toxicity. Dr. John R. Bucher, NIEHS, agreed and indicated that this possibility would be added to the discussion. Dr. Carlson pointed out the rather marked effect on lung weights in the studies and asked if more information could be given concerning possible reasons for this finding. Dr. Chan replied that it appeared to result from perivascular macrophage accumulations and edema fluid.

Dr. Davidson, another principal reviewer, said the report was well written. She asked why gavage was chosen over administration in the diet. Dr. Chan replied that the alkaloid was difficult to obtain and that gavage allowed more efficient use of the material.

Dr. Bailey said that although NOAELs are given for the test animals, it would be useful if information could be added concerning the levels of riddelliine contamination in various foods. The FDA data on riddelliine residues in foods are unpublished.

After discussion of editorial matters, Dr. Carlson accepted the report on behalf of the peer review panel.

INTRODUCTION

Physical Properties, Occurrence, and Exposure

Riddelliine is a solid with a melting point of 194° to 196° C (Molyneux *et al.*, 1991). It is soluble in chloroform, acetone, and ethanol, and is sparingly soluble in water. As a solid, it is stable at room temperature in diffuse light for 12 months or longer. Alcoholic and aqueous solutions of riddelliine are stable at room temperature when protected from light. Riddelliine is a member of a class of pyrrolizidine alkaloids (PAs). The PAs are esters of unsaturated basic alcohols or necines (*e.g.*, heliotridine, retronecine, supinidine, crotanecine, otonecine) and a necic acid (a C_5-C_{10} branched-chain unsaturated, epoxidized or hydroxylated mono- or dicarboxylic acid) (*Merck Index*, 1983). They may be monoesters with monocarboxylic acids (heliotrine, lasiocarpine) or macrocyclic diesters formed from dicarboxylic acids (monocrotaline, retrorsine). Riddelliine is a macrocyclic diester of a necine, retronecine (Figure 1), and a necic acid, riddelliic acid (Figure 2).

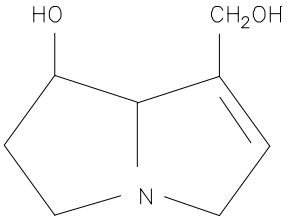

FIGURE 1 Chemical Structure of Retronecine

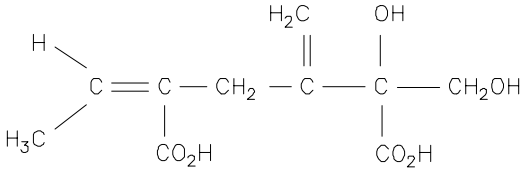

FIGURE 2 Chemical Structure of Riddelliic Acid

Riddelliine is isolated from plants of the genus *Senecio* (Molyneux *et al.*, 1979), which are found in the rangelands in the western United States. The alkaloids occur in different parts of the plants with the highest content in the seeds and flowering tops. Riddelliine and other PAs in these plants may cause economic loss to ranchers, as cattle, horses, and, to a lesser extent, sheep ingesting these plants succumb to the toxic effects. These plants may contaminate human food sources as intact plants or their seeds may contaminate commercial grains. Riddelliine residues may be found in meat animal tissues, and in products such as milk and honey (USFDA, unpublished report on riddelliine). In East India, the root of *Crotalaria juncea*, which contains PAs, is taken as a hemoptysis remedy

and the plant is used against impetigo psoriasis and as an emmenagogue. The "bush tea" used to treat children for colds in Jamaica, as well as the herbal teas used in the American Southwest, may contain riddelliine and other PAs.

Disposition and Metabolism

At present, there are no data on the absorption and metabolic fate of riddelliine. However, other pyrrolizidine alkaloids have been extensively studied and the information accumulated serves as a background for the study of the toxicity of riddelliine.

Disposition studies have been conducted with [^{3}H]-monocrotaline (Hayashi, 1966), [^{14}C]-lasiocarpine (Culvenor *et al.*, 1969), [^{14}C]-senecionine, and [^{14}C]-seneciphylline (Eastman *et al.*, 1982). In general, about 80% of the ingested PAs are excreted, rapidly and unchanged, in the urine and feces, with urine the more prevalent route. Exhaled CO_2 is a minor route of excretion, with about 10% of the ingested PAs excreted in this manner. The ingested alkaloids are concentrated in the liver and kidney; very low levels of radioactivity are found in other tissues, including the lung and spleen.

Pyrrolizidine alkaloids appear to be metabolized via common pathways (McLean, 1970; Huxtable, 1979; Peterson and Culvenor, 1983; Mattocks, 1986). The alkaloids may be hydrolyzed, converted to N-oxides, or dehydrogenated to pyrrolic derivatives. Hydrolysis and N-oxidation seem to be detoxication pathways. Dehydrogenation appears to be associated with cytotoxicity.

Hydrolysis gives rise to a mono- or dicarboxylic acid and a pyrrole ring. The acid formed is apparently of no significance in toxicity. The pyrrole ring is further oxidized to an N-oxide or dehydrogenated to a pyrrolic metabolite (Figure 3). The dehydrogenated pyrrolic metabolites, dehydroalkaloids, are powerful alkylating agents and are responsible for the toxic reaction of the alkaloids. They are very reactive and immediately upon formation attack nucleophile centers (SH, NH, and OH groups) in protein and other cell constituents (Hsu *et al.*, 1975). Covalent binding of senecionine and seneciphylline to hepatic DNA, RNA, and protein has been demonstrated (Eastman *et al.*, 1981, 1982). Hincks *et al.* (1991) showed that riddelliine cross-links cellular DNA in cultured bovine kidney epithelial cells. Dehydroalkaloids may be further hydrolyzed to dehydroamino alcohols that are carcinogenic (Peterson *et al.*, 1983) and teratogenic (Peterson and Jago, 1980) and are thought to be responsible for the development of extrahepatic lesions. It is difficult to

separate the carcinogenicity of dehydroalkaloids and the dehydroamino alcohols due to spontaneous hydrolysis of the former (Peterson *et al.*, 1983).

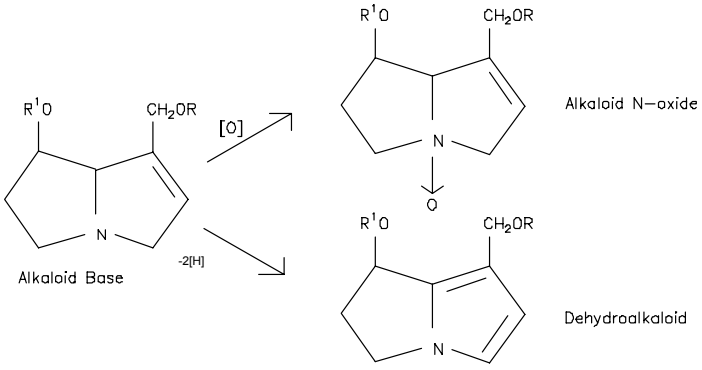

FIGURE 3 Oxidation and Dehydrogenation of an Alkaloid Base

The N-oxides are water soluble, have low toxicity, and are rapidly excreted in the urine. However, the intestinal bacterial flora can reduce them back to the parent compound (Powis *et al.*, 1979). Hepatic toxicity has been reported when indicine N-oxide was used as a chemotherapeutic agent in the treatment of cancer (Letendre *et al.*, 1981, 1984; Cook *et al.*, 1983).

The hydrolysis and dehydrogenation reactions are carried out by the liver microsomal mixed-function oxidase system. It has been shown that pretreatment of the animal with phenobarbital increases the rate of metabolic conversion and enhances toxicity (White *et al.*, 1983; Bruner *et al.*, 1986). Pretreatment with a microsomal enzyme inhibitor such as SKF 525A has the opposite effect (Allen *et al.*, 1972; Bruner *et al.*, 1986) and reduces toxicity. Thus, susceptibility to the toxic effects of pyrrolizidine alkaloids is determined by factors that affect the activity of the microsomal enzyme system at the time of PA exposure.

Other factors that affect the susceptibility to PA toxicity include species, strain, diet, age, and sex. Gerbils, hamsters, guinea pigs, goats, sheep, rabbits, and Japanese quail are resistant, while humans, monkeys, rats, mice, chicks, turkeys, cattle, pigs, and horses are sensitive to the toxicity of PAs (Cheeke, 1989). Dietary constituents rich in sulfhydryl compounds may have some protecting value against pyrrolizidine poisoning. The strong

nucleophilic nature of the presumptive toxic pyrrole makes it readily subject to adduct formation with -SH compounds. Thus, methionine has been used successfully to treat racehorses poisoned with *Senecio* (Huxtable, 1979). The toxic effects of monocrotaline in rats are ameliorated by feeding a high cysteine diet (Buckmaster *et al.*, 1976). Presumably, the sulfhydryls bind the toxic metabolites of PAs and minimize the amount of toxicant available to cause tissue damage. Administration of antioxidants such as BHA or ethoxyquin, which induce GSH S-transferases, also protects against the toxic effects of monocrotaline (Kahl and Wulff, 1979; Miranda *et al.*, 1981). Young rats are more susceptible to the toxic effects of PAs than are adult rats. The greater susceptibility is probably related to the PA-metabolizing enzyme levels and the sensitivity of the tissues in different age groups. Male rats are better able to metabolize PAs into pyrroles and are therefore more susceptible to PA toxicity than are females (Mattocks, 1972).

Toxicity

HUMAN EFFECTS

Heliotrope poisoning is endemic in central Asia, where seeds of *Heliotropium* species enter the wheat crop. The typical clinical picture is that of ascites, hepatosplenomegaly, veno-occlusive disease of the liver, and abnormal liver function (McLean, 1970; Huxtable, 1980). In South Africa, a disease known as "bread poisoning," found among poor Europeans, was traced to the inclusion of *Senecio* and *Crotalaria* seeds and flowers in whole grain processed for bread flour (Selzer and Parker, 1951). A veno-occlusive disease outbreak in Jamaica was traced to the widespread use of "bush tea," an herbal medicine used to treat children for colds (Stirling *et al.*, 1962). In the American Southwest, the popular herbal tea, gordolobo yerba, is a potential hazard for exposure to toxic PAs. The Mexican herb *Gnaphalium macounii*, used in gordolobo yerba, is easily confused with *Senecio longilobus*. Some deaths have been reported from mistaken preparation of gordolobo yerba with *S. longilobus* (Huxtable, 1980). Cases reported include a 6-month-old female infant who developed liver cirrhosis (Stillman *et al.*, 1977) after being fed gordolobo yerba, containing 70 to 147 mg/kg of PAs total, for 2 weeks and a 2-month-old boy who died after exposure to *S. longilobus* alkaloids in gordolobo yerba at 13 to 17 mg/kg daily for 4 days (Fox *et al.*, 1978). One case of hepatic veno-occlusive disease of an infant who died 38 days after birth was traced to the mother's drinking a coltsfoot tea preparation for cough during pregnancy (Roulet *et al.*, 1988). The tea preparation was found to contain leaves and flowers of *Tussilago farfara* and roots of *Petasites officinalis* (Spang, 1989), which contain senecionine and senkirkine (Westendorf, 1992).

In the acute stage of PA toxicity, severe abdominal pain is accompanied by emesis and diarrhea. Liver biopsy shows centrilobular congestion resulting in compression and disappearance of liver cell cords and necrosis. In the chronic stage, hepatosplenomegaly, ascites, hepatic veno-occlusion, fibrosis and cirrhosis, portal hypertension, lymphadenopathy, anemia, hemosiderosis, widespread hemorrhage, nephritis, and thymic cortical necrosis are noted (Peterson and Culvenor, 1983). At low levels of exposure, lung lesions rather than liver lesions occur. The toxic manifestations in the lung are similar to those described in animal studies.

ANIMAL TOXICITY

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 PAs 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 reported worldwide. The knowledge among stockmen that PA-containing plants such as *Crotalaria* and *Senecio* species were common toxic agents in animal diseases was verified by animal feeding studies in which the plants were fed whole (Mattocks, 1986). Clinical signs in poisoned animals include neurological, gastrointestinal (diarrhea), and hematologic (high blood ammonia, hemolysis) effects. Ascites is often observed.

Molyneux *et al.* (1991) reported that calves fed *Senecio riddellii*, which contains only riddelliine and its N-oxide, for 20 days showed weight loss, signs of depression, reduced feed intake, 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 PA or its metabolite(s) acts on the liver tissue inducing acute necrosis and/or a marked enlargement of hepatocytes, which has been referred to as megalocytosis. The process takes place even after a single exposure to PA. The second

stage develops in response to the initial damage. The 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 the 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) has reviewed the toxicity of the pyrrolizidine alkaloids and describes 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 of monocrotaline per day by young (45 to 50 g) rats for 21 days led to pulmonary toxicity in the absence of hepatotoxicity. Pulmonary toxicity was manifested by increased lung-to-body-weight ratios, increased lung protein, and reduction in 5-hydroxytryptamine (5-HT) and noradrenaline removal and metabolism. The young rats also developed pulmonary hypertension and right ventricular hypertrophy. The reduced capacity of monocrotaline-damaged lung endothelial cells to remove 5-HT probably allowed circulating 5-HT and other vasoconstrictor levels 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.

Raczniak *et al.* (1978) reported that monthly doses of monocrotaline administered to young *Macaca* (stump-tail) monkeys resulted in significant ultrastructural changes in the right ventricle. These changes included mitochondrial hypertrophy, streaming and clumping of Z-band material, degeneration of myofibrils, changes in ribosomes and proliferation of intercellular collagen fibers. These changes were considered to be indicative of compensation to hypertensive heart disease.

Mineral metabolism in rats has been reported to be modified by dietary intake of *Senecio* plant material. 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 test animals, an even greater accumulation of copper was found. Impairment of hematopoiesis and accelerated erythrocyte destruction were noted (Swick *et al.*, 1982).

REPRODUCTIVE TOXICITY AND TERATOGENICITY

PAs and their metabolites can cross the placenta. Heliotrine and dehydroheliotridine injected into rat dams at the fourteenth day of pregnancy were recovered in the fetuses. The PAs induced growth retardation and fetal mortality (Peterson and Jago, 1980). Administration of heliotrine to pregnant rats during organogenesis led principally 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 showed hydropic or fatty vacuolation of liver cells. Weanling rats that died or that were killed between 1 and 6 months of age showed hemorrhagic necrosis, an increase in hepatic centrilobular reticulin, thickening of centrilobular veins, hyperplastic nodules, and bile duct proliferation. The fact that hepatic lesions were observed only after birth and not in fetuses was probably due to the inability of embryonic liver to activate PAs (Mattocks, 1986).

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 PAs inhibited colony formation by cultured bovine kidney epithelial cells. The PAs were not lethal to the cultured cells, but 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 PAs, 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 PAs is not clear. It should be noted that more tumors developed following the cessation of pyrrolizidine

alkaloid intake (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). However, megalocyte development is attributed to the action of PAs, which allow the cells to go through DNA synthesis and the cell cycle to repeat without undergoing cell division (Samuel and Jago, 1975; Mattocks, 1986). Hincks *et al.* (1991) postulated that the antimitotic activity of PAs is related to their ability to cross-link DNA and thereby inactivate the section of genome that codes for the proteins of division (Samuel and Jago, 1975).

CARCINOGENICITY

The carcinogenicity of pyrrolizidine alkaloids has been studied by administration of the plant, crude extracts of the plant, or the alkaloids to rats via varying routes. Harris and Chen (1970) reported that malignant liver tumors developed in rats fed diets containing 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 carcinomas in rats. These authors also reported that monocrotaline acted synergistically with aflatoxin B_1 in hepatic carcinogenesis. Both male and female rats fed lasiocarpine at low doses (7, 15, or 30 ppm) for 2 years developed hepatic angiosarcoma, hepatocellular carcinoma, and hepatocellular adenoma. Female rats also developed hematopoietic tumors (NCI, 1978).

In drinking water studies, hepatomas were induced in rats given retrorsine or isatidine at 0.03 g/L, 3 days per week (Schoental *et al.*, 1954). Petasitenine given at 0.01% induced hemangioendothelial sarcoma and hepatic adenoma in rats (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). Liver nodules were observed in 4 of 4 male and 5 of 12 female Wistar rats given riddelliine (0.02 mg/mL) in drinking water twice weekly for 6 months, followed by three intraperitoneal injections of 25 mg/kg during the seventh month or by a single intraperitoneal injection of 30 mg/kg 1 year after the beginning of riddelliine treatment (Schoental and Head, 1957). The IARC (1976) considered the study inconclusive.

Young male Sprague-Dawley rats given biweekly subcutaneous injections of dehydroretronecine for 12 months developed rhabdomyosarcomas at the site of injection

(Allen *et al.*, 1975). Peterson *et al.* (1983) reported that dehydroheliotridine given to rats intraperitoneally produced tumors in the liver, lungs, intestines, and other organs.

GENETIC TOXICITY

The published genotoxicity data on riddelliine indicate that the chemical is genotoxic *in vitro* and *in vivo*. It induced mutations in *Salmonella typhimurium* strain TA100 when tested with rat and hamster liver S9 activation (Zeiger *et al.*, 1988), and it induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary (CHO) cells in the presence of S9 (Galloway *et al.*, 1987). Sister chromatid exchanges were also increased in CHO cells in the absence of S9, but the response was markedly lower than that observed with S9. Therefore, it appears that metabolic activation is necessary for the genotoxic activity of riddelliine. Support for this idea comes from metabolism, cytotoxicity, and DNA and protein binding studies performed with other pyrrolizidine alkaloids; results of these studies showed 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). That the liver is a site sensitive to the genotoxic action of riddelliine is supported by the observation that riddelliine induced a significant increase in unscheduled DNA synthesis (UDS) in hepatocytes of rats treated by gavage *in vivo* (Mirsalis, 1987).

The results of short-term genotoxicity tests with riddelliine have been reported in several abstracts. Positive results were reported for induction of mutations in mouse lymphoma L5178Y cells (Rudd *et al.*, 1983), UDS in Fischer 344 rat hepatocytes *in vitro* (Mirsalis *et al.*, 1983), S-phase and unscheduled DNA synthesis *in vivo* (Mirsalis, 1987), and micronuclei in peripheral blood polychromatic erythrocytes of Swiss mice treated *in vivo* (MacGregor *et al.*, 1985).

Study Rationale and Design

Riddelliine was nominated by the Food and Drug Administration and the Bureau of Foods for toxicity and carcinogenicity testing because of its economic impact on the livestock industry, the potential for human exposure, and the information available on the toxicity of other pyrrolizidine alkaloids, which suggests that riddelliine is 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. The studies performed included 2-week and 13-week studies with 14-week recovery in F344/N rats and B6C3F$_1$ mice, clinical pathology, histopathology, reproductive system evaluations, and mating trials. Riddelliine was also evaluated for mutagenicity in *S. typhimurium*, for induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, for the induction of micronuclei in mouse bone marrow cells and peripheral blood, and for the induction of S-phase DNA synthesis and unscheduled DNA synthesis in the liver of rats and mice.

MATERIALS AND METHODS

Procurement and Characterization of Riddelliine

Riddelliine (CAS Number 23246-96-0) was supplied by Dr. Russell J. Molyneux (Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture). The chemical was extracted and purified from Riddell's groundsel (*Senecio riddellii*) collected from rangeland. Two lots were shipped to the NTP. Lot U101981 was used in the 2-week studies and Lot U041483 was used in the 13-week studies.

Identity, purity, and stability analyses were conducted at Midwest Research Institute (Kansas City, MO). The study chemical, a white, crystalline solid, was identified as riddelliine by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; the spectra were consistent with the structure of riddelliine and with available literature references. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with theoretical values. Cumulative analytical data, based on nuclear magnetic resonance spectroscopy, thin layer chromatography, and high-performance liquid chromatography (HPLC) using two solvent systems, indicated approximately 95% riddelliine, 5% of the alkaloid retrorsine, and about 0.2% seneciphylline for Lot U101981 and approximately 92% riddelliine, 5% retrorsine, and 1.4% seneciphylline for Lot U041483. An additional impurity with an area of 0.3% relative to the major peak was observed in HPLC analysis of Lot U041483. Quantitation based on nonaqueous amine titration indicated a purity of 99.7% ± 0.5% riddelliine for Lot U101981 and 98.6% ± 0.7% riddelliine for Lot U041483; however, this method did not differentiate between riddelliine and other pyrrolizidine alkaloids. Water content by weight loss on drying was 0.3% for Lot U101981 and 0.065% for Lot U041483. Stability studies indicated that riddelliine was stable as the bulk chemical when stored for 2 weeks, protected from light, at temperatures up to 60± C. At the study laboratory, subsequent chemical reanalyses by infrared spectroscopy, HPLC, and titration (13-week studies only) revealed consistent purity levels for the bulk chemical relative to the reference standard over the course of these studies.

Dose Formulations

Dose formulations were prepared by dissolving the appropriate quantity of riddelliine (weighed to the nearest 0.1 mg) in 0.1 M phosphate buffer (pH 6.0) to achieve the desired concentrations, which ranged from 0 to 5 mg/mL for the studies in rats and from 0 to 2.5 mg/mL for the studies in mice. Stability studies indicated that a 4 mg/mL solution of riddelliine in 0.1 M phosphate buffer was stable for 3 weeks in the dark at 5° C and for 3 hours at room temperature open to air and light; 0.032 mg/mL riddelliine was also stable for 3 hours at room temperature open to air and light. Losses of approximately 2% to 4.5% of the chemical were observed under all other storage conditions. The dose formulations used in the 2-week and 13-week studies were stored in Teflon-sealed amber glass bottles in the dark at approximately 5° C and were used within 3 weeks of preparation. Results of analyses of dose formulations for the 2-week and 13-week studies by ultraviolet absorption spectroscopy before administration to animals were within 10% of theoretical values.

Toxicity Study Designs

Male and female F344/N rats and B6C3F$_1$ mice used in these studies were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). Rats and mice were shipped to the study laboratory at approximately 4 weeks of age, were quarantined for 2 weeks, and were 6 weeks of age when the studies began (animals used in the mating trials were quarantined for 4 weeks and were 8 weeks of age at the beginning of the studies). Rats used in the 13-week studies were received in two shipments (one for the mating trial and the other for the base, clinical pathology, and genetic toxicology studies). Mice used in the 13-week studies were received in three shipments (one for the mating trial, one for the base study, and one for the genetic toxicology study). Several mice were found dead or were killed moribund during the quarantine period preceding the 13-week studies; necropsy revealed changes consistent with stress or decreased food or water intake. Additional mice showing evidence of emaciation or stress were not used in the studies. Two male and two female rats and mice were necropsied 2 to 3 days before the 2-week studies began; five male and five female rats and mice from each shipment were necropsied 2 to 3 days before the 13-week studies began. No evidence of infectious disease or internal parasitism was observed in any of these animals. At the end of the 13-week studies, blood samples were taken from five control rats and five sentinel mice of each sex for determination of viral antibody titers; all samples were negative (Boorman *et al.*, 1986;

Rao *et al.*, 1989a,b). Additional details concerning study design and performance are listed in Table 1.

With the exception of mating trial animals, five rats were housed per cage, and mice were housed individually for the 2-week and 13-week studies. Prior to mating, mating trial males were housed individually and females were housed two per cage. Animal rooms were maintained at 72° F (ranging from 64° to 79° F) and 50% relative humidity (ranging from 20% to 78%), with more than 10 fresh air changes per hour. Fluorescent light was provided for 12 hours per day. Feed and water were available *ad libitum*.

In the 2-week studies, groups of five rats and five mice per sex were administered riddelliine in 0.1 M phosphate buffer by gavage at dose levels of 0 (vehicle control), 0.33, 1.0, 3.3, 10, and 25 mg/kg of body weight. The high dose of 25 mg/kg was based on a report by Schoental and Magee (1959) that 6 of 11 Wistar rats (30 to 60 g) died from 11 to 60 days after a gavage dose of 30 to 39 mg/kg. Another report indicated that the intravenous LD_{50} of riddelliine in mice is 105 mg/kg (Anonymous, 1949). Dose volumes were 5 mL/kg body weight for rats and 10 mL/kg for mice and were based on individual body weights taken on Day 1 for Week 1, Day 8 for Week 2, and Day 15 for the final two doses. Riddelliine was administered for 5 days per week for 2 weeks plus 2 consecutive dose days before terminal sacrifice; thus, each animal received a total of 12 treatments.

Based on the results of the 2-week studies, the high dose selected for the 13-week studies was 10 mg/kg for rats and 25 mg/kg for mice. In the 13-week studies, 20 animals per species per sex were administered riddelliine in 0.1 M phosphate buffer by gavage. Dose levels for rats were 0 (vehicle control), 0.1, 0.33, 1.0, 3.3, or 10 mg/kg body weight; dose volumes of 5 mL/kg body weight were based on individual rat body weights taken weekly. Dose levels for mice were 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg; dose volumes of 10 mL/kg body weight were based on individual body weights taken weekly. The test article was administered on weekdays, excluding holidays, for 13 weeks. At the end of the treatment period, 10 rats and 10 mice per dose group were killed. Half of the remaining animals (five rats and five mice per dose group) were killed after a 7-week recovery period and the other half were killed after a 14-week recovery period. The recovery studies were conducted because it had been reported that PAs are mitotic inhibitors and that tumors developed after the cessation of PA administration.

Complete necropsies were performed on animals in the 2-week and 13-week base studies. The following organs from rats and mice in the base studies (including animals in the recovery groups) were weighed: brain, heart (whole organ and right and left ventricles), right kidney, liver, lung, spleen, thymus, and right testis. Organs and tissues were examined for gross lesions and were fixed in 4CF-1G fixative (buffered formaldehyde-glutaraldehyde mixture). Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations of protocol-required tissues were performed on all control animals, all animals in the highest dose group with at least 60% survivors at the time of sacrifice, plus all animals in higher dose groups inclusive of early deaths and survivors. In the 2-week and 13-week studies, all gross lesions and the adrenal glands, heart, kidneys, liver, lungs, spleen, and testes of rats and mice in the lower dose groups were examined microscopically. In the 13-week studies, the mandibular and mesenteric lymph nodes, pancreas, salivary glands, and seminal vesicle of rats and the forestomach of mice were also examined in these groups. Tissues required by the protocol to be examined are listed in Table 1.

Upon completion of the laboratory pathologist's histologic evaluation, 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 sent to an independent pathology laboratory where quality assessment was performed. The results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

Supplemental Evaluations

HEMATOLOGY AND CLINICAL CHEMISTRY

Clinical pathology studies were performed on rats and mice in the 13-week gavage studies. Analyses were conducted on Days 3, 14, and 30 for rats in a special study group (five animals per dose group) and at Week 13 on selected rats and mice in the base studies (10 animals per dose group). Clinical pathology studies were not performed on base study animals during the recovery phase. Rats in the special study group received 0, 0.1, 1.0, or 10 mg/kg riddelliine; the doses were given by gavage in a 0.1 M phosphate buffer up

to a final dose volume of 5 mL/kg. Doses were administered daily, excluding weekends and holidays, for 30 days.

At all time points, rats and mice used for clinical pathology evaluations were anesthetized with CO_2:O_2 (60:40) and bled from the retroorbital sinus. Blood for hematology analyses was collected in Vacutainers® (Becton-Dickinson and Co., Rutherford, NJ) containing potassium EDTA. Blood for serum chemistry analyses was collected in containers without anticoagulant, allowed to clot at room temperature and centrifuged. Clinical pathology studies were limited to hematology for the mice due to animal size and lack of adequate blood volume. Parameters evaluated and methods used are listed in Table 1.

REPRODUCTIVE SYSTEM EVALUATIONS

Sperm Morphology and Vaginal Cytology in Rats and Mice

Vaginal cytology and sperm morphology evaluations were performed on rats and mice from the 13-week base studies. Ten male rats from each of the vehicle control, 0.1, 1.0, and 3.3 mg/kg groups were evaluated; 10 females rats from each of the vehicle control, 0.1, 1.0, and 10 mg/kg groups were evaluated. Mice from the vehicle control, 0.33, 3.3, and 25 mg/kg groups (10 animals per dose group) were also evaluated. Methods were those described by Morrissey *et al.* (1988). Briefly, beginning 7 days prior to sacrifice, the vaginal vaults of 10 females of each species were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and were used to ascertain stages of the estrous cycle (*i.e.*, diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The right epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Tyrode's buffer (mice) or test yolk (rats) was applied to slides and a small incision was made at the distal border of the epididymal tail. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were evaluated using a videomicroscopic system.

Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered 0.9% saline solution. Cauda were gently minced and the tissue was incubated

in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer.

Mating Trials in Rats and Mice

Mating trials were conducted on 20 male and 40 female rats and mice per dose level. Rats received 0 (vehicle control), 0.1, 1.0, or 10 mg/kg riddelliine by gavage at a dose volume of 5 mL/kg body weight; mice received 0 (vehicle control), 0.33, 3.3, or 25 mg/kg riddelliine by gavage at a dose volume of 10 mL/kg body weight. Doses were selected based on body weight gains and survival in the 2-week studies. Rats and mice were dosed once per day, 5 days per week; males and females were dosed prior to and throughout mating; females were also dosed during gestation and lactation. Dose volumes were adjusted weekly on the basis of individual body weights. Because of excessive mortality in rats receiving 10 mg/kg riddelliine, the rats receiving this dose were not mated; the surviving rats in this dose group were killed after 10 weeks of treatment. After 10 weeks (rats) or 10½ weeks (mice) of treatment, two females and one male from each dose group were placed in the same cage for 5 days, were separated for 2 days, and were housed together again for up to 5 more days. Females were checked each morning for sperm-positive vaginal smears (rats) or sperm plugs (mice). Females were separated from the males when there was evidence of a sperm-positive mating or when the 2-week mating period had elapsed; gestation Day 0 was defined as the day when a female was separated from the male. At the end of the mating period, males were killed without necropsy. Females were weighed on gestation Days 0 and 18 (mice) or 19 (rats). Dams were allowed to deliver and pups were examined for any gross malformations. The number of live and dead pups was recorded and live pups were sexed and weighed individually. Dam and pup weights were determined on postpartum Days 0, 5, 14, and 21. Females that did not deliver a litter by gestation Day 23 (rats) or Day 22 (mice) were killed and the uterus was examined for evidence of unsuccessful pregnancy; if there was no evidence of pregnancy, the uterus was stained with 10% sodium sulfide and was examined microscopically for early implantation sites. Dams and pups were killed following body weight determinations on postpartum Day 21. Necropsies were not performed.

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Gavage Studies of Riddelliine

EXPERIMENTAL DESIGN	
Study Laboratory	SRI International, Menlo Park, CA
Size of Study Groups	2-Week Studies: Five males and five females per species per dose group 13-Week Studies: Base Studies: 20 males and 20 females per species per dose group Clinical Pathology Study: five male and five female rats per dose group Mating Trial Studies: 20 males and 40 females per species per dose group
Route of Administration	Gavage with 1 mL syringes with stainless steel ball-tipped needles
Dose Volume	Rats: 5 mL/kg body weight Mice: 10 mL/kg body weight
Concentration of Dose Formulations	2-Week Studies: Rats: 0, 0.066, 0.2, 0.66, 2.0, 5.0 mg/mL Mice: 0, 0.033, 0.1, 0.33, 1.0, 2.5 mg/mL 13-Week Studies: Rats: 0, 0.02, 0.066, 0.2, 0.66, 2.0 mg/mL Mice: 0, 0.033, 0.1, 0.33, 1.0, 2.5 mg/mL
Doses/Duration of Dosing	2-Week Studies: Rats: 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg; 5 days per week plus 2 dose days, for a total of 12 doses Mice: 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg; 5 days per week plus 2 dose days, for a total of 12 doses 13-Week Studies: Base Studies: Rats: 0, 0.1, 0.33, 1.0, 3.3, or 10 mg/kg; weekdays, excluding holidays, for 13 weeks Mice: 0, 0.33, 1.0, 3.3, 10, or 25 mg/kg; weekdays, excluding holidays, for 13 weeks Clinical Pathology Study: Rats: 0, 0.1, 1.0, or 10 mg/kg; weekdays, excluding holidays, for 30 days Mating Trial Studies: Rats: 0, 0.1, 1.0, or 10 mg/kg; weekdays, excluding holidays, prior to and during the mating period (males and females) and during gestation and lactation (females) Mice: 0, 0.33, 3.3, or 25 mg/kg; weekdays, excluding holidays, prior to and during the mating period (males and females) and during gestation and lactation (females)
Date of First Dose	2-Week Studies: Rats: 3 November 1986 Mice: 5 November 1986 13-Week Studies: Rats: 18 March 1987 Mice: 11 March 1987
Date of Last Dose	2-Week Studies: Rats: 18 November 1986 Mice: 20 November 1986 13-Week Studies: Base Studies: Rats: 17 June 1987 (males), 18 June 1987 (females) Mice: 10 June 1987 (males), 11 June 1987 (females) Clinical Pathology Study: Rats: 16 April 1987 Mating Trial Studies: Rats: 31 May 1987 (males and females in the 10 mg/kg group), 14 June 1987 (all other males), Day 19 postpartum or Gestation Day 22 (all other females) Mice: 7 June 1987 (males), Day 20 postpartum or Gestation Day 21 if no signs of pregnancy were observed (females)

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Gavage Studies of Riddelliine (continued)

Necropsy Dates	2-Week Studies: Rats: 19 November 1986 Mice: 21 November 1986 13-Week Studies: Base Studies: Rats: 18 June 1987 (males), 19 June 1987 (females) Mice: 11 June 1987 (males), 12 June 1987 (females) 7-Week Recovery: Rats: 6 August 1987 (males), 7 August 1987 (females) Mice: 30 July 1987 (males), 31 July 1987 (females) 14-Week Recovery: Rats: 24 September 1987 (males), 25 September 1987 (females) Mice: 17 September 1987 (males), 18 September 1987 (females)
Type and Frequency of Observation	2-Week Studies: Animals were observed twice daily and were weighed at initiation of dosing, weekly thereafter, and at necropsy. Clinical observations were recorded daily. 13-Week Studies: Base Studies: Animals were observed twice daily. Body weight and clinical observations were recorded weekly. Clinical Pathology Study: Same as base studies Mating Trial Studies: Animals were observed twice daily. Clinical observations were recorded once per week for the first 4 weeks of treatment, then at 4 week intervals (rats) or at 3 week intervals (mice). Animals were weighed once per week for the first 10 weeks (rats) or 10½ weeks (mice) of treatment, then at end of mating. Females were weighed on gestation Day 0, Day 18 (mice), Day 19 (rats), and on Days 0, 5, 14, and 21 postpartum. Offspring were sexed and were observed for malformations. Offspring were also weighed on Days 0, 5, 14, and 21 postpartum.
Necropsy and Histologic Examinations	Complete necropsies were performed on all animals in the base studies. The protocol required that tissues be examined microscopically in all control animals, all animals in the highest dose group with at least 60% survivors, and all animals in higher dose groups, including animals that died early. The following tissues were examined: adrenal glands, bone (sternebrae, including marrow), brain (three sections), esophagus, eyes (if grossly abnormal), gallbladder (mice), gross lesions, heart, large intestine (cecum, colon, rectum), kidneys, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland (including surface skin), nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial or clitoral glands (rats), prostate gland, salivary glands, seminal vesicles, small intestine (duodenum, jejunum, ileum), spinal cord and sciatic nerve (if neurological signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (13-week study rats and mice in the vaginal cytology groups only). Selected organs and gross lesions were examined in the lower dose groups.
Supplemental Evaluations	Clinical Pathology Study (13-Week Study): Hematologic evaluations were performed on rats on Days 14 and 30 and on rats and mice at Week 13. Clinical chemistry studies were performed on rats at all time points (Days 3, 14, and 30 and Week 13). Evaluation of erythrocyte (RBC) and leukocyte (WBC) counts, hemoglobin (HGB) concentration, hematocrit (HCT), and mean cell volume (MCV) were performed with a S560 Whole Blood Analyzer (Coulter Electronics, Hialea, FL). Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were manually calculated. Platelet counts and determination of mean platelet volume (MPV) were performed using a Baker's Series 810 Whole Blood Platelet Analyzer (Baker Instruments Corp., Allentown, PA). Leukocyte differential and reticulocyte counts were determined by microscopic examination of blood smears using modified Romanowsky and new methylene blue stains, respectively.

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Gavage Studies of Riddelliine (continued)

Supplemental Evaluations (continued)	Clinical Pathology Study (continued): Clinical chemistry analyses, including determinations of activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and sorbitol dehydrogenase (SDH), were performed using a Gemini Miniature Centrifugal Analyzer (Electronucleonics, Inc., Fairfield, NJ). Mating Trials (13-Week Studies): Breeding of rats began on 1 June 1987 and ended on 13 June 1987. Breeding of mice began on 25 May 1987 and ended on 6 June 1987 after 10 weeks of treatment (rats) or 10.5 weeks of treatment (mice). Females were allowed to litter and the offspring were examined for malformations. Body weights of dams and offspring, litter size, sex ratio, and survival of offspring were recorded. Sperm Morphology and Vaginal Cytology (13-Week Studies): Male rats from the base study that were dosed with the vehicle control, 0.1, 1.0, or 3.3 mg/kg riddelliine (10 animals per dose group) and male mice from the base study that were dosed with the vehicle control, 0.33, 3.3, or 25 mg/kg riddelliine (10 animals per dose group) were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Female rats from the base study that were dosed with the vehicle control, 0.1, 1.0, or 10 mg/kg riddelliine (10 animals per dose group) and female mice from the base study dosed with the vehicle control, 0.33, 3.3, or 25 mg/kg riddelliine (10 animals per dose group) were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages.
ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N Rats B6C3F$_1$ Mice
Animal Source	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Study	2-Week Studies: 2 weeks 13-Week Studies: Base Studies: 2 weeks Clinical Pathology Study: 2 weeks (rats only) Mating Trial Studies: 4 weeks
Age When Placed on Study	2-Week Studies: 6 weeks 13-Week Studies: Base Studies: 6 weeks Clinical Pathology Study: 6 weeks (rats only) Mating Trial Studies: 8 weeks
Age When Killed	2-Week Studies: 8 weeks 13-Week Studies: Base Studies: 19 weeks, 26 weeks, or 33 weeks Clinical Pathology Study: 10 weeks (rats only)
Method of Animal Distribution	Animals were weighed and were randomized using a computer program.
Diet	NIH-07 Open Formula Pellets (Zeigler Brothers, Inc., Gardners, PA) and deionized water (filtered and ultraviolet-treated) available *ad libitum*
Animal Room Environment	Rats were housed five animals per cage by sex and mice housed individually for all base studies. For mating trial studies, male animals were housed individually and females were housed two animals per cage until the mating period began. After mating, females were housed individually and males were killed. Temperature was maintained at 64° to 79° F and relative humidity at 20% to 78%, with more than 10 air changes per hour. Fluorescent light was provided for 12 hours per day.

Genetic Toxicity Studies

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. 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 and 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 of at least five doses of riddelliine. The high dose was limited by toxicity. All positive trials were repeated under the conditions that elicited the positive response.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). Riddelliine was supplied as a coded aliquot. 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. A single flask per dose was used.

In the SCE test without S9, CHO cells were incubated for 25 to 26 hours with riddelliine in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. 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, and 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. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level, except for the 10 and 30 µg/mL groups in the trial conducted with S9. For these two dose groups, fewer cells were scored due to the extremely high numbers of SCEs, which made scoring very difficult. Because significant chemical-induced cell cycle delay was seen at the 100 µg/mL dose level in the trial with 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.

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 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 with S9 was based on the cell cycle information obtained in the SCE test; because 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).

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in MacGregor *et al.* (1990). Riddelliine, dissolved in sodium phosphate buffer, was administered by gavage to male and female mice five times per week for either 4 or 13 weeks. At the end of the study, blood 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/

pyronine Y (MacGregor *et al.*, 1983) and coded. Slides were scanned at 630 or 1000 x magnification using a semi-automated image analysis system to determine the frequency of micronuclei in 2000 polychromatic erythrocytes (PCEs; 13-week study only) and 10,000 normochromatic erythrocytes (NCEs) in each of 10 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 and orange with 540 nm UV 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.

BONE MARROW MICRONUCLEUS TEST PROTOCOL

Preliminary range finding studies were performed. Factors affecting dose selection included chemical solubility, toxicity, and the extent of cell cycle delay induced by riddelliine. Based on the results of these studies, male mice to be tested for induction of bone marrow micronuclei were administered a single gavage treatment of riddelliine dissolved in sodium phosphate buffer; the dosing volume was 0.4 mL. Solvent control animals were gavaged with 0.4 mL buffer only, and the positive control mice received 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. Smears were air-dried, fixed, and stained; 2000 PCEs were scored for frequency of micronucleated cells in each of eight animals per dose group, per tissue. In addition, the percentage of PCEs among the total erythrocyte population in the peripheral blood and the bone marrow was scored for each dose group as a measure of toxicity.

UNSCHEDULED DNA SYNTHESIS AND S-PHASE DNA SYNTHESIS TEST PROTOCOLS

For unscheduled DNA synthesis and S-phase DNA synthesis assays, livers from rats and mice treated with riddelliine 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 (WE) supplemented with 2 mM *l*-glutamine, 50 µg/mL gentamycin sulfate (Sigma), 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 (dWE) 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 WE 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/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 1000 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 3000 cells per animal. The percentage of cells in S-phase was calculated for each dose group.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed with the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry, hematology, and sperm motility data, which typically have skewed distributions, were analyzed with the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

ANALYSIS OF MATING TRIAL DATA

Jonckheere's test was used to assess the significance of dose-response trends for average litters per male, dam weights, length of gestation, and survival and mean body weights of pups. If a trend was present ($P \leq 0.10$), Shirley's test was used to test for significance; otherwise, Dunn's test was used. The Cochran-Armitage trend test was used to assess trends in male fertility.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *S. typhimurium* test was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF CHINESE HAMSTER OVARY CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (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≤0.05), in the absence of any responses reaching 20% above background, led to a call of equivocal.

Chromosomal aberration data were presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1987). For a single trial, a statistically significant (P≤0.05) difference for one dose point and a significant trend (P≤0.015) was considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose point, led to an equivocal call (Galloway *et al.*, 1987).

ANALYSIS OF MOUSE PERIPHERAL BLOOD MICRONUCLEUS DATA

Log transformation of the NCE data, testing for normality by the Shapiro-Wilk test, and testing for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was analyzed by analysis of variance using the SAS GLM procedure. The NCE data for each dose group were compared with the concurrent solvent control using Student's *t*-test. 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 (1970) binomial test. The percentage of PCEs among total erythrocytes was analyzed by an analysis of variance on ranks (classed by sex) and individual dose groups were compared with the concurrent solvent control using a *t*-test on ranks.

ANALYSIS OF BONE MARROW MICRONUCLEUS DATA

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. Statistical analyses were performed on the micronucleated PCE and percent PCE data as described in the peripheral blood micronucleus protocol above.

Quality Assurance

The animal studies of riddelliine were performed in compliance with Food and Drug Administration's Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of SRI International performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Gavage Study in F344/N Rats

Four males in the 25 mg/kg group died or were killed moribund before the end of the study (Table 2). Dose-related decreases in the mean final body weight and mean body weight gain were observed in males receiving 10 or 25 mg/kg riddelliine by gavage. Mean final body weights and body weight gains of female rats receiving riddelliine were similar to control values.

TABLE 2 Survival and Weight Gain of F344/N Rats in the 2-Week Gavage Study of Riddelliine

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Vehicle Controls[4] (%)
		Initial[2]	Final	Change[3]	
MALE					
Vehicle	5/5	105	169	64	
0.33	5/5	105	169	64	100
1.0	5/5	107	180	74	107
3.3	5/5	104	162	58	96
10	5/5	105	151	46	90
25	1/5[5]	107	114[6]	9[6]	68[6]
FEMALE					
Vehicle	5/5	95	126	31	
0.33	5/5	93	132	39	105
1.0	5/5	93	134	41	106
3.3	5/5	92	127	35	101
10	5/5	94	126	32	100
25	5/5	95	121	26	96

1 Number surviving at 16 days/number of animals per dose group.
2 Final body weights were recorded on Day 14.
3 Mean weight change of the animals in each dose group surviving to Day 16.
4 (Dosed group mean/vehicle control group mean) x 100.
5 Day of death: 13, 14, 15, 15. Three of these animals were killed moribund (Days 13, 15, and 15).
6 n=3.

Administration of riddelliine produced a dose-related salivation response in both male and female rats, ranging from profuse salivation in animals treated with 25 mg/kg riddelliine to slight salivation in the 3.3 mg/kg groups. This response was initially noted on Day 3 of dosing and occurred immediately after dosing throughout the entire treatment period for those animals receiving 10 or 25 mg/kg riddelliine and on all except the last 2 dose days for animals receiving 3.3 mg/kg riddelliine. Clinical signs of toxicity in male rats appeared on Day 10 of dosing. All five male rats receiving 25 mg/kg appeared hunched and hypoactive and had ruffled fur. On Day 12, these animals were slightly ataxic and exhibited moderate dyspnea; by Day 13, four of these animals were emaciated. One female in the 25 mg/kg group had hunched posture and nasal discharge during Week 2 of the study.

Marked dose-related increases were noted for absolute and relative lung and spleen weights for both males and females receiving riddelliine. A mild increase in mean relative liver weight was noted for male rats given 1.0 to 25 mg/kg riddelliine and for females given 1.0 or 3.3 mg/kg riddelliine, but positive trends with dose were not apparent. Relative kidney weights were also notably greater than control values for males administered 10 or 25 mg/kg riddelliine and for females administered 25 mg/kg.

Dose-related decreases in absolute and relative left ventricular weights were noted in both males and females dosed with riddelliine; statistically significant or notable decreases occurred for males given 3.3 to 25 mg/kg and for females given 10 or 25 mg/kg. Significant negative trends with dose also occurred for absolute and relative right ventricle weights of male and female rats and the absolute heart weight of females.

Gross necropsy observations in riddelliine-treated rats were indicative of hepatotoxicity. Liver lesions, variably described as enlarged, firm, mottled, or reddened, were seen at doses of 1.0 mg/kg and higher. There was a dose-dependent increase in the incidence of this finding; all males and females in the 10 and 25 mg/kg groups were affected. Further evidence of gross hepatotoxicity was the presence of generalized icterus in four of five males and in one of five females in the 25 mg/kg groups. Darkened and/or enlarged lymph nodes were a common necropsy finding in many male and female rats administered 25 mg/kg riddelliine, and a darkened thymus was frequently observed in animals receiving doses of 1.0 mg/kg or greater. The only other consistently reported gross finding was edema of the pancreas in four of five male rats in the 25 kg/mg group.

Microscopically, the most significant effects of riddelliine treatment were in the liver (Table 3). The most severe liver effect observed was hemorrhagic centrilobular necrosis, seen in the four male rats in the high-dose (25 mg/kg) group that died early; hepatic necrosis was the apparent cause of death in these rats. The remaining viable, periportal hepatocytes in these animals were characterized by cytologic changes consisting of karyomegaly and increased amounts of homogeneous to granular eosinophilic cytoplasm, which were collectively diagnosed as cytologic alteration. This change, unaccompanied by overt necrosis but associated with marked centrilobular congestion, was also present in the single high-dose male rat that survived to the end of the study. Diffuse cytologic change of lesser severity, characterized primarily by cytoplasmic granularity and eosinophilia, was seen in all males administered 1.0 mg/kg or greater and in all females administered 3.3 mg/kg or greater.

Histopathologic effects of riddelliine administration were also noted in the lung and spleen of both sexes and in the pancreas of males (Table 3). In the lung, focal hemorrhage and/or edema, generally perivascular in location, occurred at the high-dose level in both sexes; perivascular edema was also observed at dose levels as low as 1.0 mg/kg in males and 3.3 mg/kg in females. Increased hematopoietic cell proliferation in the spleen was present in all high-dose male and female rats and in four of five males receiving 10 mg/kg. Separation of pancreatic lobules by interstitial hemorrhage and edema was present in most high-dose male rats, and edema alone occurred in the 10 mg/kg group. In multiple lymph nodes of most animals in the high-dose groups, congestion and associated erythrophagocytosis by medullary histiocytes were observed. No lesions that were clearly treatment-related were seen in the heart or in other organs examined.

Based on depressed body weight gains, mortality, and histopathology, the dose levels set for the 13-week study of male and female rats were 0, 0.1, 0.33, 1.0, 3.3, and 10 mg/kg riddelliine.

TABLE 3 Incidence and Severity of Selected Lesions in F344/N Rats in the 2-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)					
	Vehicle Control	0.33	1.0	3.3	10	25
MALE						
Liver						
Necrosis	0	0	0	0	0	5 (4.0)
Cytologic alteration	0	0	5 (2.2)	5 (2.6)	5 (3.0)	5 (3.0)
Lung						
Hemorrhage	0	0	1 (1.0)	0	0	5 (2.2)
Edema	0	0	3 (1.3)	5 (1.0)	5 (1.8)	4 (2.0)
Spleen						
Increased hematopoiesis	0	0	0	0	4 (2.0)	5 (2.6)
Pancreas						
Edema	0	0	0	0	4 (1.7)	5 (2.4)
Hemorrhage	0	0	0	0	0	3 (1.7)
FEMALE						
Liver						
Cytologic alteration	0	0	0	5 (2.0)	5 (2.8)	5 (3.0)
Lung						
Hemorrhage	0	0	0	0	0	2 (1.5)
Edema	0	0	0	5 (1.4)	5 (2.0)	5 (2.0)
Spleen						
Increased hematopoiesis	0	0	0	0	0	5 (2.0)

[1] The incidence is the number of animals with lesions from groups of 5. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

13-Week Gavage Study in F344/N Rats

Nineteen of 20 males in the 10 mg/kg group died or were killed moribund between Day 58 and Day 92 of the base study. No female rats died during the 13-week base study; however, 5 of 10 female rats in the 10 mg/kg recovery groups died between Days 124 and 173 (Tables 4-6).

Dose-related decreases in mean final body weight and body weight gain were noted for male and female rats given riddelliine by gavage for 13 weeks (Tables 4-6 and Figure 4). The decrease was more pronounced for male rats than for female rats; base study males given 3.3 mg/kg riddelliine weighed 15% less than controls after 13 weeks of treatment and the single surviving male given 10 mg/kg weighed 46% less than controls, while females in these dose groups weighed 8% less than controls at the end of the base study.

Dose-related decreases in mean body weights and body weight gains for males and females in the recovery groups were also noted after 13 weeks of riddelliine administration (Tables 5 and 6). However, the weight gains for treated males and females during the 7-week and 14-week recovery periods were similar to control weight gains, with the exception of female rats in the high-dose (10 mg/kg) group, which gained notably more weight than females in other dose groups. After the 14-week recovery period, the mean body weights of females in the 1.0 and 3.3 mg/kg groups only were slightly lower than the mean body weight of the controls.

The majority of the clinical observations related to the treatment with riddelliine occurred in male and female rats in the 10 mg/kg groups. The most commonly observed clinical signs were jaundice, abnormal posture, ruffled fur, discolored urine, urine-stained fur, diarrhea, emaciation, and alopecia, which occurred mainly in the inguinal and vaginal areas.

TABLE 4 Survival and Weight Gain of F344/N Rats in the 13-Week Base Study of Riddelliine Administered by Gavage

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Vehicle Controls[4] (%)
		Initial[2]	Final	Change[3]	
MALE					
Vehicle	10/10	113	361	248	
0.1	10/10	106	347	241	96
0.33	10/10	101	339	239	94
1.0	10/10	107	332	225	92
3.3	10/10	104	308	204	85
10	1/10[5]	106	195	95	54
FEMALE					
Vehicle	10/10	91	209	117	
0.1	10/10	89	205	115	98
0.33	10/10	92	203	111	97
1.0	10/10	87	197	110	94
3.3	10/10	88	192	104	92
10	10/10	89	193	104	92

1 Number surviving at 13 weeks/number of animals per dose group.

2 Body weights were measured at Day 1. Subsequent calculations are based on animals surviving to the end of the base study.

3 Mean weight change of the survivors.

4 (Dosed group mean/vehicle control group mean) x 100.

5 Week of death: all animals died between Weeks x9 and 13 (on the last day of dosing).

TABLE 5 Survival and Weight Gain of F344/N Rats in the 13-Week Gavage Study of Riddelliine Followed by a 7-Week Recovery Period

Dose (mg/kg)	Survival[1]	Mean Body Weight (g) Initial[2]	Week 13	Change[3]	Week 13 Wt Relative to Controls[4] (%)	Mean Body Wt (g) Week 20	Change[5]	Final Weight Relative to Controls[4] (%)
MALE								
Vehicle	5/5	106	358	252		415	57	
0.1	5/5	109	348	240	97	401	52	97
0.33	5/5	107	338	231	94	385	48	93
1.0	5/5	101	327	226	91	376	49	91
3.3	5/5	101	319	218	89	375	57	90
10	0/5[6]	109	–	–	–	–	–	–
FEMALE								
Vehicle	5/5	93	211	118		232	21	
0.1	5/5	94	210	115	99	235	25	101
0.33	5/5	91	212	121	100	232	20	100
1.0	5/5	90	205	115	97	230	25	99
3.3	5/5	91	191	100	90	208	17	90
10	4/5[7]	98	198	100	94	225	32	97

1 Number surviving at 20 weeks/number of animals per dose group.

2 Body weights were measured at Day 1. Subsequent calculations are based on animals surviving to the end of the evaluation period (Week 13 or Week 20). For groups with no survivors, no Week 13 or Week 20 body weights or body weight changes are given.

3 Mean weight change of the survivors from Week 0 to Week 13.

4 (Dosed group mean/vehicle control group mean) x 100.

5 Mean weight change of the survivors from Week 13 to Week 20.

6 No males in the 10 mg/kg group survived past Week 13.

7 One 7-week recovery female died between Weeks 18 and 20.

TABLE 6 Survival and Weight Gain of F344/N Rats in the 13-Week Gavage Study of Riddelliine Followed by a 14-Week Recovery Period

Dose (mg/kg)	Survival[1]	Mean Body Weight (g) Initial[2]	Week 13	Change[3]	Week 13 Wt Relative to Controls[4] (%)	Mean Body Wt (g) Week 27	Change[5]	Final Weight Relative to Controls[4] (%)
MALE								
Vehicle	5/5	96	331	235		413	82	
0.1	5/5	99	348	249	105	428	80	104
0.33	5/5	105	352	247	106	439	87	106
1.0	5/5	106	326	220	99	414	88	100
3.3	5/5	99	311	212	94	398	86	96
10	0/5[6]	99	–	–	–	–	–	–
FEMALE								
Vehicle	5/5	100	222	122		253	31	
0.1	5/5	94	207	114	93	243	35	96
0.33	5/5	96	206	110	93	244	38	96
1.0	5/5	94	200	106	90	227	28	90
3.3	5/5	88	188	100	85	216	27	85
10	1/5[7]	89	194	105	87	255	50	101

1 Number surviving at 27 weeks/number of animals per dose group.

2 Body weights were measured at Day 1. Subsequent calculations are based on animals surviving to the end of the evaluation period (Week 13 or Week 27). For groups with no survivors, no week 13 or Week 27 body weights or body weight changes are given.

3 Mean weight change of the survivors from Week 0 to Week 13.

4 (Dosed group mean/vehicle control group mean) x 100.

5 Mean weight change of the survivors from Week 13 to Week 27.

6 No males in the 10 mg/kg group survived past Week 13.

7 14-week recovery females died between Weeks 18 and 25.

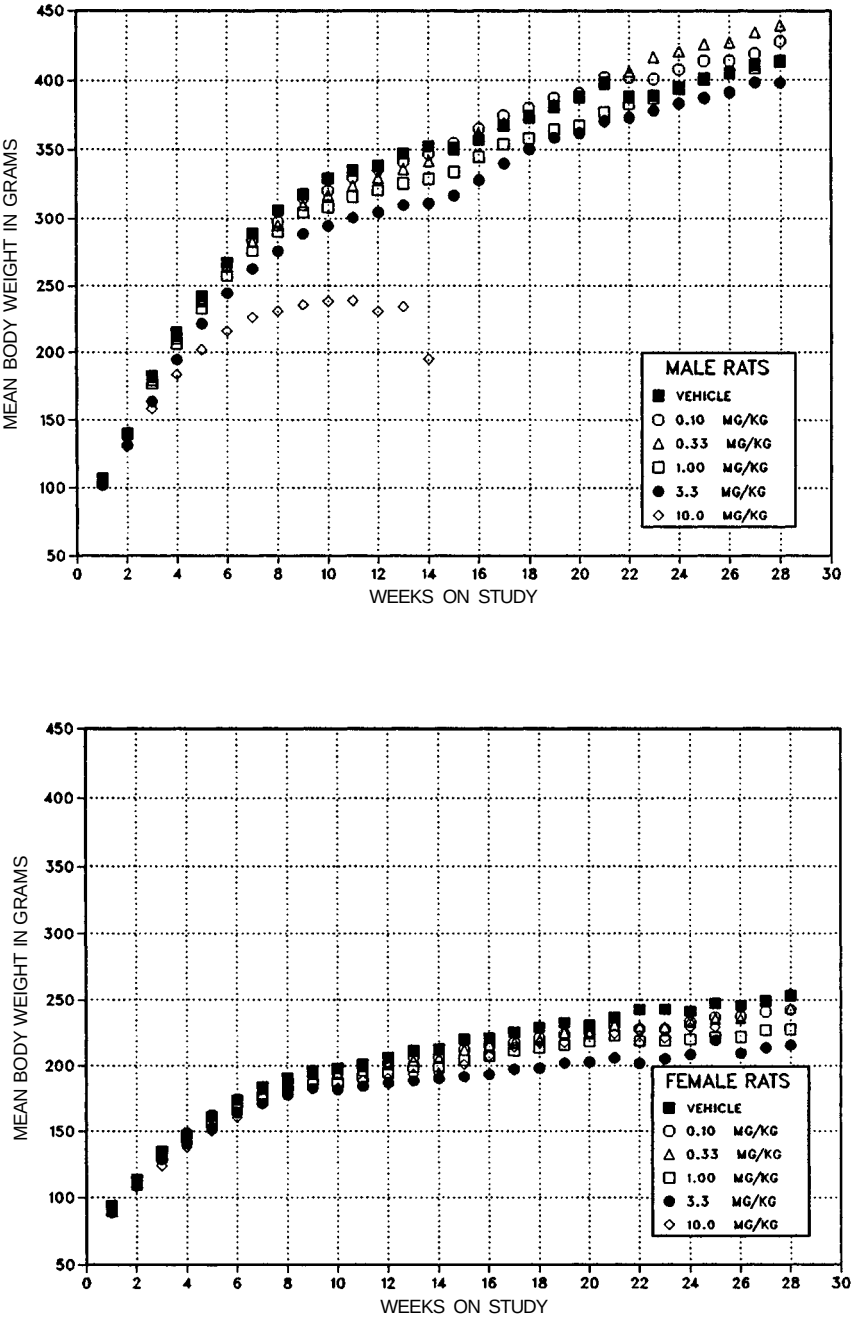

FIGURE 4 Body Weights of F344/N Rats Administered Riddelliine by Gavage for 13 Weeks

A statistically significant positive trend with dose occurred in the 13-week base study for mean absolute and relative weights of lung and spleen and for relative weights of brain and right testis in male rats (Tables 7 and A1). In females, a significant positive trend with dose occurred for absolute and relative brain, right kidney, lung, and spleen weights and for relative heart and liver weights. The absolute and relative heart and liver weights for male rats showed a statistically significant negative trend with dose. Absolute and relative thymus and absolute left ventricle weights showed a statistically significant decrease with increasing dose for female rats.

During the 7-week and 14-week recovery periods, body weight gains of animals in the dosed groups began to recover toward control levels, and organ weights in general became more appropriate for body weights (with the exception of high-dose females, which continued to die during the recovery period) (Tables 7 and A1). Relative and/or absolute spleen weights remained elevated for males and markedly elevated for females. Heart weights were uniformly lower after the 7-week recovery period than immediately following dosing, or after 14 weeks of recovery in females. The reason for this is not apparent. The relative heart weights of male and female rats were slightly greater than control values after 14 weeks of recovery; the increase was significant for females in the 3.3 mg/kg group. Absolute and/or relative right ventricular weights of treated males were similar to those of the controls, but those of females treated with 3.3 mg/kg were significantly increased 14 weeks after cessation of riddelliine administration.

TABLE 7 Selected Organ Weights of F344/N Rats Administered Riddelliine by Gavage for 13 Weeks[1]

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10[2]
MALE						
Base Study						
n	10	10	10	10	10	1
Necropsy body weight	365	351	340*	336**	310**	201
Brain weight	2.018	1.978	1.956	1.869	1.957	1.830
Relative brain weight	5.54	5.66	5.78	5.57	6.34**	9.10
Heart weight	1.110	1.049	1.010**	1.016**	0.884**	0.755
Relative heart weight	3.04	2.99	2.97	3.02	2.86**	3.75
Right kidney weight	1.227	1.190	1.166	1.191	1.067**	1.190
Relative right kidney weight	3.36	3.39	3.42	3.54	3.45	5.92
Liver weight	14.799	14.025	14.420	14.002	11.431**	4.380
Relative liver weight	40.59	39.94	42.26	41.58	36.75**	21.78
Lung weight	1.799	1.845	1.761	1.870	2.243**	1.530
Relative lung weight	4.93	5.25	5.15	5.56	7.21**	7.61
Spleen weight	0.860	0.851	0.880	0.936*	1.062**	1.330
Relative spleen weight	2.36	2.43	2.59	2.78**	3.46**	6.61
Right testis weight	1.519	1.478	1.383*	1.466*	1.404*	0.152
Relative right testis weight	4.17	4.22	4.07	4.36*	4.53**	0.76
Left ventricle weight	0.477	0.510	0.486	0.470	0.428	0.328
Relative left ventricle weight	1.31	1.46	1.42	1.40	1.38	1.63
Right ventricle weight	0.154	0.147	0.139	0.142	0.145	0.094
Relative right ventricle weight	0.42	0.42	0.41	0.42	0.47	0.47
7-Week Recovery						
n	5	5	5	5	5	
Necropsy body weight	420	411	388*	379*	378**	
Brain weight	2.002	2.096	2.036	2.020	2.002	
Relative brain weight	4.78	5.11	5.27*	5.34*	5.32*	
Heart weight	1.145	1.264	1.161	1.111	1.098	
Relative heart weight	2.73	3.08*	3.00	2.94	2.92	
Liver weight	15.622	15.906	15.308	15.238	12.898**	
Relative liver weight	37.27	38.73	39.55	40.28*	34.13*	

1 Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

2 Weights are not available for males in the 10 mg/kg groups of the 7-week and 14-week recovery studies.

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

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

TABLE 7 Selected Organ Weights of F344/N Rats Administered Riddelliine by Gavage for 13 Weeks (continued)

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
MALE (continued)						
7-Week Recovery (continued)						
Spleen weight	0.938	0.956	0.998	0.988	1.096*	
Relative spleen weight	2.24	2.33	2.57	2.61	2.93**	
Left ventricle weight	0.526	0.563	0.506	0.496	0.493	
Relative left ventricle weight	1.26	1.37	1.31	1.31	1.31	
Right ventricle weight	0.155	0.148	0.158	0.158	0.144	
Relative right ventricle weight	0.37	0.36	0.41	0.42	0.38	
14-Week Recovery						
n	5	5	5	5	5	
Necropsy body weight	419	432	445	417	400	
Heart weight	1.198	1.258	1.316	1.211	1.241	
Relative heart weight	2.86	2.92	2.96	2.91	3.10	
Liver weight	16.992	18.046	19.120	15.918	14.446	
Relative liver weight	40.54	41.79	42.95	38.13	36.05*	
Spleen weight	1.002	0.988	1.020	0.982	1.072	
Relative spleen weight	2.40	2.28	2.29	2.36	2.68*	
Left ventricle weight	0.391	0.373	0.372	0.367	0.371	
Relative left ventricle weight	0.93	0.87	0.83	0.88	0.93	
Right ventricle weight	0.142	0.144	0.161	0.139	0.152	
Relative right ventricle weight	0.34	0.34	0.36	0.33	0.38	
FEMALE						
Base Study						
n	10	10	10	10	10	10
Necropsy body weight	211	207	205	199*	196**	193**
Brain weight	1.790	1.823	1.840	1.818	1.864*	1.844*
Relative brain weight	8.51	8.82	9.01*	9.17**	9.54**	9.56**
Heart weight	0.681	0.697	0.715	0.696	0.729	0.695
Relative heart weight	3.23	3.37	3.50	3.51	3.73**	3.59**
Right kidney weight	0.668	0.715	0.752	0.693	0.723	0.915**
Relative right kidney weight	3.16	3.45**	3.67**	3.49**	3.69**	4.73**
Liver weight	6.835	7.499	7.212	7.143	7.631*	6.368
Relative liver weight	32.42	36.31*	35.23*	35.91*	39.01*	32.96*
Lung weight	1.152	1.201	1.192	1.245	1.646**	1.680**
Relative lung weight	5.46	5.81	5.82	6.25	8.38**	8.68**

TABLE 7 Selected Organ Weights of F344/N Rats Administered Riddelliine by Gavage for 13-Weeks (continued)

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
FEMALE (continued)						
Base Study (continued)						
Spleen weight	0.516	0.559	0.520	0.535	0.623**	1.353**
Relative spleen weight	2.46	2.71	2.55	2.69	3.19**	7.00**
Thymus weight	0.249	2.244	0.252	0.230	0.212*	0.189**
Relative thymus weight	1.18	1.18	1.24	1.15	1.09	0.98**
Left ventricle weight	0.371	0.353	0.375	0.357	0.315*	0.348*
Relative left ventricle weight	1.76	1.71	1.83	1.80	1.61	1.79
Right ventricle weight	0.109	0.101	0.104	0.102	0.083*	0.121
Relative right ventricle weight	0.51	0.48	0.51	0.51	0.42	0.62*
7-Week Recovery						
n	5	5	5	5	5	4
Necropsy body weight	236	238	235	233	209	228
Brain weight	1.842	1.886	1.838	1.850	1.856	1.893
Relative brain weight	7.84	8.01	7.85	7.97	8.89*	8.32
Heart weight	0.534	0.511	0.504	0.499	0.427**	0.525*
Relative heart weight	2.28	2.15	2.15	2.14	2.05	2.30
Right kidney weight	0.882	0.868	0.922	0.886	0.820	1.205**
Relative right kidney weight	3.75	3.65	3.92	3.81	3.93	5.29**
Liver weight	7.862	7.532	8.598	8.856	6.998	5.995**
Relative liver weight	33.37	31.65	36.59	38.05*	33.57	26.39**
Lung weight	1.202	1.158	1.174	1.308	1.168	1.418
Relative lung weight	5.08	4.90	5.01	5.60	5.59	6.23**
Spleen weight	0.592	0.598	0.598	0.598	0.584	1.683**
Relative spleen weight	2.52	2.52	2.55	2.57	2.79	7.37**
Thymus weight	0.189	0.173	0.187	0.177	0.110**	0.075**
Relative thymus weight	0.80	0.75	0.80	0.76	0.53**	0.33**
Left ventricle weight	0.336	0.315	0.301	0.350	0.291	0.377
Relative left ventricle weight	1.43	1.34	1.29	1.50	1.40	1.65
Right ventricle weight	0.096	0.090	0.114	0.109	0.087	0.118
Relative right ventricle weight	0.41	0.38	0.49	0.47	0.42	0.52*

TABLE 7 Selected Organ Weights of F344/N Rats Administered Riddelliine by Gavage for 13-Weeks (continued)

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
FEMALE (continued)						
14-Week Recovery						
n	5	5	5	5	5	1
Necropsy body weight	255	245	249	231	217	257
Brain weight	1.914	1.826	1.880	1.888	1.860	1.970
Relative brain weight	7.53	7.46	7.56	8.18*	8.61**	7.66
Heart weight	0.828	0.773	0.826	0.768	0.786	1.122
Relative heart weight	3.26	3.15	3.33	3.33	3.62**	4.36
Right kidney weight	0.994	0.978	0.986	0.900*	0.888*	1.330
Relative right kidney weight	3.91	4.00	3.96	3.90	4.10	5.17
Liver weight	9.460	9.048	9.912	9.042	8.270*	7.330
Relative liver weight	37.20	36.88	39.91	39.07	38.07	28.49
Spleen weight	0.596	0.658	0.636	0.602	0.636	1.950
Relative spleen weight	2.35	2.69*	2.55	2.61	2.93**	7.58
Left ventricle weight	0.266	0.248	0.256	0.264	0.254	0.379
Relative left ventricle weight	1.05	1.01	1.03	1.14	1.17	1.47
Right ventricle weight	0.112	0.116	0.118	0.103	0.1112	0.168
Relative right ventricle weight	0.44	0.47	0.48	0.45	0.51*	0.65

Changes were noted in several hematology and clinical chemistry parameters at various doses and time points (Table B1). Some changes were minor and sporadic and did not demonstrate treatment-related trends. Other changes that suggested a treatment relationship are described below. The severity of the changes was dose dependent, and male rats were more affected than female rats. By Week 13, only one male rat in the 10 mg/kg group survived, and results from this animal were not included in the statistical comparison. However, there were large differences in hematology and serum chemistry values between this survivor and the controls.

Changes in hematology parameters were noted on Days 14 and 30 and at Week 13 in male and female rats (Table 8). On Day 14, significant increases in erythrocyte count and hematocrit occurred in male and female rats in the 1.0 and 10 mg/kg groups. Hemoglobin concentration was also significantly increased for female rats in these two dose groups. By Day 30, these values were similar to or lower than control values for both males and females; the decreases in hemoglobin concentration and erythrocyte count were significant

for males in the 10 mg/kg group. Additionally, the hemoglobin concentration for male rats receiving 0.1 mg/kg was significantly decreased. At Week 13, the erythrocyte count, hematocrit, and hemoglobin concentration were similar to control values or were elevated again for male and female rats in the 10 mg/kg groups. Increases occurred in several other dose groups for male rats, including the 0.1, 1.0, and 3.3 mg/kg groups. There was an exception in that the surviving male rat in the 10 mg/kg group had an erythrocyte count that was less than the mean for the control animals. The reticulocyte counts were elevated in male rats in the 10 mg/kg group at Days 14 and 30 and Week 13. The reticulocyte count was also elevated for males in the 3.3 mg/kg group at Week 13. Significantly increased reticulocyte counts in female rats were noted only at Week 13 and involved all dose groups. Mean cell volume increased in all treated groups of male rats on Day 30 and Week 13, except for males receiving 0.1 mg/kg at Week 13. At Day 14, these hematology findings were consistent with a hemoconcentration related to dehydration. By Day 30, the results were consistent with an anemia and at Week 13, hemoconcentration again. A regenerative response, evidenced by increased numbers of reticulocytes, occurred as early as Day 14 in high-dose males. By Week 13, this response involved both sexes and multiple dose groups. At Day 30, three of five high-dose female rats were hematologically anemic, yet no increase in reticulocyte count occurred. This would be consistent with a slower development of anemia in the female rats and a delay in the regenerative response.

Platelet counts were markedly decreased in the high-dose male rats at all time points. There was also a decrease in platelet count in the 3.3 mg/kg group by Week 13, but this was not statistically significant. In female rats, platelet counts were significantly decreased in the high-dose group at Week 13. Increases in mean platelet volume paralleled changes in the platelet counts.

Leukocyte counts were significantly increased in high-dose female rats on Day 14 and at Week 13. In male rats, leukocyte counts were increased at Week 13 in all dose groups except the 0.33 mg/kg group. The leukocytosis was produced by an increase in the lymphocyte count. This was true for the high-dose (10 mg/kg) female rats on Day 14 and Week 13 and the male rats in the 1.0 and 3.3 mg/kg groups at Week 13. At Week 13, the surviving male rat in the 10 mg/kg group had an increased number of segmented neutrophils, but not lymphocytes, which would be consistent with an inflammatory response. Mild increases in monocyte numbers occurred in high-dose male and female rats on Day 14.

TABLE 8 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
MALE						
n (Days 14 and 30)	5	5	–[2]	5	–[2]	5
n (Week 13)	10	10	10	10	10	1
Hematocrit (%)						
Day 14	43.9 ± 0.6	43.4 ± 1.1	–	47.2 ± 0.9*	–	48.9 ± 1.8*
Day 30	48.9 ± 0.6	47.7 ± 0.9	–	49.6 ± 0.6	–	47.4 ± 1.1
Week 13	47.0 ± 0.5	49.1 ± 0.3**	46.8 ± 1.0	49.4 ± 0.3**	51.1 ± 0.6**	50.0
Hemoglobin (g/dL)						
Day 14	15.6 ± 0.3	15.3 ± 1.1[3]	–	15.8 ± 0.2	–	16.9 ± 0.7
Day 30	17.4 ± 0.2	16.6 ± 0.2*	–	17.3 ± 0.2	–	16.2 ± 0.3**
Week 13	16.6 ± 0.1	16.9 ± 0.2*	16.5 ± 0.2	17.2 ± 0.1**	17.8 ± 0.2**	16.8
Erythrocytes (10^6/µL)						
Day 14	6.71 ± 0.10	6.80 ± 0.25	–	7.35 ± 0.12*	–	7.71 ± 0.36*
Day 30	8.22 ± 0.13	7.81 ± 0.14	–	7.99 ± 0.15	–	7.57 ± 0.23*
Week 13	9.08 ± 0.09	9.38 ± 0.06*	8.74 ± 0.11	9.22 ± 0.04	9.68 ± 0.09**	8.04
Reticulocytes (10^6/µL)						
Day 14	0.34 ± 0.03	0.40 ± 0.03	–	0.38 ± 0.04	–	0.57 ± 0.07**
Day 30	0.20 ± 0.02	0.17 ± 0.01	–	0.18 ± 0.01	–	0.42 ± 0.04*
Week 13	0.14 ± 0.02	0.16 ± 0.03	0.14 ± 0.03	0.18 ± 0.03	0.26 ± 0.04*	0.61
Mean cell volume (fL)						
Day 30	58.4 ± 0.5	60.2 ± 0.5*	–	60.8 ± 0.6**	–	61.2 ± 1.0*
Week 13	50.7 ± 0.3	51.0 ± 0.2	51.6 ± 0.2*	52.3 ± 0.4**	51.5 ± 0.2*	62.0
Mean cell hemoglobin (pg)						
Day 14	23.0 ± 0.3	22.0 ± 0.6[3]	–	21.8 ± 0.5	–	22.0 ± 0.0*
Week 13	18.1 ± 0.2	17.9 ± 0.2	19.0 ± 0.2*	18.4 ± 0.2	18.1 ± 0.1	21.0
Mean cell hemoglobin concentration (g/dL)						
Day 30	35.6 ± 0.2	34.6 ± 0.2*	–	34.6 ± 0.2*	–	34.2 ± 0.4**
Platelets (10^3/µL)						
Day 14	994.0 ± 22.8	998.0 ± 23.6	–	890.0 ± 95.3	–	243.6 ± 36.6**
Day 30	756.4 ± 114.9	905.2 ± 27.9	–	950.4 ± 9.2	–	79.6 ± 9.6
Week 13	685.1 ± 23.1	729.5 ± 16.8	676.9 ± 20.1	764.7 ± 14.3	598.6 ± 26.7[4]	48.0
Mean platelet volume (µm^3)						
Day 14	7.26 ± 0.07	7.10 ± 0.08	–	7.32 ± 0.11	–	8.10 ± 0.21*
Day 30	7.62 ± 0.31	7.16 ± 0.18	–	7.40 ± 0.13	–	10.96 ± 0.41*
Week 13	7.53 ± 0.10	7.60 ± 0.07	7.69 ± 0.09	7.75 ± 0.11	7.93 ± 0.10**	9.50
Leukocytes (10^3/µL)						
Week 13	8.91 ± 0.35	9.73 ± 0.30*	9.65 ± 0.31	11.45 ± 0.18**	10.38 ± 0.19**	13.60
Segmented neutrophils (10^3/µL)						
Week 13	1.17 ± 0.07	1.51 ± 0.18	1.51 ± 0.14	1.50 ± 0.15	1.27 ± 0.11	4.35
Lymphocytes (10^3/µL)						
Week 13	7.40 ± 0.34	7.82 ± 0.23	7.58 ± 0.19	9.42 ± 0.26**	8.43 ± 0.21**	8.02
Monocytes (10^3/µL)						
Day 14	0.05 ± 0.03[5]	0.12 ± 0.06	–	0.21 ± 0.04*	–	0.61 _ 0.04**

1 Data are given as mean ± standard error.

2 No animals were designated for the clinical pathology study for this dose group.

3 n=3.

4 n=9.

5 n=4.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE 8 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Dose (mg/kg)					
	Vehicle Control	**0.1**	**0.33**	**1.0**	**3.3**	**10**
FEMALE						
n (Days 14 and 30)	5	5	–[2]	5	–[2]	5
n (Week 13)	10	10	10	10	10	10
Hematocrit (%)						
Day 14	43.9 ± 0.6	43.8 ± 0.9	–	48.5 ± 0.7*	–	52.6 ± 1.7**
Day 30	47.2 ± 1.2[5]	49.3 ± 0.5	–	46.0 ± 1.7	–	40.7 ± 3.2
Week 13	48.0 ± 0.6	43.9 ± 0.7	45.8 ± 0.4	45.4 ± 0.5	46.5 ± 0.9	51.5 ± 0.6
Hemoglobin (g/dL)						
Day 14	15.8 ± 0.1	16.3 ± 0.4[5]	–	16.9 ± 0.2**	–	18.7 ± 0.6**[5]
Day 30	17.6 ± 0.5[5]	17.0 ± 0.1	–	16.4 ± 0.5	–	15.4 ± 1.1
Week 13	17.0 ± 0.2	16.7 ± 0.3	16.5 ± 0.1	17.1 ± 0.2	17.2 ± 0.3	18.1 ± 0.2*
Erythrocytes (10^6/µL)						
Day 14	6.71 ± 0.11	6.70 ± 0.19	–	7.24 ± 0.13	–	8.22 ± 0.27**
Day 30	7.73 ± 0.21[5]	7.58 ± 0.09	–	7.25 ± 0.30	–	6.69 ± 0.53
Week 13	8.19 ± 0.08	8.09 ± 0.11	7.98 ± 0.05	8.24 ± 0.10	8.41 ± 0.15	8.88 ± 0.12**
Reticulocytes (10^6/µL)						
Day 14	0.23 ± 0.03	0.30 ± 0.03	–	0.30 ± 0.02	–	0.22 ± 0.02
Day 30	0.13 ± 0.03[5]	0.13 ± 0.02	–	0.12 ± 0.01	–	0.13 ± 0.02[5]
Week 13	0.09 ± 0.01	0.20 ± 0.02**	0.12 ± 0.02*	0.12 ± 0.01*	0.13 ± 0.02	0.49 ± 0.10**
Mean cell volume (fL)						
Week 13	57.3 ± 0.4	53.3 ± 0.3**	55.8 ± 0.5	53.8 ± 0.1**	54.1 ± 0.5**	56.9 ± 0.5
Mean cell hemoglobin concentration (g/dL)						
Week 13	35.5 ± 0.3	38.1 ± 0.2**	36.0 ± 0.2	37.4 ± 0.2**	37.1 ± 0.3*	35.0 ± 0.2
Platelets (10^3/µL)						
Day 14	945.2 ± 34.6	987.2 ± 15.2	–	1020.0 ± 41.1	–	920.0 ± 38.3
Day 30	798.5 ± 27.5[5]	844.8 ± 11.5	–	840.0 ± 16.6	–	700.0 ± 51.3
Week 13	813.6 ± 27.9[4]	843.6 ± 86.6	765.8 ± 24.1	828.8 ± 17.0	767.3 ± 16.0[4]	150.7 ± 8.5**
Mean platelet volume (µm^3)						
Week 13	7.06 ± 0.29	7.25 ± 0.10	7.18 ± 0.08	7.26 ± 0.07	7.45 ± 0.13	8.95 ± 0.12**
Leukocytes (10^3/µL)						
Day 14	7.90 ± 0.90	9.00 ± 0.95	–	8.56 ± 0.93	–	12.96 ± 1.11*
Day 30	9.05 ± 0.73[5]	10.52 ± 0.48	–	16.02 ± 5.15	–	14.00 ± 2.49
Week 13	7.28 ± 0.44	8.34 ± 0.74	7.04 ± 0.22	7.96 ± 0.33	8.42 ± 0.40	11.52 ± 0.25**[4]
Segmented neutrophils (10^3/µL)						
Day 14	0.50 ± 0.13	1.18 ± 0.04*	–	0.72 ± 0.17	–	0.91 ± 0.24
Week 13	0.71 ± 0.09	1.32 ± 0.06**[4]	1.03 ± 0.08**	1.02 ± 0.09*	1.21 ± 0.08**	1.71 ± 0.21**
Lymphocytes (10^3/µL)						
Day 14	6.91 ± 0.81	7.60 ± 0.97	–	7.60 ± 0.83	–	11.20 ± 0.88**
Day 30	7.95 ± 0.77[5]	9.56 ± 0.51	–	14.29 ± 4.69	–	12.20 ± 2.06
Week 13	6.21 ± 0.37	6.30 ± 0.36	5.82 ± 0.20	6.76 ± 0.29	6.86 ± 0.39	7.77 ± 0.74*
Monocytes (10^3/µL)						
Day 14	0.10 ± 0.07	0.13 ± 0.02	–	0.14 ± 0.03	–	0.56 ± 0.09**
Eosinophils (10^3/µL)						
Week 13	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.14 ± 0.04**

There were changes in clinical chemistry parameters in male and female rats on Days 3, 14, and 30 and at Week 13 (Table 9). Increased serum alkaline phosphatase activity occurred in high-dose male rats at all time points. This change was also noted on Day 14 for males receiving 1.0 mg/kg and at Week 13 for males in the 0.33 and 3.3 mg/kg groups. In female rats, serum alkaline phosphatase activity increased in the two highest dose groups (3.3 and 10 mg/kg) at Week 13. An increase in the activity of alanine aminotransferase (ALT) occurred in the high-dose male and female rats on Days 14 and 30; the increase in ALT for female rats on Day 14 was not statistically significant but was consistent with increases at other time points. In the female rats, ALT activity was also elevated in the 1.0 and 3.3 mg/kg groups on Day 30 and at Week 13, respectively. There was an increase in sorbitol dehydrogenase (SDH) activity in males and females in the 10 mg/kg groups on Day 14 and at Week 13. For male rats, this increase also occurred in the 10 mg/kg group on Day 30; in females, SDH activity was also increased in the 1.0 and 10 mg/kg groups on Day 3 and in the 0.1, 0.33, 1.0, and 3.3 mg/kg groups at Week 13.

A number of gross necropsy findings were considered to be related to treatment with riddelliine. The most consistent findings included fluid in the abdominal cavity (ascites); yellow discoloration of the carcass (icterus); firm and granular livers; dark/red and enlarged lymph nodes; pancreatic edema; and firm, granular, and enlarged spleens. These observations were noted most frequently in the 19 male and 5 female high-dose rats that died during the study. Mottled hearts and darkened loops of small bowel were also common necropsy findings in these animals that died early. In general, similar findings were made at both the 13-week study necropsy and the 7-week and 14-week recovery necropsies, although the gross lesions occurred less frequently in the recovery animals.

TABLE 9 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
MALE						
n (Days 3, 14, and 30)	5	5	_[2]	5	_[2]	5
n (Week 13)	10	10	10	10	10	1
Alanine aminotransferase (IU/L)						
Day 14	26 ± 4	33 ± 7	_	34 ± 3	_	109 ± 21**
Day 30	35 ± 3	29 _ 4	_	42 ± 3	_	136 ± 13**
Week 13	96 ± 10	60 ± 7*[3]	87 ± 12	61 ± 4*[3]	94 ± 10	283
Alkaline phosphatase (IU/L)						
Day 3	758 ± 32	893 ± 73	_	854 ± 68	_	969 ± 31*
Day 14	653 ± 17	688 ± 28	_	836 ± 67*	_	1321 ± 43**
Day 30	522 ± 15	475 ± 14	_	557 ± 43	_	1288 ± 90*[4]
Week 13	246 ± 10	261 ± 7	286 ± 22*	268 ± 10	374 ± 29**	302
Creatine phosphokinase (IU/L)						
Day 14	139 ± 13[4]	280 ± 98	_	391 ± 86*	_	511 ± 215[5]
Week 13	76 ± 5	177 ± 33**	103 ± 10	191 ± 43**	97 ± 11[3]	61
Creatine phosphokinase BB (IU/L)						
Day 14	105 ± 14[4]	188 ± 35*	_	280 ± 32**	_	268 ± 107*[5]
Day 30	276 ± 15[6]	264 ± 61[5]	_	280 ± 64[6]	_	99 ± 27*[4]
Glutamyl dehydrogenase (IU/L)						
Day 14	5 ± 1	6 ± 1	_	6 ± 0	_	25 ± 6*
Day 30	9 ± 2[4]	15 ± 1*[4]	_	17 ± 2**	_	49 ± 7**[4]
Week 13	39 ± 11[3]	19 ± 2[3]	28 ± 7[7]	13 ± 1*[3]	54 ± 9[3]	106
Sorbitol dehydrogenase (IU/L)						
Day 14	19 ± 2	15 ± 0	_	19 ± 1	_	66 ± 12*
Day 30	20 ± 1	18 ± 2	_	22 ± 2	_	63 ± 3**
Week 13	39 ± 4[3]	33 ± 6[3]	42 ± 2	34 ± 3	41 ± 6[3]	66
FEMALE						
n (Days 3, 14, and 30)	5	5	_[2]	5	_[2]	5
n (Week 13)	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 14	22 ± 2	25 ± 1	_	25 ± 7	_	48 ± 15
Day 30	25 ± 6	28 ± 2	_	40 ± 6*	_	74 ± 11**
Week 13	42 ± 3	37 ± 3	48 ± 5[3]	39 ± 2	60 ± 7*	152 ± 6**
Alkaline phosphatase (IU/L)						
Week 13	190 ± 9	205 ± 4[3]	188 ± 9	195 ± 7	247 ± 11**	334 ± 35**[8]
Glutamyl dehydrogenase (IU/L)						
Day 30	14 ± 3	18 ± 2	_	18 ± 2	_	25 ± 4*
Week 13	36 ± 6	29 ± 7	22 ± 3	17 ± 4*[3]	19 ± 3[3]	48 ± 6
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 0	14 ± 0	_	15 ± 1*	_	18 ± 2**
Day 14	20 ± 1	15 ± 0	_	32 ± 7	_	72 ± 18*
Day 30	30 ± 6	21 ± 1	_	22 ± 4	_	39 ± 2
Week 13	15 ± 1[9]	23 ± 2**[3]	22 ± 2**[10]	21 ± 2*	22 ± 3*[9]	53 ± 5**[7]

[1] Data are given as mean ± standard error.

[2] No animals were designated for the clinical pathology study for this dose group.

[3] n=9. [5] n=3. [7] n=7. [9] n=6.

[4] n=4. [6] n=2. [8] n=5. [10] n=8.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

Microscopically, the most significant effects of riddelliine treatment were in the liver (Table 10). A spectrum of liver effects was present at the 10 mg/kg dose level, with the major findings being necrosis and cytologic alteration of hepatocytes, proliferative lesions of hepatocytes, and bile duct hyperplasia. Necrosis was more severe in males, all but one of which died prior to the end of the 13-week study. It consisted of coagulative changes or drop-out of hepatocytes, primarily in centrilobular areas, with associated congestion and/or hemorrhage (Plate 1). The remaining viable parenchymal cells had cytologic changes consisting of markedly enlarged, prominent vesicular nuclei (karyomegaly) and increased amounts of eosinophilic, glassy cytoplasm (cytomegaly); cellular enlargement resulted in disruption of the hepatic cord architecture. Scattered hepatocytes had distended, vacuolated cytoplasm. In females treated with 10 mg/kg, these cytologic alterations were the predominant change with less severe, usually single-cell necroses. Other hepatic lesions of mild to moderate severity observed in most of the rats in the 10 mg/kg groups included hyperplasia of bile ductules, usually in the periportal area and extending into surrounding parenchyma, and mixed inflammatory cell infiltration that included numerous macrophages containing pigment (hemosiderin).

In high-dose females that were allowed to recover for 7 or 14 weeks, the hepatotoxic effects of riddelliine described above persisted or progressed in severity. Hepatocytologic alterations of karyocytomegaly and cytoplasmic eosinophilia occurred with similar incidences and severity. A noteworthy finding seen in all females examined during the recovery periods was a markedly increased severity of bile duct proliferation relative to that present at Week 13. At the later time points, this change had progressed to a diffuse proliferation of ductules, which dissected and disrupted hepatic cords and isolated small clusters of hepatocytes (Plate 2).

TABLE 10 Incidences of Liver Lesions in F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
MALE						
Karyomegaly						
13 weeks	0	0	10	10	10	19[2]
7-week recovery	0	0	5	5	5	–[3]
14-week recovery	0	0	5	5	5	–
Cytomegaly						
13 weeks	0	0	0	0	10	19[2]
7-week recovery	0	0	0	0	5	–
14-week recovery	0	0	0	0	5	–
Necrosis, hepatocyte						
13 weeks	0	0	1	0	0	19[2]
7-week recovery	0	0	1	0	1	–
14-week recovery	0	0	0	2	1	–
Bile duct hyperplasia						
13 weeks	0	0	0	0	1	19[2]
7-week recovery	0	0	0	0	1	–
14-week recovery	0	0	0	0	1	–
Foci of cellular alteration						
13 weeks	0	0	1	0	1	0[2]
7-week recovery	0	0	0	3	2	–
14-week recovery	0	0	0	1	3	–
FEMALE						
Karyomegaly						
13 weeks	0	0	7	10	10	10
7-week recovery	0	0	5	5	5	8[4]
14-week recovery	0	0	5	5	5	2[5]
Cytomegaly						
13 weeks	0	0	0	0	1	10
7-week recovery	0	0	0	0	0	8[4]
14-week recovery	0	0	0	0	0	2[5]
Necrosis, hepatocyte						
13 weeks	0	0	0	0	0	10
7-week recovery	0	0	0	0	0	8[4]
14-week recovery	0	0	0	0	0	2[5]
Bile duct hyperplasia						
13 weeks	0	0	0	0	0	10
7-week recovery	0	0	0	0	1	8[4]
14-week recovery	0	0	0	0	0	2[5]
Foci of cellular alteration						
13 weeks	0	0	0	0	1	2
7-week recovery	0	0	0	0	0	1[4]
14-week recovery	0	0	0	0	0	0[5]

TABLE 10

Incidences of Liver Lesions in F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Dose (mg/kg)					
	Vehicle Control	0.1	0.33	1.0	3.3	10
FEMALE (continued)						
Nodular hyperplasia						
13 weeks	0	0	0	0	0	1
7-week recovery	0	0	0	0	0	2[4]
14-week recovery	0	0	0	0	0	2[5]
Adenoma						
13 weeks	0	0	0	0	0	2
7-week recovery	0	0	0	0	0	0[4]
14-week recovery	0	0	0	0	0	1[5]

[1] The incidence is the number of animals with lesions from groups of 10 (13-week base study) or 5 (7-week and 14-week recovery studies) unless otherwise noted.
[2] n=20, including 19 early deaths and one terminal sacrifice.
[3] No male survivors for recovery studies in the 10 mg/kg group.
[4] n=8, including four scheduled sacrifices and four early deaths during the 7-week recovery period.
[5] n=2, including one scheduled sacrifice and one early death during the 7-week to 14-week recovery period.

Two of 10 high-dose female rats examined at 13 weeks had hepatocellular adenomas; one of these animals had multiple adenomas. Adenomas were characterized by distinctly expansile nodules that compressed adjacent parenchyma or bulged the capsular surface (Plates 3 and 4). The cells composing the adenomas were generally well differentiated, but there was usually some degree of cellular hypertrophy and/or pleomorphism. Three other female rats from this group had proliferative hepatocellular lesions diagnosed as foci of cellular alteration or focal nodular hyperplasia. Altered foci were small, spherical lesions composed of hepatocytes with altered staining characteristics, which blended into the surrounding parenchymal cells. Nodular hyperplasia was a larger, more clearly demarcated and expansile lesion consisting of slightly pleomorphic cells that caused some compression. Foci of nodular hyperplasia were more apparent in female rats during the recovery periods. In one female that died during the 14-week recovery period, multiple adenomas were present in a complex histologic picture of admixed cholangiocellular and multifocal, nodular, hepatocellular hyperplasia.

At doses less than 10 mg/kg, riddelliine exposure was associated with variable cytologic alterations of hepatocytes. Diffuse karyomegaly and cytomegaly were prominent in the 3.3 mg/kg groups; nuclei were enlarged and vesicular, and the cells had increased amounts of glassy eosinophilic cytoplasm. These changes were more pronounced in males

than in females, which had a lack of obvious cellular swelling at this dose level. The cytoplasm of scattered hepatocytes was distended by multiple clear vacuoles. At decreasing doses, slight nuclear enlargement and eosinophilia of the cytoplasm, without appreciably increased cell size, were the primary changes. As in the 10 mg/kg groups, hepatocytologic effects occurring at lower doses persisted into the recovery periods in both males and females. Foci of cellular alteration were a particularly common finding in recovery period males in the 1.0 and 3.3 mg/kg groups.

Accumulations of intravascular cells identified as macrophages were observed in the lung and kidney of most male and female rats in the 10 mg/kg groups after 13 weeks of riddelliine treatment and in the lung, kidney, and liver of female rats during the recovery periods (Table 11). These cells occurred in aggregates that appeared free in the vessel lumen or closely adherent to the underlying endothelium. They were most frequently detected in the large veins of the renal cortex and in variably sized vessels in the lung (Plate 5). The cells had abundant, faint, eosinophilic cytoplasm that usually contained iron-positive golden-brown pigment (hemosiderin); occasionally, phagocytized erythrocytes could be seen within the cells. In the lung, these cells were indistinguishable from alveolar macrophages. Light microscopic morphology, evidence of phagocytic capability, and ultrastructural demonstration of numerous cytoplasmic phagolysosomes and ruffled surface membranes (Plate 6) were criteria for identification of these cells as macrophages. In addition to the frequent occurrence of this finding in the high-dose (10 mg/kg) groups, sporadic occurrences were noted in the lungs and kidneys of males in the 3.3 mg/kg group.

Several lesions were observed in the spleen in high-dose males and females. There was mild to moderate hematopoietic cell proliferation in the red pulp. Lymphoid depletion of the white pulp was seen as a loss of primarily marginal zone lymphocytes. Both of these findings were generally more severe in animals that died early than in survivors. A minimal change observed in all rats was the presence of focal fibrous thickenings containing accumulations of lymphocytes in the splenic capsule. In high-dose males only, there was a mild increase in intracellular pigment (hemosiderin) within macrophages.

TABLE 11 Incidences of Intravascular Macrophage Accumulation in F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)			
	Vehicle Control	1.0	3.3	10
MALE				
Liver				
13 weeks	0	0	0	0[2]
7-week recovery	0	0	0	–[3]
14-week recovery	0	0	0	–
Lung				
13 weeks	0	0	1	20[2]
7-week recovery	0	0	0	–
14-week recovery	0	0	0	–
Kidney				
13 weeks	0	0	2	18[2]
7-week recovery	0	0	0	–
14-week recovery	0	0	0	–
FEMALE				
Liver				
13 weeks	0	0	0	0
7-week recovery	0	0	0	3[4]
14-week recovery	0	0	0	1[5]
Lung				
13 weeks	0	0	0	10
7-week recovery	0	0	0	8[4]
14-week recovery	0	0	0	2[5]
Kidney				
13 weeks	0	0	0	9
7-week recovery	0	0	0	2[4]
14-week recovery	0	0	0	0[5]

1 The incidence is the number of animals with lesions from groups of 10 (13-week base study) or 5 (7-week and 14-week recovery studies) unless otherwise noted.

2 n=20, including 19 early deaths and one terminal sacrifice.

3 No male survivors in the 10 mg/kg group for recovery studies.

4 n=8, including four scheduled sacrifices and four early deaths during the 7-week recovery period.

5 n=2, including one scheduled sacrifice and one early death during the 7-week to 14-week recovery period.

Bone marrow hyperplasia consisted of replacement of the normal marrow fat with hematopoietic cells. This lesion was found only at the 10 mg/kg dose level, in both the males and females that died early, as well as in the surviving females of the base study. This was generally a mild change that involved both myeloid and erythroid components.

A spectrum of changes including moderate to marked congestion, erythrophagocytosis, and accumulation of hemosiderin-laden macrophages was observed in multiple lymph nodes of high-dose rats. In many cases, red blood cells filled the subcapsular and medullary sinuses where they were frequently phagocytosed within macrophages. These effects were

more prominent in the males, which died prior to the end of the 13-week study, but were also common findings in the females at Week 13 and also in females that died or were killed during the recovery periods. The mesenteric node was most commonly affected.

Treatment of rats with 10 mg/kg was also associated with hemorrhagic lesions of the heart; these lesions were present in all males and in most females that died early and accounted for the mottled hearts noted at necropsy. The affected areas were large, were typically located in the subepicardial myocardium of the ventricles, and were characterized by the separation of myofibers by the accumulation of red blood cells; leukocytes were also sometimes admixed with the extravasated red cells. Myofibers within these foci of hemorrhage had glassy, eosinophilic cytoplasm, but lacked overt evidence of necrosis.

A lesion of the pancreas, interstitial edema, which was characterized by widening of the interstitial spaces, was found in both sexes of rats in the high-dose groups. In the interstitial spaces, there was a variable presence of faintly staining edema fluid and/or mixed inflammatory cells, frequently hemosiderin-containing macrophages. Edema was a minimal to mild change and was accompanied in a few instances by necrosis of adjacent fat with mineralization and fibrosis. Edema persisted into the 7-week and 14-week recovery periods, as indicated by its presence in all high-dose females examined at these time points; the lesion also occurred in males in the 3.3 mg/kg group.

Several lesions in the kidney were considered to be related to riddelliine treatment. Minimal to moderate mineralization was found in the renal tubules of all males in the high-dose group. The incidences of foci of tubule regeneration (minimal) were slightly increased in treated females at Week 13 and during the recovery period. Hydropic degeneration of the transitional epithelium of the renal pelvis and the presence of iron-positive pigment (hemosiderin) in the tubules, minimal to mild changes, respectively, were observed primarily in high-dose males. Collectively, these findings suggest some toxic effect of riddelliine on the kidney.

Luminal, mucosal, or submucosal hemorrhage at various sites along the gastrointestinal tract occurred frequently in high-dose male rats and corresponded to gross necropsy findings of darkened loops of bowel. Submucosal edema was a common finding in the lower intestines of these animals as well.

Atrophic changes that affected various tissues in high-dose animals and that were attributed to debilitation included atrophy of the testis, epididymis, seminal vesicle, prostate gland, and preputial gland in males and atrophy of the ovary (lack of corpora lutea) in females. Cortical atrophy of the thymus was another high-dose effect in both sexes and was interpreted to be secondary to stress.

Sperm morphology evaluations were performed on base study male rats in the vehicle control, 0.1, 1.0, and 3.3 mg/kg groups, and vaginal cytology evaluations were performed on base study female rats in the vehicle control, 0.1, 1.0, and 10 mg/kg groups. Male rats in the 0.1, 1.0, and 3.3 mg/kg groups had statistically significantly lower right epididymal weights compared to the vehicle control group and males in the 1.0 and 3.3 mg/kg groups also had significantly lower right testis weights compared to the control group; however, the organ weights were appropriate for the body weights of animals in these groups (Table C1). There were no significant differences between treated groups and the control group for any of the spermatozoal measurements. Female rats receiving 10 mg/kg riddelliine by gavage differed markedly from controls in the relative frequency of time spent in the various estrous stages; females in this dose group spent more time in estrus and less time in the other stages than did controls or females in the 0.1 and 1.0 mg/kg groups (Table C2). Indeed, for all 10 females in this group, estrous cycle length could not be determined. The rats were effectively held in constant estrus.

2-Week Gavage Study in B6C3F$_1$ Mice

All animals survived to the end of the 2-week study (Table 12). Mean final body weights and mean body weight gains of treated animals were similar to those of controls.

TABLE 12 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Gavage Study of Riddelliine

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Vehicle Controls[4] (%)
		Initial[2]	Final	Change[3]	
MALE					
Vehicle	5/5	24.1	27.0	2.9	
0.33	5/5	24.6	27.4	2.8	101
1.0	5/5	23.4	26.5	3.0	98
3.3	5/5	24.4	27.5	3.1	102
10	5/5	23.4	27.0	3.6	100
25	5/5	23.0	26.2	3.2	97
FEMALE					
Vehicle	5/5	19.4	22.7	3.3	
0.33	5/5	20.5	23.1	2.6	102
1.0	5/5	19.8	22.9	3.1	101
3.3	5/5	20.2	22.7	2.5	100
10	5/5	20.2	22.9	2.7	101
25	5/5	19.1	22.1	3.0	98

1 Number surviving at 16 days/number of animals per dose group.
2 Initial group mean body weight. Subsequent calculations are based on animals surviving to the end of the study.
3 Mean body weight change of the survivors.
4 Dosed group mean/vehicle control group mean x 100.

Only one male mouse receiving riddelliine (10 mg/kg group) exhibited clinical signs of toxicity; this animal exhibited slight emaciation on Day 5 of treatment but appeared normal later. In addition, one female in the 10 mg/kg group appeared hunched on Day 3 of treatment and appeared emaciated on Day 4, but was noted to be normal later. No animals in the 0.33, 1.0, 3.3, or 25 mg/kg groups showed any clinical signs of toxicity.

Statistically significant, dose-related increases in mean liver weight and liver-weight-to-body-weight ratio were noted in both males and females given riddelliine by gavage for 2 weeks. Absolute and relative liver weights were significantly greater than control values for males given 10 or 25 mg/kg riddelliine and for females given 3.3, 10, or 25 mg/kg; absolute liver weights alone were significantly increased for females in the 0.33 and

1.0 mg/kg groups. In addition, the relative thymus weight of female mice showed a significant positive trend with dose, but the difference from control was statistically significant for the high-dose (25 mg/kg) group only.

No treatment-related gross lesions were noted at necropsy following 2 weeks of treatment with riddelliine by gavage. Microscopic examination revealed an effect in the liver in both male and female mice at the highest dose level. This change, diagnosed as cytomegaly, was characterized by enlargement of centrilobular hepatocytes due to increased amounts of pale-staining, finely granular cytoplasm. The no-effect level for this finding was 0.33 mg/kg.

Based on the absence of body weight depression and mortality and on the lack of life-threatening histopathologic lesions, the dose levels selected for the 13-week study were the same as those used in the 2-week study.

13-Week Gavage Study in B6C3F$_1$ Mice

Three males and 10 females died during the first 3 weeks of dosing; none of the deaths were considered to be related to riddelliine treatment (Tables 13-15). One male in each of the vehicle control, 0.33 mg/kg, and 3.3 mg/kg groups assigned to the 7-week recovery study died or was killed moribund (Table 14). Histopathologic evaluation confirmed two of these deaths (vehicle control and 0.33 mg/kg groups) to be dosing accidents, but the cause of death for the animal in the 3.3 mg/kg group was unknown. For females, three deaths in the vehicle control group and two deaths in each of the 0.33 and 25 mg/kg groups were attributed to gavage accidents. Two female mice in the vehicle control group and one female in the 1.0 mg/kg group died within the first 3 weeks of the study due to unknown causes.

At the end of the 13-week dosing period, mean body weights and body weight gains for male and female mice in the 10 and 25 mg/kg groups were notably lower than the vehicle control values (Tables 13-15; Figure 5). Very little recovery from body weight depression occurred during the 7-week and 14-week recovery periods, and weight gains remained less than those of controls, particularly in the 10 and 25 mg/kg groups.

No clinical observations that were clearly treatment related were noted during the periods of dosing or recovery.

TABLE 13 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Base Study of Riddelliine Administered by Gavage

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Vehicle Controls[4] (%)
		Initial[2]	Final	Change[3]	
MALE					
Vehicle	10/10	23.3	34.0	10.7	
0.33	10/10	24.0	37.3	13.3	110
1.0	10/10	22.9	35.9	13.0	106
3.3	10/10	23.5	34.8	11.4	102
10	10/10	23.5	31.0	7.5	91
25	10/10	23.6	28.2	4.6	83
FEMALE					
Vehicle	8/10[5]	19.7	29.9	10.5	
0.33	10/10	18.5	28.1	9.7	94
1.0	10/10	19.7	32.5	12.8	109
3.3	10/10	19.0	30.2	11.2	101
10	10/10	17.5	25.1	7.6	84
25	10/10[6]	19.1	23.6	4.5	79

1 Number surviving at 13 weeks/number of animals per dose group.

2 Body weights were measured at Day 1. Subsequent calculations are based on animals surviving to the end of the base study.

3 Mean weight change of the survivors.

4 (Dosed group mean/vehicle control group mean) x 100.

5 Week of death: Both animals died by Week 3.

6 Body weights were recorded for only nine animals.

TABLE 14 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine Followed by a 7-Week Recovery Period

Dose (mg/kg)	Survival[1]	Mean Body Weight (g) Initial[2]	Week 13	Change[3]	Week 13 Wt Relative to Controls[4] (%)	Mean Body Wt (g) Week 20	Change[5]	Final Weight Relative to Controls[4] (%)
MALE								
Vehicle	4/5[6]	23.4	36.1	12.2		45.1	9.0	
0.33	4/5[7]	23.5	32.7	9.0	91	38.0	5.3	84
1.0	5/5	24.0	35.3	11.3	98	40.9	5.6	91
3.3	4/5[8]	22.6	33.3	10.4	92	39.9	6.6	88
10	5/5	24.0	33.4	9.4	93	39.2	5.8	87
25	5/5	24.1	29.1	5.0	81	35.7	6.6	79
FEMALE								
Vehicle	3/5[9]	18.9	29.1	9.9		33.4	4.3	
0.33	4/5[9]	19.4	32.0	12.5	110	37.0	5.0	111
1.0	4/5[9]	19.0	32.1	13.2	110	36.5	4.4	109
3.3	5/5	19.1	31.4	12.3	108	37.1	5.7	111
10	5/5	18.1	26.3	8.3	91	29.1	2.8	87
25	4/5[9]	20.2	24.5	4.4	84	25.7	1.2	77

1 Number surviving at 20 weeks/number of animals per dose group.

2 Body weights were measured at Day 1. Subsequent calculations are based on animals surviving to the end of the evaluation period (Week 13 or Week 20).

3 Mean weight change of the survivors from Week 0 to Week 13.

4 (Dosed group mean/vehicle control group mean) x 100.

5 Mean weight change of the survivors from Week 13 to Week 20.

6 Week of death: 2.

7 Week of death: 3.

8 Week of death: 1.

9 All deaths in females occurred by Week 3.

TABLE 15 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine Followed by a 14-Week Recovery Period

Dose (mg/kg)	Survival[1]	Mean Body Weight (g) Initial[2]	Week 13	Change[3]	Week 13 Wt Relative to Controls[4] (%)	Mean Body Wt (g) Week 27	Change[5]	Final Weight Relative to Controls[4] (%)
MALE								
Vehicle	5/5	24.8	37.4	12.6		45.1	7.8	
0.33	5/5	23.8	36.2	12.4	97	44.4	8.2	98
1.0	5/5	22.8	35.5	12.7	95	43.0	7.5	95
3.3	5/5	23.5	34.1	10.6	91	40.2	6.1	89
10	5/5	22.9	30.1	7.2	81	35.5	5.3	79
25	5/5	22.9	27.4	4.5	73	33.4	6.0	74
FEMALE								
Vehicle	4/5[6]	19.1	31.7	12.4		38.9	7.2	
0.33	4/5[6]	18.9	29.8	10.8	94	35.8	6.0	92
1.0	5/5	18.8	31.0	12.2	98	36.3	5.3	93
3.3	5/5	18.6	27.9	9.3	88	35.8	7.8	92
10	5/5	18.5	26.0	7.5	82	31.1	5.1	80
25	4/5[6]	18.2	23.3	5.2	74	26.8	3.4	69

1 Number surviving at 27 weeks/number of animals per dose group.

2 Body weights were measured at Day 1. Subsequent calculations are based on animals surviving to the end of the evaluation period (Week 13 or Week 27).

3 Mean weight change of the survivors from Week 0 to Week 13.

4 (Dosed group mean/Vehicle control group mean) x 100.

5 Mean weight change of the survivors from Week 13 to Week 27.

6 All deaths occurred by Week 3.

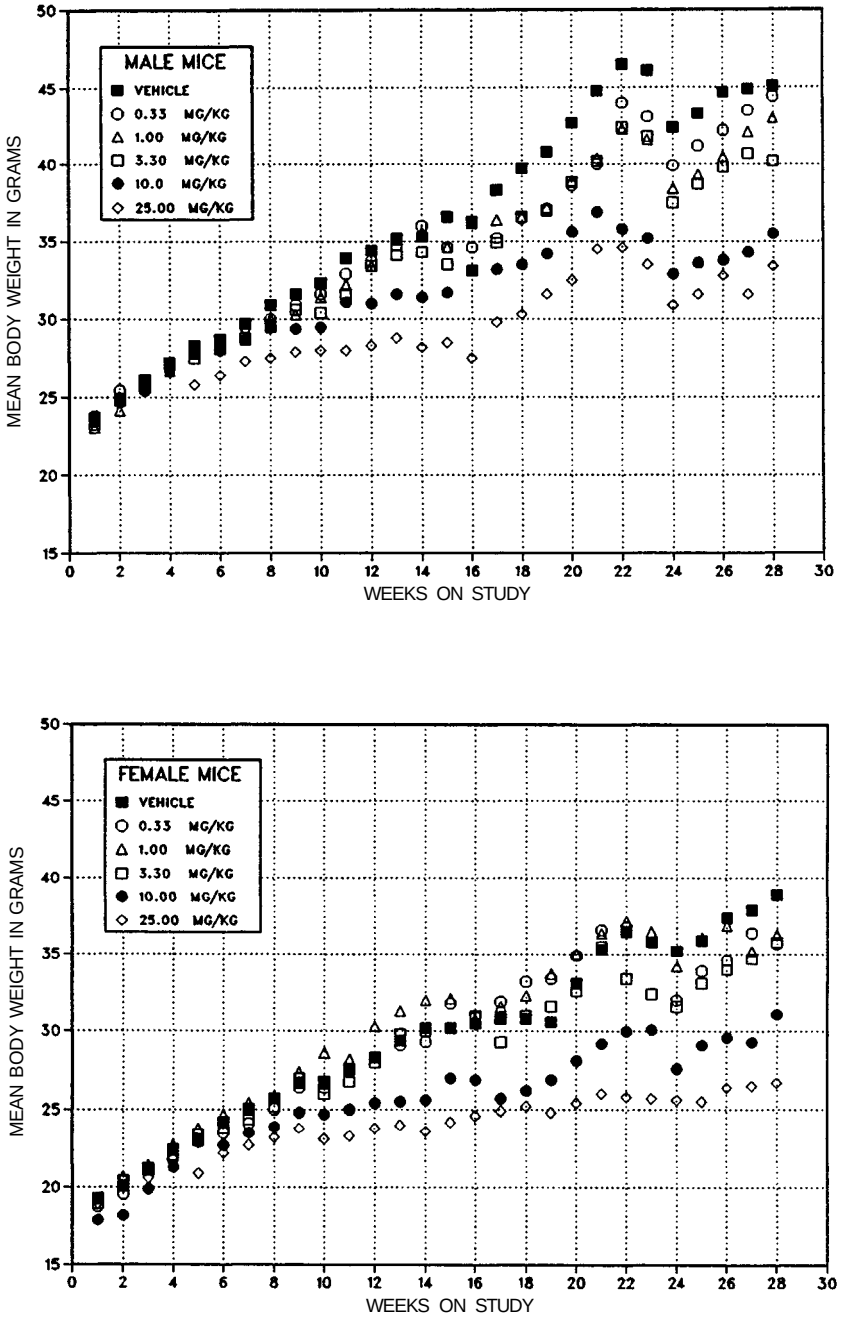

FIGURE 5 Body Weights of B6C3F$_1$ Mice Administered Riddelliine by Gavage for 13 Weeks

A statistically significant positive trend with dose occurred in the 13-week base study for mean organ-weight-to-body-weight ratios for the brain, lung, and spleen in male and female mice, the right testis for male mice, and the liver for female mice (Tables 16 and A2). A statistically significant negative trend with dose was noted for the absolute heart and liver weights for both male and female mice, the absolute kidney weights of males, and the absolute brain and thymus weights for female mice. The absolute lung weights of male mice and the absolute spleen weights for both males and females also showed a positive trend with dose. Statistically significant differences from control values occurred most frequently for mice in the two highest dose groups (10 and 25 mg/kg) (Table 16).

During the 7-week and 14-week recovery periods, there was little evidence that the organs that showed inappropriate weights relative to body weight at the end of the dosing period were changing to become more appropriate for body weight. The same general pattern of differences in relative and absolute organ weights was seen. This is in contrast to the findings in the 13-week rat study, in which evidence of recovery to more normal organ weights was noted.

TABLE 16 Selected Organ Weights of B6C3F$_1$ Mice Administered Riddelliine by Gavage for 13 Weeks[1]

	Dose (mg/kg)					
	Vehicle Control	0.33	1.0	3.3	10	25
MALE						
Base Study						
n	10	10	10	10	10	10
Necropsy body weight	33.6	36.3	35.2	34.1	31.3	28.6**
Brain weight	0.456	0.462	0.461	0.470	0.472	0.466
Relative brain weight	13.94	12.81	13.18	13.92	15.13	16.30**
Heart weight	0.178	0.171	0.174	0.183	0.157*	0.140**
Relative heart weight	5.40	4.75	4.99	5.36	5.02	4.90
Right kidney weight	0.304	0.291	0.303	0.291	0.275*	0.250**
Relative right kidney weight	9.15	8.03**	8.67	8.56	8.81	8.74
Liver weight	1.762	1.621	1.995	1.690	1.552**	1.612**
Relative liver weight	53.19	44.72	59.96	49.80	49.67	56.44
Lung weight	0.343	0.387	0.310	0.371	0.397*	0.428**
Relative lung weight	10.35	10.72	8.82	10.93	12.72**	15.00**
Spleen weight	0.081	0.076	0.075	0.080	0.084	0.106**
Relative spleen weight	2.45	2.10	2.14	2.36	2.69	3.70**
Right testis weight	0.122	0.123	0.127	0.127	0.123	0.118
Relative right testis weight	3.71	3.42	3.64	3.75	3.92	4.14*
Left ventricle	0.071	0.073	0.056	0.074	0.066	0.049**
Relative left ventricle weight	2.15	2.02	1.62	2.18	2.12	1.71**
Right ventricle	0.025	0.025	0.022	0.023	0.021*	0.021*
Relative right ventricle weight	0.76	0.70	0.62**	0.69	0.68	0.75
7-Week Recovery						
n	4	4	5	4	5	5
Necropsy body weight	45.1	39.0	42.2	40.9	40.6	37.0*
Brain weight	0.473	0.473	0.472	0.480	0.478	0.488
Relative brain weight	10.61	12.14	11.22	11.87	11.93	13.29**
Heart weight	0.190	0.166	0.167	0.164	0.161*	0.150**
Relative heart weight	4.23	4.25	3.96	4.03	4.00	4.09
Liver weight	2.430	2.090	2.332	2.220	2.098	1.782**
Relative liver weight	53.67	53.61	55.35	54.36	51.85	48.14**

1 Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
2 n=3.
3 n=4.
* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

TABLE 16 Selected Organ Weights of B6C3F$_1$ Mice Administered Riddelliine by Gavage for 13 Weeks (continued)

	Dose (mg/kg)					
	Vehicle Control	0.33	1.0	3.3	10	25
MALE (continued)						
7-Week Recovery (continued)						
Lung weight	0.225	0.208	0.226	0.218	0.278	0.288*
Relative lung weight	5.06	5.30	5.36	5.35	6.91*	7.90**
Spleen weight	0.095	0.093	0.084	0.100	0.098	0.110
Relative spleen weight	2.12	2.38	2.00	2.44	2.43	2.99**
Right testis weight	0.135	0.121*	0.129	0.130	0.134	0.128
Relative right testis weight	3.03	3.10	3.07	3.20	3.32	3.49
Left ventricle weight	0.081	0.074	0.074	0.070	0.075	0.064**
Relative left ventricle weight	1.80	1.89	1.76	1.71	1.85	1.75
14-Week Recovery						
n	5	5	5	5	5	5
Necropsy body weight	45.5	44.3	43.4	41.3	35.6**	33.9**
Brain weight	0.488	0.478	0.484	0.486	0.488	0.464
Relative brain weight	10.86	10.89	11.42	12.03	13.77*	13.78*
Heart weight	0.184	0.200	0.193	0.190	0.189	0.156
Relative heart weight	4.08	4.58	4.50	4.61	5.32*	4.64*
Right kidney weight	0.406	0.406	0.400	0.426	0.398	0.365
Relative right kidney weight	8.94	9.28	9.36	10.40*	11.21**	10.53**
Liver weight	2.174	2.246	2.244	2.120	1.846	1.660*
Relative liver weight	47.50	50.78	51.69	51.39	52.06	49.15
Lung weight	0.254	0.272	0.250	0.250	0.290	0.263[2]
Relative lung weight	5.59	6.16	5.82	6.09	8.17**	7.50**[2]
Spleen weight	0.092	0.082	0.096	0.094	0.092	0.092
Relative spleen weight	2.04	1.86	2.21	2.32	2.60	2.77*
Right testis weight	0.137	0.133	0.133	0.129	0.126	0.121*
Relative right testis weight	3.03	3.04	3.07	3.18	3.56*	3.60**
Left ventricle	0.087	0.0810	0.085	0.074	0.077	0.067**
Relative left ventricle weight	1.91	1.84	1.99	1.81	2.17	1.99

TABLE 16 Selected Organ Weights of B6C3F$_1$ Mice Administered Riddelliine by Gavage for 13 Weeks (continued)

	Dose (mg/kg)					
	Vehicle Control	0.33	1.0	3.3	10	25
FEMALE						
Base Study						
n	8	10	10	10	10	9
Necropsy body weight	30.0	27.7	32.5	30.0	25.2**	24.2**
Brain weight	0.495	0.493	0.495	0.489	0.466**	0.472**
Relative brain weight	16.58	17.94	15.32	16.38	18.49**	19.52**
Heart weight	0.145	0.138	0.147	0.143	0.121**	0.108**
Relative heart weight	4.85	5.02	4.56	4.78	4.79	4.46
Liver weight	1.465	1.300	1.776	1.468	1.288**	1.274**
Relative liver weight	48.98	46.92	54.74	49.02	51.01	52.62*
Lung weight	0.364	0.334	0.371	0.362	0.360	0.374
Relative lung weight	12.21	12.12	11.45	12.14	14.25**	15.49**
Spleen weight	0.105	0.092	0.106	0.101	0.106	0.171**
Relative spleen weight	3.54	3.33	3.27	3.38	4.21	7.04**
Thymus weight	0.055	0.055	0.060	0.058	0.051	0.041**
Relative thymus weight	1.85	2.01	1.85	1.95	2.03	1.69
Left ventricle weight	0.059	0.056	0.065	0.058	0.053	0.046**
Relative left ventricle weight	1.98	2.03	2.02	1.93	2.11	1.91
7-Week Recovery						
n	3	4	4	5	5	4
Necropsy body weight	33.3	38.0	37.0	36.0	29.7	27.5*
Brain weight	0.490	0.498	0.495	0.488[3]	0.486	0.485
Relative brain weight	14.91	13.10	13.68	13.64[3]	16.36	17.65*
Heart weight	0.171	0.157	0.158	0.145	0.134*	0.123**
Relative heart weight	5.11	4.11	4.40	4.03	4.51	4.46
Right kidney weight	0.240	0.243	0.245	0.254	0.238	0.255
Relative right kidney weight	7.28	6.36	6.72	7.09	8.01	9.29**
Lung weight	0.207	0.193	0.188	0.196	0.218	0.283**
Relative lung weight	6.25	5.06	5.10	5.44	7.33	10.24**
Spleen weight	0.103	0.098	0.090	0.112	0.104	0.163**
Relative spleen weight	3.12	2.56	2.46	3.13	3.50	5.92**

TABLE 16 Selected Organ Weights of B6C3F$_1$ Mice Administered Riddelliine by Gavage for 13 Weeks (continued)

	Dose (mg/kg)					
	Vehicle Control	0.33	1.0	3.3	10	25
FEMALE (continued)						
14-Week Recovery						
n	4	4	5	5	5	4
Necropsy body weight	39.9	37.5	37.8	36.8	30.3*	27.7**
Brain weight	0.488	0.508	0.504	0.492	0.490	0.490
Relative brain weight	12.53	13.83	13.52	13.74	16.45*	17.72**
Heart weight	0.151	0.162	0.162	0.156	0.158	0.143
Relative heart weight	3.84	4.36	4.33	4.37	5.28**	5.16*
Right kidney weight	0.278	0.270	0.282	0.270	0.252	0.245
Relative right kidney weight	7.02	7.36	7.55	7.55	8.38	8.86*
Liver weight	1.818	1.843	1.986	1.928	1.612	1.400*
Relative liver weight	46.32	49.77	52.90	53.38	52.88	50.51
Lung weight	0.263	0.225	0.230	0.240	0.248	0.253
Relative lung weight	6.64	6.21	6.14	6.74	8.23	9.11*
Spleen weight	0.148	0.105*	0.112*	0.130	0.104*	0.173
Relative spleen weight	3.80	2.86	2.99	3.70	3.42	6.22**

Hematology evaluations were performed at Week 13. Differences in values of erythrocyte and platelet parameters similar to those found in rats treated for 13 weeks were noted (Table B2). As with the rats, the differences in mice were more pronounced in males.

Erythrocyte count, hematocrit, and hemoglobin concentration increased significantly in male mice in the 0.33, 10, and 25 mg/kg groups (Table 17). No similar differences were noted in female mice. Reticulocyte counts were increased in female mice in the 10 and 25 mg/kg groups. An increase in reticulocytes was noted in male mice treated with 25 mg/kg, but this increase was not statistically significant. A slight increase in mean cell volume was noted in male mice in the 10 and 25 mg/kg groups and in female mice in the 25 mg/kg group. Platelet counts were significantly decreased in males treated with 10 or 25 mg/kg and in females in all dose groups except the 1.0 mg/kg group; this difference was most pronounced in the high-dose groups. The mean platelet volume was increased in males and females in the 25 mg/kg groups and in males in the 0.33 mg/kg group. Minor differences in values of leukocyte parameters were observed, but these did not suggest a treatment-related trend.

TABLE 17 Selected Hematology Data for B6C3F$_1$ Mice after 13 Weeks of Treatment in the 13-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)					
	Vehicle Control	0.33	1.0	3.3	10	25
MALE						
n	10	9	10	10	9	9
Hematocrit (%)	55.5 ± 0.9	59.4 ± 1.0*	54.9 ± 0.7	56.0 ± 1.1	62.9 ± 1.7**	66.1 ± 1.20**
Hemoglobin (g/dL)	18.0 ± 0.3	19.3 ± 0.4**	18.2 ± 0.2	18.0 ± 0.4	19.8 ± 0.6**	21.1 ± 0.5**
Erythrocytes (10^6/µL)	10.81 ± 0.20	11.76 ± 0.17**	10.82 ± 0.16	10.90 ± 0.25	11.90 ± 0.30**	12.37 ± 0.30**
Reticulocytes (10^6/µL)	0.20 ± 0.02	0.25 ± 0.04	0.25 ± 0.02	0.21 ± 0.02	0.21 ± 0.02	0.27 ± 0.03
Mean cell volume (fL)	50.2 ± 0.4	49.4 ± 0.3	49.8 ± 0.3	50.3 ± 0.4	51.7 ± 0.2**	52.4 ± 0.3**
Platelets (10^3/µL)	1070.7 ± 25.8	1131.8 ± 63.9[2]	1139.8 ± 69.7	1091.8 ± 32.2	673.8 ± 58.9**[2]	394.7 ± 29.6**
Mean platelet volume (µm^3)	5.63 ± 0.04	6.62 ± 0.25**[2]	5.80 ± 0.09	5.67 ± 0.03	5.83 ± 0.15[2]	6.06 ± 0.11**
Leukocytes (10^3/µL)	5.02 ± 0.34	3.11 ± 0.20**	5.14 ± 0.32	3.80 ± 0.37	4.41 ± 0.30	5.22 ± 0.42
FEMALE						
n	8	10	10	10	10	8
Hematocrit (%)	62.4 ± 1.4	61.6 ± 1.1	58.6 ± 1.5	63.3 ± 1.6	65.6 ± 1.3	63.9 ± 2.7
Hemoglobin (g/dL)	19.7 ± 0.4	19.5 ± 0.3	19.5 ± 0.5	20.2 ± 0.4	20.8 ± 0.4	19.9 ± 0.8
Erythrocytes (10^6/µL)	11.68 ± 0.23	11.45 ± 0.20	11.42 ± 0.29	11.93 ± 0.29	12.28 ± 0.24	11.45 ± 0.53
Reticulocytes (10^6/µL)	0.16 ± 0.02[5]	0.22 ± 0.02	0.26 ± 0.05	0.24 ± 0.03	0.28 ± 0.03*[3]	0.23 ± 0.02*[5]
Mean cell volume (fL)	52.3 ± 0.5	52.3 ± 0.3	50.3 ± 0.2	52.0 ± 0.3	52.3 ± 0.3	54.8 ± 0.4**
Platelets (10^3/µL)	944.6 ± 27.4	833.6 ± 26.7*	917.4 ± 35.7	706.2 ± 34.4**	546.5 ± 61.4**	323.5 ± 44.8**[2]
Mean platelet volume (µm^3)	5.80 ± 0.08	5.56 ± 0.05	5.85 ± 0.07	5.80 ± 0.04	6.00 ± 0.12	6.53 ± 0.11**[2]
Leukocytes (10^3/µL)	5.01 ± 0.25	4.16 ± 0.31	4.69 ± 0.20	4.63 ± 0.44	4.09 ± 0.23	5.19 ± 0.86

[1] Data are given as mean ± standard error.
[2] n=10.
[3] n=9.
[4] n=8.
[5] n=7.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

No gross necropsy findings were attributed to riddelliine administration after 13 weeks of treatment or following a 7-week or 14-week recovery period.

Microscopic effects were present in the liver and forestomach of treated mice (Table 18). Liver lesions consisted of centrilobular cytomegaly, characterized by increased amounts of pale-staining, finely granular cytoplasm in centrilobular hepatocytes. This effect was evident in high-dose males and females in the base study; however, the severity of the change was slightly greater in females (mild to moderate) than in the males (minimal to mild). Centrilobular cytomegaly persisted through the recovery periods in females, but

not in males. In addition, minimal bile duct hyperplasia was noted in most high-dose females at the end of the 7-week and 14-week recovery periods (Plate 7), indicating possible progression of the effect of riddelliine over time.

Hyperplasia of the stratified squamous epithelium of the forestomach was observed in mice receiving 10 or 25 mg/kg (Table 18). Hyperplasia was typically a mild, focal lesion of increased epithelial thickness caused by an increase in the number of cell layers. Hyperplasia was sometimes accompanied by a slight increase in the amount of keratin overlying the lesion or by submucosal inflammation and/or edema. Minimal erosion or ulceration was an infrequent finding in the hyperplastic lesions. In general, forestomach hyperplasia seemed to regress during the recovery period; no appreciable hyperplastic changes were evident in any mice in the 7-week recovery study, and after 14 weeks of recovery, involvement was minimal and occurred in only one male and two females in the high-dose groups.

No histologic findings in the lungs or spleen corresponding to the dose-dependent increases in weights of these tissues were noted.

Sperm morphology and vaginal cytology evaluations were performed on base study mice in the vehicle control, 0.33, 3.3, and 25 mg/kg groups. There were no biologically significant findings in males (Table C3). However, females in the 25 mg/kg group showed a marked prolongation of estrous cycle length. For 7 of the 10 females in this group, distinct stages of the cycle could not be scored and the total length of the cycle appeared to be longer than 7 days (Table C4).

TABLE 18 Incidences of Liver and Forestomach Lesions in B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine[1]

	Dose (mg/kg)			
	Vehicle Control	3.3	10	25
MALE				
Liver				
Centrilobular cytomegaly				
13 weeks	0	0	0	10
7-week recovery	0	0	0	0
14-week recovery	0	0	0	0
Forestomach				
Epithelial hyperplasia				
13 weeks	0	0	8	8
7-week recovery	0	0	0	0
14-week recovery	0	0	0	1
FEMALE				
Liver				
Centrilobular cytomegaly				
13 weeks	0	0	0	10
7-week recovery	0	0	0	4
14-week recovery	0	0	0	4
Bile duct hyperplasia				
13 weeks	0	0	0	0
7-week recovery	0	0	0	3
14-week recovery	0	0	0	3
Forestomach				
Epithelial hyperplasia				
13 weeks	0	0	1	2
7-week recovery	0	0	0	0
14-week recovery	0	0	0	2

[1] The incidence is the number of animals with lesions from groups of 10 (13-week base study) or 5 (7-week and 14-week recovery studies).

Mating Trial Results

Administration of riddelliine to rats at doses of 0.1 or 1.0 mg/kg prior to and during mating and gestation had no effect on fertility. Length of gestation was not affected by riddelliine administration (Table C5). The body weights of treated dams were significantly lower than the body weight of the controls on Day 0 of gestation in the 0.1 and 1.0 mg/kg groups and on Day 19 in the 1.0 mg/kg group; however, the weight gains of treated dams and controls were similar during pregnancy. Weights of treated dams during lactation were significantly lower than control values on lactation Days 0, 5, 14, and 21. There were

no significant differences between treated groups and the controls in the number of litters, litter size, percentage of pups born alive, pup survival, or male pup weights (Table C6). However, on Days 14 and 21 of lactation, the female pup weights for the 1.0 mg/kg group were significantly lower than the control values. No gross malformations were observed in any of the pups.

In the mating trial in mice, one control male and one control female died due to dosing accidents within the first 3 weeks of treatment. One female in the 25 mg/kg group was killed moribund after 8 weeks of dosing. Riddelliine administration had no significant effect on fertility. For mice treated with riddelliine by gavage, there was no effect on the length of gestation (Table C7). Females in the 25 mg/kg group weighed significantly less than the controls on gestation Day 0; the body weight of dams in this group was also significantly lower than that of the controls on lactation Days 0, 5, 14, and 21, as was the dam weight for the 3.3 mg/kg group on lactation Day 21. The percentage of pups born alive and pup weights on Days 0, 5, 14, and 21 of lactation were significantly lower than control values for the 25 mg/kg group (Table C8). Pup survival was also significantly reduced by riddelliine administered at a dose of 25 mg/kg; by Day 14, 95% of control pups survived, whereas only 85% of the pups in the 25 mg/kg group survived.

Genetic Toxicity

Riddelliine (100 to 5000 µg/plate) was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of 30% induced rat and hamster liver S9 (Table D1); it was not mutagenic with 10% S9, and results of mutagenicity testing were negative in strains TA97, TA98, and TA1535 with and without S9 activation. A small, dose-related increase in mutant colonies was observed with strain TA97 in the presence of induced rat S9, but this response was judged to be equivocal. Riddelliine induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells with and without S9; the response observed in the presence of S9 was very strong (Table D2; 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 chromosomal aberrations in CHO cells in the presence of S9 at all three dose levels scored (Table D3; Galloway *et al.*, 1987). No increase in aberrations was observed in the absence of S9.

Riddelliine was tested in mice for induction of micronuclei in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) of peripheral blood and bone marrow. No increases in micronucleated NCEs or PCEs were observed in peripheral blood samples (Tables D4 and D5). However, a single-dose gavage micronucleus assay did show a slight but significant increase in micronucleated PCEs both in bone marrow and peripheral blood samples collected 24 and 48 hours, respectively, after treatment (Table D6). The doses used in the single-treatment study (up to 270 mg/kg) were higher than those used in the 4-week and 13-week studies (up to 25 mg/kg), and this may account for the difference in results between the chronic and the acute micronucleus assays.

Unscheduled DNA synthesis (UDS) and S-phase DNA synthesis were measured in cultured hepatocytes from F344/N rats and B6C3F$_1$ mice following 5 and 30 days of treatment by gavage with riddelliine. An increase in UDS, a measure of DNA damage and repair, was observed at both time points in at least one dose group each in male rats and in male and female mice (Tables D7 and D8). 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 dose group each 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 either 5 or 30 days, S-phase synthesis appeared inhibited (*i.e.*, less than control values) at the highest doses (10 and 25 mg/kg) of riddelliine. These data suggest that the hepatotoxicity of riddelliine may be due, in part, to its 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 the interpretation of the results.

PLATE 1

Liver from a male rat in the 10-mg/kg group in the 13-week gavage study of riddelliine. There is necrosis and loss of centrilobular hepatocytes (arrows); remaining periportal hepatocytes (asterisks) are enlarged and disorganized. H&E 82x.

PLATE 2

Liver from a female rat in the 10-mg/kg group in the 13-week gavage study of riddelliine, after a 14-week recovery period. There is hyperplasia of bile ductule cells (arrows), which have dissected and disrupted the hepatic cords. Note hepatocytes with increased amounts of cytoplasm and enlarged nuclei. H&E165x.

PLATE 3

Hepatocellular adenoma in the liver of a female rat in the 10mg/kg group in the 13-week gavage study of riddelliine. The neoplasm is well demarcated from surrounding parenchyma (arrows) and protrudes above the capsular surface. H&E 33x.

PLATE 4

Higher magnification of hepatocellular adenoma showing compressed hepatocytes (H) adjacent to more pleomorphic adenoma cells (A). H&E165x.

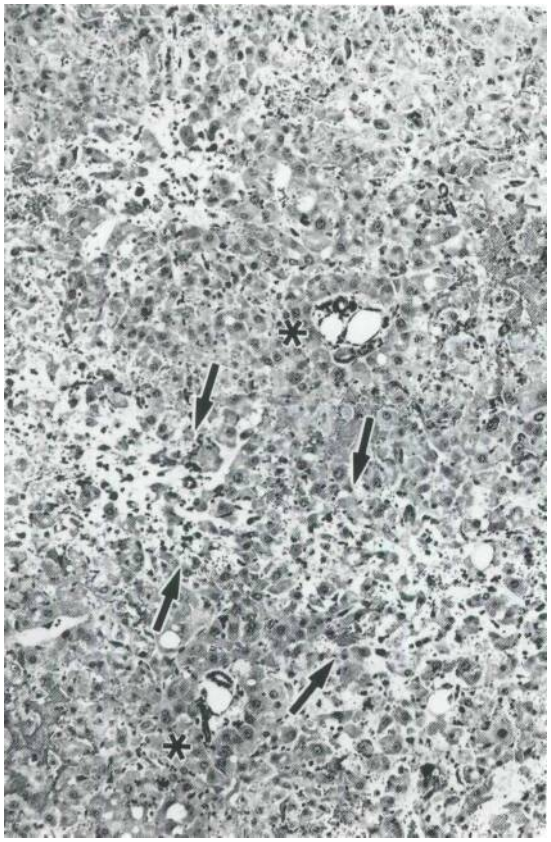

PLATE 1

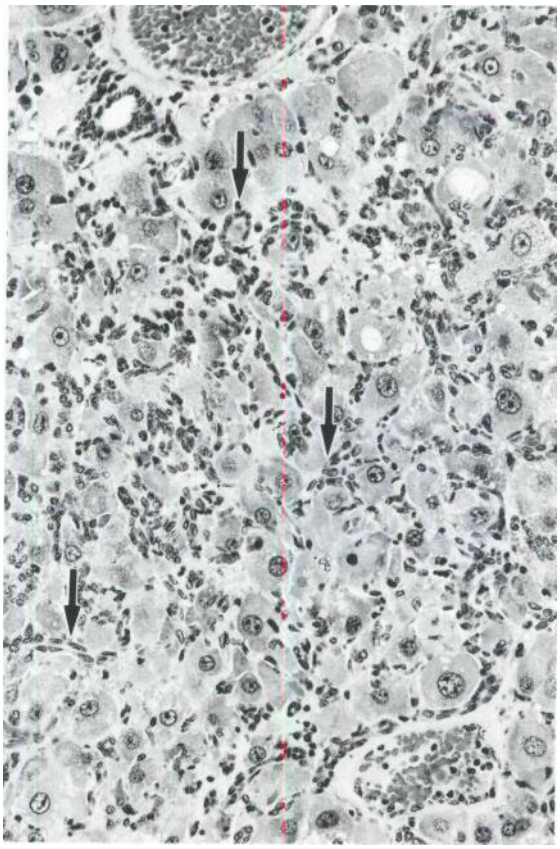

PLATE 2

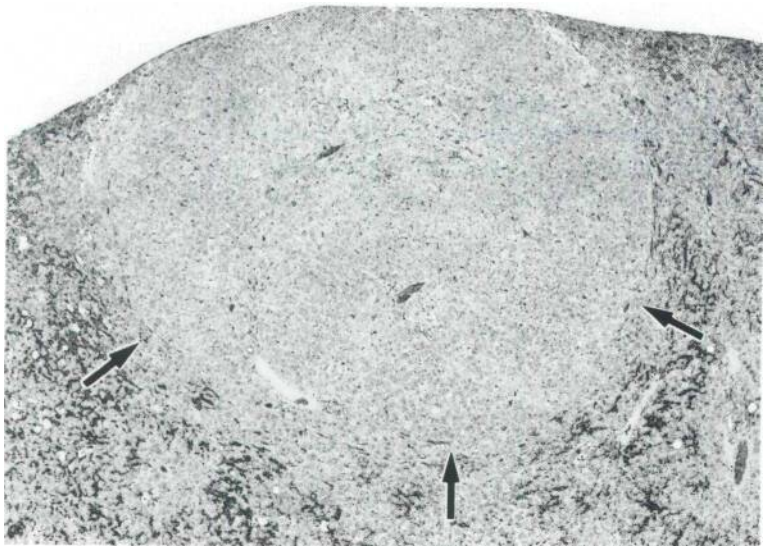

PLATE 3

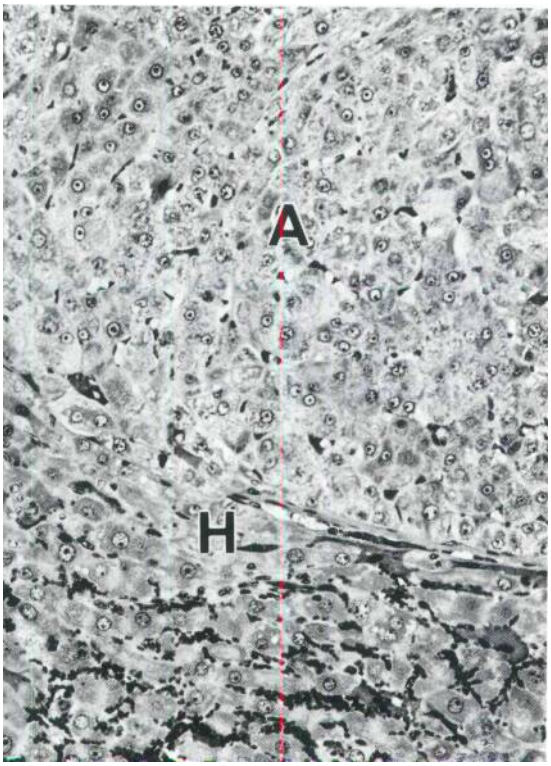

PLATE 4

PLATE 5

Kidney (top) and lung (bottom) from a male rat in the 10mg/kg group in the 13-week gavage study of riddelliine. Clusters of intraluminal cells (arrows) appear attached to the wall of interstitial vessels. H&E 165x.

PLATE 6

Electron micrograph of intravascular macrophages in the lung of a male rat in the 10mg/kg group of the 13-week gavage study of riddelliine. Macrophages are resting on the endothelium (E) of a pulmonary vessel; they have interdigitating plasma membranes and abundant cytoplasm containing lysosomal structures (arrows). 3750x.

PLATE 7

Centrilobular hepatocytes from a control female mouse (left) and a female mouse in the 25mg/kg group (right) in the 13-week gavage study of riddelliine, after a 7-week recovery period. Enlarged hepatocytes have increased amounts of granular cytoplasm (arrows). Oval nuclei of hyperplastic bile ductules (arrowheads) are interspersed between hepatocytes. H&E165x.

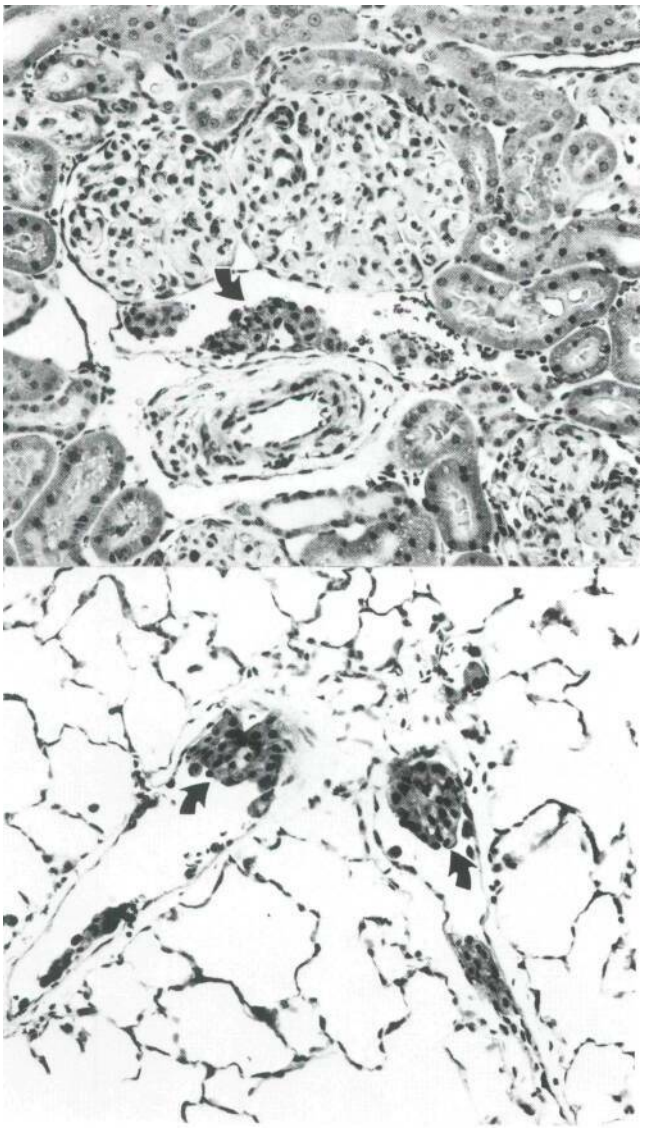

PLATE 5

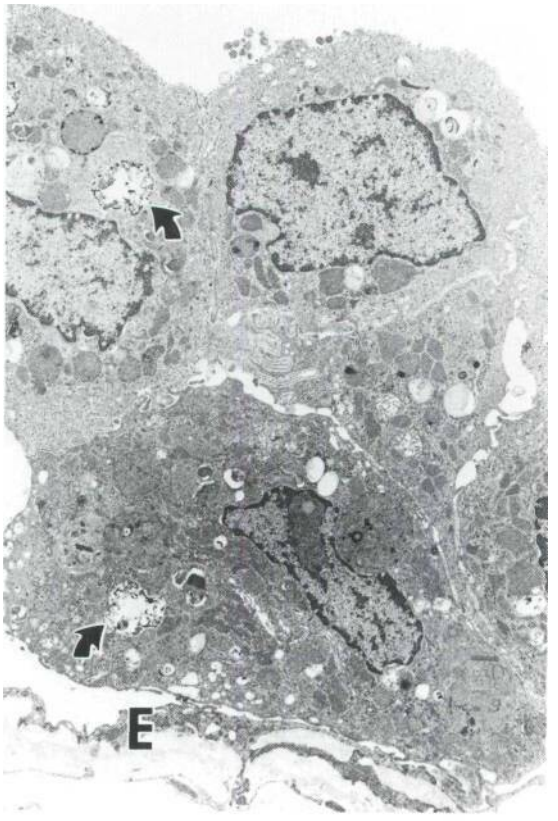

PLATE 6

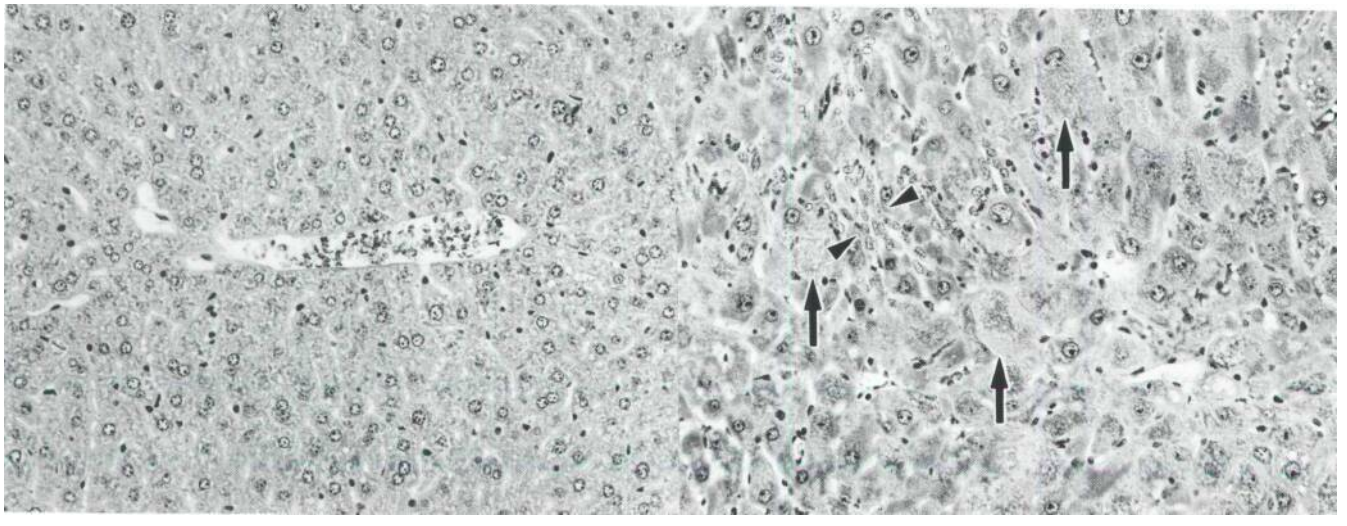

PLATE 7

DISCUSSION

The administration of riddelliine to rats resulted in deaths, lower body weight gains, and neoplastic and nonneoplastic pathologic changes. In the 2-week study, male rats were killed moribund or died after receiving 25 mg/kg riddelliine. In the 13-week study, 19 of 20male rats became moribund after receiving 10 mg/kg riddelliine for 2 months or longer. During the recovery period following 13 weeks of riddelliine administration, several females in the 10 mg/kg group died, but in other groups, signs of recovery were evident, and body weight gains of surviving dosed animals were similar to those of the controls.

The liver was the primary site for riddelliine toxicity in rats. Vascular lesions in the lung and kidney, consisting of accumulations of macrophages attached to blood vessel walls, were also observed in most high-dose rats after 13 weeks of riddelliine administration. There were additional lesions in the heart, spleen, kidney, and pancreas in the 13-week study. In both the 2-week and 13-week studies, female rats exhibited histopathologic changes that were similar to but less severe than those observed in male rats. Thus, female rats appeared to be less sensitive than males to the toxic effects of riddelliine.

In the 2-week study, hepatic hemorrhagic centrilobular necrosis (males) and cytologic alteration (males and females) were observed. In the 13-week study, hepatocyte cytomegaly and karyomegaly, cytoplasmic vacuolization, centrilobular necrosis, bile duct hyperplasia, and mixed inflammatory cell infiltration were the primary findings in rats. These observations are in accordance with the hepatotoxic effects of pyrrolizidine alkaloids (for reviews, see McLean, 1970, and WHO, 1988). In particular, hemorrhagic centrilobular necrosis and the appearance of markedly enlarged hepatocytes, referred to as "megalocytosis," are considered histologic hallmarks of pyrrolizidine toxicity. Another liver effect associated with toxicity by pyrrolizidine alkaloids, particularly in humans, is "veno-occlusive disease," characterized by fibrous scarring and occlusion of hepatic veins. Occlusive lesions, which are less readily reproduced in experimental animals than are necrotic and megalocytic effects, did not occur in the toxicity studies of riddelliine. This may be a reflection of dose and duration of exposure, as it has been observed that single large doses of alkaloids are more effective than repeated doses in producing hepatic lesions similar to veno-occlusive disease.

Hepatocellular adenomas were observed in two high-dose female rats after 13 weeks of riddelliine administration, and one high-dose female rat examined after the 14-week recovery period had an adenoma. No liver neoplasms have been observed in control female rats of similar age in previous NTP 13-week studies.

The changes in the serum chemistry parameters that occurred in rats in the 13-week study are consistent with the liver damage seen histologically. The increases in activities of alanine aminotransferase and sorbitol dehydrogenase indicate hepatocellular pathology, caused by a loss of cell membrane integrity or cellular necrosis with subsequent enzyme release. Both enzymes are liver specific and are primarily found in the cytosol of hepatocytes. Alkaline phosphatase is an enzyme found in biliary epithelium, and an increase in serum activity is consistent with damage to the biliary tree as occurs with inflammation, obstruction/constriction, proliferation, and/or neoplasia. The increase in alkaline phosphatase activity is consistent with the biliary hyperplasia seen histologically. The changes in the enzyme activities were pronounced in the high-dose male and female rats by Day 14 of exposure, indicating that liver damage occurred relatively early in the study.

An increase in liver weight was noted in male and female rats in the 2-week study and in female rats in the lower dose groups in the 13-week study. However, absolute liver weights were decreased or unchanged in dosed male rats and high-dose female rats in the 13-week study. There was a marked, dose-related enhancement of S-phase DNA replication in female rats after 5 days of treatment *in vivo*; the only group of males that showed comparable stimulation in DNA replication was the low-dose group. DNA replication, while still increased over controls, was lower at the 30-day time point than at 5days in all groups of dosed males and females. The DNA replication data (presumably representing cell replication) did not correspond directly with the changes in liver weight and indicated that the predominant effect of riddelliine at all doses was to stimulate cell replication in rats. However, the liver weight and S-phase DNA synthesis findings may not be inconsistent, in view of the previously reported antimitotic effect of pyrrolizidine alkaloids at higher doses or with prolonged exposure (Mattocks, 1986). Riddelliine apparently was toxic to the hepatocytes, causing DNA damage and necrosis, but at the same time it induced DNA synthesis. Megalocytosis development was the result of the antimitotic effect of riddelliine. Dosed male and female rats had increased lung weights

which persisted through the 14-week recovery period. Congestion, hemorrhage, and intravascular macrophage accumulation were observed, but no hypertrophy occurred.

Pyrrolizidine alkaloids have been reported to cause tumors in experimental animals (Mattocks, 1986). In the present studies, riddelliine-treated rats and mice were examined 7 weeks and 14 weeks after 13-week treatment with riddelliine to determine whether a possible release from an antimitotic effect would allow more lesions to develop during the recovery periods. In both male and female rats, karyomegaly, cytomegaly, and cytoplasmic vacuolization of hepatocytes persisted 14 weeks after cessation of riddelliine administration. Bile duct hyperplasia markedly increased in severity during the recovery period in female rats, and the incidences of foci of hepatocellular alteration and/or focal hepatocellular hyperplasia were increased in males in the 1.0 and 3.3 mg/kg groups and in females in the high-dose (10 mg/kg) group. One high-dose female examined during the 14-week recovery period had a hepatocellular adenoma. Thus, for some lesions, there was evidence of further development after dosing was stopped, and this included potentially preneoplastic hepatocellular foci. However, it was not possible to discern an effect on the tumor incidence because it was so low, and the sample size was small.

Cardiopulmonary effects of pyrrolizidine alkaloids have been well established (Mattocks and Driver, 1983; Mattocks, 1986; WHO, 1988). Monocrotaline is the most active and widely studied of the pneumotoxic alkaloids and has been shown to cause pulmonary endothelial damage with associated platelet sequestration and pulmonary vascular inflammation, thrombosis, and hypertrophy; these changes presumably lead to increased pulmonary arterial pressure (pulmonary hypertension) and resultant right ventricular hypertrophy (cor pulmonale). Similar effects were not conclusively shown in the current studies with riddelliine. Morphologic changes in the lungs suggestive of endothelial damage were limited to perivascular edema and hemorrhage in the 2-week study in rats; these changes were not reproduced in the 13-week studies. Although some increases in lung weights were observed, no consistent corresponding increases in heart weights suggestive of cor pulmonale were found. The absence of significant cardiopulmonary effects by riddelliine is perhaps not unexpected, given the fact that pyrrolizidine alkaloids may vary considerably in their relative degree of pneumotoxicity. However, the accumulation of intravascular macrophages in the riddelliine-treated rats suggests the possible development of cardiopulmonary effects if riddelliine were administered for more than 13 weeks.

In a review of the literature concerning the toxicity of pyrrolizidine alkaloids (McLean, 1970), the frequent finding of cellular aggregates within vessels of the liver and lung has been noted. These accumulations have sometimes been assumed to be proliferative endothelial cells, although in other studies they were interpreted to be macrophages. In the current studies of riddelliine, intravascular cells found in the kidney, lung, and liver were identified as macrophages by demonstration of erythrophagocytosis, hemosiderosis, and ultrastructural features. The pathogenesis of these macrophage accumulations is unknown. The cells appeared to adhere to the underlying endothelium, suggesting that they are a consequence of alkaloid-induced endothelial damage. Because platelets are known to interact with damaged endothelium, some supporting evidence for riddelliine-associated endothelial toxicity may be in the hematologic finding of thrombocytopenia. A marked thrombocytopenia occurred in high-dose male rats at all time points (Days 14 and 30 and Week 13); thrombocytopenia occurred later in the female rats and was observed in high-dose animals at Week 13. Endothelial damage and platelet aggregation may be expected to result in a hypercoagulable state and widespread thrombosis, a finding that was not seen in the riddelliine studies. However, as has been demonstrated with monocrotaline pyrrole (Schultze *etal.*, 1991), exposure to toxic metabolites of pyrrolizidine alkaloids does not necessarily result in systemic hypercoagulability and disseminated thrombosis. Sequestration of platelets in the lungs occurs following treatment with monocrotaline pyrrole (White and Roth, 1988) and is presumably involved in maintenance of vessel wall integrity; such interaction with altered endothelium without overt thrombosis could be postulated for riddelliine-treated rats, contributing to the observed thrombocytopenia.

Changes in erythrocyte parameters indicated a regenerative anemia with a concomitant dehydration. At Day 14, increases in erythrocyte count, hematocrit, and hemoglobin concentration in animals in the 1.0 and 10 mg/kg groups were consistent with hemoconcentration, suggesting dehydration. At Day 30, these parameters were decreased in male and female rats treated with 10 mg/kg and were consistent with an anemia. By Week 13, erythrocyte count, hematocrit, and hemoglobin concentration were elevated, again indicating hemoconcentration, which can mask the presence and severity of existing anemia. Increased reticulocyte counts were consistent with a regenerative response to an anemia. The reticulocyte response occurred by Day 14 in high-dose male rats and increased in magnitude through Week 13; occurrence of the regenerative response was not observed until Week 13 in female rats. This hematologic finding was supported

histologically by extramedullary hematopoiesis in the spleen and bone marrow hyperplasia in the high-dose male and female rats. An additional microscopic finding which indicated possible red blood cell damage and increased turnover was erythrophagocytosis in lymph nodes and by intravascular macrophages. However, the lack of an associated hemosiderosis does not support a hemolytic process, and the cause of regenerative anemia in riddelliine-treated rats is unclear.

In general, mice appeared to be less sensitive than rats to the toxicity of riddelliine. Administration of up to 25 mg/kg for 13 weeks did not affect survival. At dose levels of 10 and 25 mg/kg, riddelliine decreased body weight gains of male and female mice. Body weights did not return to normal during the 7-week and 14-week recovery periods.

In dosed male and female mice, the relative weights of lung and spleen were elevated and these changes persisted 14 weeks after cessation of riddelliine administration. However, no corresponding microscopic lesions were found. Liver effects of riddelliine in mice were less severe than those in rats, being limited to relatively mild enlargement of centrilobular hepatocytes in both sexes and bile duct hyperplasia in females during the recovery period. Lesions of the forestomach in mice were likely due to a direct irritant effect of riddelliine as a result of gavage exposure.

In the rat reproductive toxicity evaluations, spermatozoal measurements in males receiving 3.3mg/kg were not affected even though decreased right epididymal and right testis weights were observed. At 10 mg/kg (a dose that was lethal to the males), riddelliine caused a persistent estrus in females. However, there were no effects on fertility, length of gestation, weight gain of the dams during gestation, litter size, or percentage of live pups in the mating trials, in which 1.0 mg/kg was the high dose. At 14 and 21 days of age, suckling female pups had lower body weights than control pups. However, the body weights of suckling male pups were similar to those of the controls at these times. PAs have been detected in milk of lactating rats (Schoental, 1959, Candrian *etal.*, 1984) and cows (Dickinson *etal.*, 1976).

In the reproductive toxicity studies in mice, riddelliine did not affect male reproductive performance; however, females in the 25 mg/kg group showed a marked prolongation of estrous cycle length. Despite this, dosed dams were able to conceive and progress with pregnancy. Gestation length was normal, but litter size was reduced, and pups of treated

dams had lower body weights at birth than control pups. The body weights of pups in the high-dose group never equaled those of the controls during the 21 days of suckling. Apparently, riddelliine at high doses (25.0mg/kg) was able to pass through the placenta and affect the development of the fetuses.

Green and Christie (1961) reported a dose-related increase in the incidence of fetal abnormalities in rat pups after a single intraperitoneal injection of 15 to 300 mg/kg of heliotrine to pregnant rats during the second week of gestation. Abnormalities were seen only in litters of dams exposed to 50 mg/kg or higher. Dose-related fetal resorptions were also reported by Persaud and Hoyte (1974) in pregnant rats administered fulvine. In the current studies with riddelliine, there was no increase seen in resorptions (although litter size was reduced and the number of live births decreased for mice) or in gross malformations. The pups were not evaluated microscopically.

Riddelliine was mutagenic in *Salmonella typhimurium* strain TA100 with S9, but results were negative in strains TA97, TA98, and TA1535. It induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. The results indicated that riddelliine, like other PAs, requires metabolic activation.

Riddelliine also induced unscheduled DNA synthesis (UDS) in hepatocytes of rats and mice treated *in vivo*. A dose-related positive trend (up to 25 mg/kg) for UDS was observed in the mouse hepatocyte cultures at the 5-day and 30-day time points. No neoplastic changes were observed in the mice in these studies at these doses. A dose-related positive trend (up to 3.3 mg/kg) was observed for unscheduled DNA synthesis in hepatocytes from female rats treated for 5 days but not 30 days, and hepatocellular adenomas were found in three females in the 10mg/kg group. The usefulness of unscheduled DNA synthesis as a marker for predicting the carcinogenicity of riddelliine is difficult to determine from these data due to the lack of a 10 mg/kg group in the UDS studies and the short duration of exposure to riddelliine (13 weeks), which may have been insufficient for a neoplastic response to develop in the mice or in the male rats.

The failure of riddelliine, which was clastogenic *in vitro*, to induce micronuclei in mouse erythrocytes after 4 or 13 weeks of administration may be because the activated metabolite of riddelliine, the reactive pyrrole produced in the liver, was short lived and was removed by binding to the endothelial cells in the heart and lung before reaching the bone marrow.

As a result, very little may have reached the bone marrow; only a weakly positive response was seen in peripheral blood and bone marrow of male mice administered a single high dose of riddelliine. This explanation is supported by results of studies with dehydromonocrotaline and dehydroretrorsine. These dehydroalkaloids produced local reactions without liver or lung lesions when given intraperitoneally. When administered intravenously, they appeared to react with macromolecules of the first organ reached (Culvenor *etal.*, 1969; Butler *etal.*, 1970). Dehydromonocrotaline (Hooson and Grasso, 1976) or dehydroheliotridine (Allen *etal.*, 1975) given subcutaneously to rats induced sarcomas at the site of injection.

In summary, the administration of riddelliine to rodents by gavage for up to 13 weeks resulted in a spectrum of neoplastic and nonneoplastic effects similar to those previously described for other pyrrolizidine alkaloids. Rats were found to be somewhat more sensitive than mice, and males more sensitive than females, to the toxic effects of riddelliine. The no-observed-adverse-effect level (NOAEL) for histopathologic changes in the 13-week studies was 3.3 mg/kg body weight for mice and 0.1 mg/kg body weight for rats. The liver was the primary target of riddelliine-induced injury that resulted in lesions characterized by cytomegaly and cytologic alteration in rats and mice and also by marked necrotic and proliferative changes in rats. Riddelliine is carcinogenic to female F344/N rats, based on the occurrence of hepatocellular adenomas.

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APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

	Vehicle Control	0.1mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
MALE						
13-Week Base Study						
n	10	10	10	10	10	1[2]
Necropsy body wt	365 ± 5	351 ± 7	340 ± 9*	336 ± 5**	310 ± 8**	201
Brain						
Absolute	2.018 ± 0.025	1.978 ± 0.018	1.956 ± 0.025	1.869 ± 0.098	1.957 ± 0.016	1.830
Relative	5.54 ± 0.07	5.66 ± 0.10	5.78 ± 0.17	5.57 ± 0.30	6.34 ± 0.15**	9.10
Heart						
Absolute	1.110 ± 0.024	1.049 ± 0.023	1.010 ± 0.030**	1.016 ± 0.022**	0.884 ± 0.019**	0.755
Relative	3.04 ± 0.05	2.99 ± 0.04	2.97 ± 0.05	3.02 ± 0.04	2.86 ± 0.05**	3.75
Right kidney						
Absolute	1.227 ± 0.025	1.190 ± 0.029	1.166 ± 0.045	1.191 ± 0.024	1.067 ± 0.026**	1.190
Relative	3.36 ± 0.04	3.39 ± 0.04	3.42 ± 0.06	3.54 ± 0.04	3.45 ± 0.11	5.92
Liver						
Absolute	14.799 ± 0.247	14.025 ± 0.426	14.420 ± 0.634	14.002 ± 0.376	11.431 ± 0.470**	4.380
Relative	40.59 ± 0.51	39.94 ± 0.60	42.26 ± 1.03	41.58 ± 0.62	36.75 ± 0.94**	21.78
Lungs						
Absolute	1.799 ± 0.065	1.845 ± 0.084	1.761 ± 0.095	1.870 ± 0.058	2.243 ± 0.125**	1.530
Relative	4.93 ± 0.17	5.25 ± 0.19	5.15 ± 0.17	5.56 ± 0.16	7.21 ± 0.33**	7.61
Spleen						
Absolute	0.860 ± 0.016	0.851 ± 0.020	0.880 ± 0.022	0.936 ± 0.019*	1.062 ± 0.033**	1.330
Relative	2.36 ± 0.04	2.43 ± 0.02	2.59 ± 0.05	2.78 ± 0.04**	3.46 ± 0.20**	6.61
Right testis						
Absolute	1.519 ± 0.018	1.478 ± 0.027	1.383 ± 0.035*	1.466 ± 0.023*	1.404 ± 0.040*	0.152
Relative	4.17 ± 0.03	4.22 ± 0.04	4.07 ± 0.06	4.36 ± 0.06*	4.53 ± 0.08**	0.76
Thymus						
Absolute	0.308 ± 0.017	0.311 ± 0.012	0.319 ± 0.019	0.283 ± 0.011	0.284 ± 0.006	0.032
Relative	0.84 ± 0.04	0.89 ± 0.04	0.94 ± 0.05	0.84 ± 0.04	0.92 ± 0.03	0.16
Left ventricle						
Absolute	0.477 ± 0.025	0.510 ± 0.016	0.486 ± 0.025	0.470 ± 0.016	0.428 ± 0.012	0.328
Relative	1.31 ± 0.06	1.46 ± 0.05	1.42 ± 0.05	1.40 ± 0.04	1.38 ± 0.02	1.63
Right ventricle						
Absolute	0.154 ± 0.007	0.147 ± 0.004	0.139 ± 0.006	0.142 ± 0.004	0.145 ± 0.010	0.094
Relative	0.42 ± 0.02	0.42 ± 0.01	0.41 ± 0.02	0.42 ± 0.01	0.47 ± 0.04	0.47

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

2 No standard error calculated due to insufficient number of animals.

3 No data are available for male rats in the 10.0 mg/kg group.

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

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

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg
MALE (continued)					
7-Week Recovery[3]					
n	5	5	5	5	5
Necropsy body wt	420 ± 7	411 ± 10	388 ± 11*	379 ± 11*	378 ± 10**
Brain					
Absolute	2.002 ± 0.026	2.096 ± 0.025	2.036 ± 0.039	2.020 ± 0.036	2.002 ± 0.019
Relative	4.78 ± 0.08	5.11 ± 0.09	5.27 ± 0.23*	5.34 ± 0.13*	5.32 ± 0.18*
Heart					
Absolute	1.145 ± 0.017	1.264 ± 0.059	1.161 ± 0.025	1.111 ± 0.036	1.098 ± 0.021
Relative	2.73 ± 0.02	3.08 ± 0.12*	3.00 ± 0.06	2.94 ± 0.06	2.92 ± 0.09
Right kidney					
Absolute	1.480 ± 0.031	1.546 ± 0.049	1.440 ± 0.038	1.410 ± 0.040	1.442 ± 0.036
Relative	3.53 ± 0.10	3.77 ± 0.11	3.72 ± 0.13	3.72 ± 0.04	3.84 ± 0.18
Liver					
Absolute	15.622 ± 0.103	15.906 ± 0.365	15.308 ± 0.281	15.238 ± 0.275	12.898 ± 0.614**
Relative	37.27 ± 0.51	38.73 ± 0.50	39.55 ± 1.06	40.28 ± 0.55*	34.13 ± 1.13*
Lungs					
Absolute	1.608 ± 0.074	1.634 ± 0.083	1.600 ± 0.090	1.522 ± 0.038	1.616 ± 0.086
Relative	3.83 ± 0.13	3.97 ± 0.12	4.12 ± 0.17	4.03 ± 0.12	4.28 ± 0.19
Spleen					
Absolute	0.938 ± 0.022	0.956 ± 0.024	0.998 ± 0.042	0.988 ± 0.051	1.096 ± 0.074*
Relative	2.24 ± 0.04	2.33 ± 0.02	2.57 ± 0.04	2.61 ± 0.13	2.93 ± 0.28**
Right testis					
Absolute	1.500 ± 0.026	1.459 ± 0.156	1.517 ± 0.041	1.457 ± 0.021	1.335 ± 0.042
Relative	3.58 ± 0.06	3.53 ± 0.33	3.92 ± 0.10	3.86 ± 0.09	3.54 ± 0.05
Thymus					
Absolute	0.231 ± 0.015	0.204 ± 0.018	0.174 ± 0.025	0.200 ± 0.005	0.185 ± 0.013
Relative	0.55 ± 0.04	0.50 ± 0.04	0.44 ± 0.05	0.53 ± 0.02	0.49 ± 0.04
Left ventricle					
Absolute	0.526 ± 0.023	0.563 ± 0.018	0.506 ± 0.015	0.496 ± 0.031	0.493 ± 0.025
Relative	1.26 ± 0.07	1.37 ± 0.04	1.31 ± 0.05	1.31 ± 0.06	1.31 ± 0.07
Right ventricle					
Absolute	0.155 ± 0.007	0.148 ± 0.013	0.158 ± 0.007	0.158 ± 0.010	0.144 ± 0.003
Relative	0.37 ± 0.01	0.36 ± 0.02	0.41 ± 0.02	0.42 ± 0.03	0.38 ± 0.02

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg
MALE (continued)					
14-Week Recovery[3]					
n	5	5	5	5	5
Necropsy body wt	419 ± 20	432 ± 21	445 ± 11	417 ± 11	400 ± 7
Brain					
Absolute	2.032 ± 0.054	2.094 ± 0.029	2.030 ± 0.058	2.026 ± 0.045	1.998 ± 0.106
Relative	4.87 ± 0.16	4.90 ± 0.30	4.56 ± 0.11	4.87 ± 0.10	4.99 ± 0.25
Heart					
Absolute	1.198 ± 0.059	1.258 ± 0.036	1.316 ± 0.032	1.211 ± 0.028	1.241 ± 0.048
Relative	2.86 ± 0.06	2.92 ± 0.08	2.96 ± 0.08	2.91 ± 0.06	3.10 ± 0.13
Right kidney					
Absolute	1.534 ± 0.075	1.594 ± 0.062	1.642 ± 0.044	1.524 ± 0.104	1.634 ± 0.065
Relative	3.66 ± 0.04	3.70 ± 0.12	3.69 ± 0.09	3.65 ± 0.18	4.08 ± 0.16
Liver					
Absolute	16.992 ± 0.810	18.046 ± 1.119	19.120 ± 0.614	15.918 ± 0.784	14.446 ± 0.713
Relative	40.54 ± 0.38	41.79 ± 2.01	42.95 ± 0.92	38.13 ± 0.98	36.05 ± 1.38*
Lungs					
Absolute	1.656 ± 0.065	1.830 ± 0.089	1.920 ± 0.111	1.676 ± 0.048	2.026 ± 0.264
Relative	3.97 ± 0.14	4.24 ± 0.10	4.30 ± 0.16	4.04 ± 0.18	5.05 ± 0.63
Spleen					
Absolute	1.002 ± 0.060	0.988 ± 0.063	1.020 ± 0.041	0.982 ± 0.030	1.072 ± 0.032
Relative	2.40 ± 0.13	2.28 ± 0.05	2.29 ± 0.05	2.36 ± 0.05	2.68 ± 0.05*
Right testis					
Absolute	1.547 ± 0.048	1.558 ± 0.025	1.582 ± 0.059	1.559 ± 0.042	1.486 ± 0.040
Relative	3.70 ± 0.07	3.63 ± 0.15	3.55 ± 0.07	3.74 ± 0.05	3.71 ± 0.09
Thymus					
Absolute	0.220 ± 0.023	0.227 ± 0.051	0.256 ± 0.029	0.202 ± 0.041	0.228 ± 0.041
Relative	0.53 ± 0.06	0.52 ± 0.10	0.57 ± 0.06	0.48 ± 0.09	0.57 ± 0.10
Left ventricle					
Absolute	0.391 ± 0.015	0.373 ± 0.023	0.372 ± 0.026	0.367 ± 0.018	0.371 ± 0.052
Relative	0.93 ± 0.02	0.87 ± 0.06	0.83 ± 0.05	0.88 ± 0.03	0.93 ± 0.14
Right ventricle					
Absolute	0.142 ± 0.008	0.144 ± 0.004	0.161 ± 0.016	0.139 ± 0.007	0.152 ± 0.010
Relative	0.34 ± 0.03	0.34 ± 0.02	0.36 ± 0.04	0.33 ± 0.02	0.38 ± 0.03

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
FEMALE						
13-Week Base Study						
n	10	10	10	10	10	10
Necropsy body wt	211 ± 5	207 ± 4	205 ± 3	199 ± 4*	196 ± 3**	193 ± 3**
Brain						
Absolute	1.790 ± 0.024	1.823 ± 0.019	1.840 ± 0.017	1.818 ± 0.019	1.864 ± 0.023*	1.844 ± 0.017*
Relative	8.51 ± 0.14	8.82 ± 0.21	9.01 ± 0.17*	9.17 ± 0.13**	9.54 ± 0.15**	9.56 ± 0.16**
Heart						
Absolute	0.681 ± 0.017	0.697 ± 0.013	0.715 ± 0.012	0.696 ± 0.013	0.729 ± 0.034	0.695 ± 0.015
Relative	3.23 ± 0.05	3.37 ± 0.06	3.50 ± 0.09	3.51 ± 0.06	3.73 ± 0.19**	3.59 ± 0.05**
Right kidney						
Absolute	0.668 ± 0.024	0.715 ± 0.018	0.752 ± 0.017	0.693 ± 0.013	0.723 ± 0.017	0.915 ± 0.022**
Relative	3.16 ± 0.06	3.45 ± 0.06**	3.67 ± 0.05**	3.49 ± 0.04**	3.69 ± 0.07**	4.73 ± 0.08**
Liver						
Absolute	6.835 ± 0.162	7.499 ± 0.216	7.212 ± 0.165	7.143 ± 0.198	7.631 ± 0.174*	6.368 ± 0.307
Relative	32.42 ± 0.28	36.31 ± 1.37*	35.23 ± 0.64*	35.91 ± 0.54*	39.01 ± 0.73*	32.96 ± 1.51*
Lungs						
Absolute	1.152 ± 0.032	1.201 ± 0.050	1.192 ± 0.042	1.245 ± 0.065	1.646 ± 0.134**	1.680 ± 0.112**
Relative	5.46 ± 0.08	5.81 ± 0.24	5.82 ± 0.18	6.25 ± 0.24	8.38 ± 0.62**	8.68 ± 0.53**
Spleen						
Absolute	0.516 ± 0.015	0.559 ± 0.021	0.520 ± 0.010	0.535 ± 0.018	0.623 ± 0.007**	1.353 ± 0.035**
Relative	2.46 ± 0.09	2.71 ± 0.12	2.55 ± 0.07	2.69 ± 0.08	3.19 ± 0.06**	7.00 ± 0.14**
Thymus						
Absolute	0.249 ± 0.006	0.244 ± 0.009	0.252 ± 0.010	0.230 ± 0.011	0.212 ± 0.012*	0.189 ± 0.014**
Relative	1.18 ± 0.03	1.18 ± 0.04	1.24 ± 0.05	1.15 ± 0.04	1.09 ± 0.06	0.98 ± 0.07**
Left ventricle						
Absolute	0.371 ± 0.015	0.353 ± 0.008	0.375 ± 0.009	0.357 ± 0.012	0.315 ± 0.007*	0.348 ± 0.018*
Relative	1.76 ± 0.06	1.71 ± 0.05	1.83 ± 0.03	1.80 ± 0.06	1.61 ± 0.04	1.79 ± 0.08
Right ventricle						
Absolute	0.109 ± 0.006	0.101 ± 0.009	0.104 ± 0.005	0.102 ± 0.003	0.083 ± 0.005*	0.121 ± 0.009
Relative	0.51 ± 0.02	0.48 ± 0.04	0.51 ± 0.03	0.51 ± 0.02	0.42 ± 0.02	0.62 ± 0.04*

TABLE A1 Organ
Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats
in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
FEMALE (continued)						
7-Week Recovery						
n	5	5	5	5	5	4
Necropsy body wt	236 ± 9	238 ± 13	235 ± 7	233 ± 6	209 ± 3	228 ± 3
Brain						
Absolute	1.842 ± 0.032	1.886 ± 0.019	1.838 ± 0.015	1.850 ± 0.014	1.856 ± 0.014	1.893 ± 0.046
Relative	7.84 ± 0.30	8.01 ± 0.39	7.85 ± 0.24	7.97 ± 0.17	8.89 ± 0.17*	8.32 ± 0.19
Heart						
Absolute	0.534 ± 0.010	0.511 ± 0.025	0.504 ± 0.008	0.499 ± 0.019	0.427 ± 0.013**	0.525 ± 0.014*
Relative	2.28 ± 0.10	2.15 ± 0.02	2.15 ± 0.07	2.14 ± 0.04	2.05 ± 0.06	2.30 ± 0.06
Right kidney						
Absolute	0.882 ± 0.023	0.868 ± 0.053	0.922 ± 0.034	0.886 ± 0.025	0.820 ± 0.015	1.205 ± 0.037**
Relative	3.75 ± 0.17	3.65 ± 0.11	3.92 ± 0.07	3.81 ± 0.02	3.93 ± 0.11	5.29 ± 0.12**
Liver						
Absolute	7.862 ± 0.267	7.532 ± 0.441	8.598 ± 0.427	8.856 ± 0.299	6.998 ± 0.279	5.995 ± 0.385**
Relative	33.37 ± 1.15	31.65 ± 0.27	36.59 ± 1.29	38.05 ± 0.50*	33.57 ± 1.68	26.39 ± 1.92**
Lungs						
Absolute	1.202 ± 0.059	1.158 ± 0.026	1.174 ± 0.025	1.308 ± 0.112	1.168 ± 0.034	1.418 ± 0.078
Relative	5.08 ± 0.11	4.90 ± 0.17	5.01 ± 0.13	5.60 ± 0.34	5.59 ± 0.13	6.23 ± 0.34**
Spleen						
Absolute	0.592 ± 0.009	0.598 ± 0.036	0.598 ± 0.010	0.598 ± 0.017	0.584 ± 0.014	1.683 ± 0.177**
Relative	2.52 ± 0.07	2.52 ± 0.11	2.55 ± 0.07	2.57 ± 0.04	2.79 ± 0.03	7.37 ± 0.71**
Thymus						
Absolute	0.189 ± 0.008	0.173 ± 0.019	0.187 ± 0.004	0.177 ± 0.006	0.110 ± 0.008**	0.075 ± 0.013**
Relative	0.80 ± 0.02	0.75 ± 0.11	0.80 ± 0.03	0.76 ± 0.02	0.53 ± 0.04**	0.33 ± 0.06**
Left ventricle						
Absolute	0.336 ± 0.013	0.315 ± 0.015	0.301 ± 0.014	0.350 ± 0.026	0.291 ± 0.030	0.377 ± 0.018
Relative	1.43 ± 0.04	1.34 ± 0.08	1.29 ± 0.08	1.50 ± 0.08	1.40 ± 0.14	1.65 ± 0.06
Right ventricle						
Absolute	0.096 ± 0.005	0.090 ± 0.006	0.114 ± 0.008	0.109 ± 0.007	0.087 ± 0.003	0.118 ± 0.011
Relative	0.41 ± 0.02	0.38 ± 0.01	0.49 ± 0.03	0.47 ± 0.04	0.42 ± 0.02	0.52 ± 0.04*

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
FEMALE (continued)						
14-Week Recovery						
n	5		5	5	5	12
Necropsy body wt	255 ± 7		245 ± 8	249 ± 6	231 ± 6*	217 ± 9**	257
Brain						
Absolute	1.914 ± 0.020		1.826 ± 0.037	1.880 ± 0.031	1.888 ± 0.037	1.860 ± 0.035	1.970
Relative	7.53 ± 0.18		7.46 ± 0.16	7.56 ± 0.12	8.18 ± 0.24*	8.61 ± 0.28**	7.66
Heart						
Absolute	0.828 ± 0.020		0.773 ± 0.025	0.826 ± 0.010	0.768 ± 0.008	0.786 ± 0.041	1.122
Relative	3.26 ± 0.11		3.15 ± 0.02	3.33 ± 0.06	3.33 ± 0.08	3.62 ± 0.12**	4.36
Right kidney						
Absolute	0.994 ± 0.021		0.978 ± 0.035	0.986 ± 0.027	0.900 ± 0.016*	0.888 ± 0.035*	1.330
Relative	3.91 ± 0.07		4.00 ± 0.16	3.96 ± 0.04	3.90 ± 0.11	4.10 ± 0.10	5.17
Liver						
Absolute	9.460 ± 0.160		9.048 ± 0.357	9.912 ± 0.327	9.042 ± 0.343	8.270 ± 0.480*	7.330
Relative	37.20 ± 0.67		36.88 ± 0.59	39.91 ± 1.51	39.07 ± 1.10	38.07 ± 1.55	28.49
Lungs						
Absolute	1.270 ± 0.031		1.212 ± 0.065	1.196 ± 0.031	1.138 ± 0.046	1.168 ± 0.041	1.460
Relative	5.00 ± 0.14		4.93 ± 0.15	4.82 ± 0.17	4.93 ± 0.23	5.40 ± 0.22	5.67
Spleen						
Absolute	0.596 ± 0.024		0.658 ± 0.021	0.636 ± 0.020	0.602 ± 0.007	0.636 ± 0.037	1.950
Relative	2.35 ± 0.11		2.69 ± 0.08*	2.55 ± 0.03	2.61 ± 0.09	2.93 ± 0.12**	7.58
Thymus						
Absolute	0.182 ± 0.014		0.191 ± 0.006	0.182 ± 0.015	0.165 ± 0.014	0.149 ± 0.010	0.149
Relative	0.71 ± 0.04		0.78 ± 0.03	0.74 ± 0.07	0.71 ± 0.06	0.69 ± 0.03	0.58
Left ventricle						
Absolute	0.266 ± 0.016		0.248 ± 0.013	0.256 ± 0.013	0.264 ± 0.010	0.254 ± 0.010	0.379
Relative	1.05 ± 0.05		1.01 ± 0.04	1.03 ± 0.06	1.14 ± 0.05	1.17 ± 0.04	1.47
Right ventricle						
Absolute	0.112 ± 0.003		0.116 ± 0.005	0.118 ± 0.004	0.103 ± 0.005	0.112 ± 0.008	0.168
Relative	0.44 ± 0.02		0.47 ± 0.02	0.48 ± 0.02	0.45 ± 0.03	0.51 ± 0.02*	0.65

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine[1]

	Vehicle Control	0.33mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
MALE						
13-Week Base Study						
n	10	10	10	10	10	10
Necropsy body wt	33.6 ± 1.9	36.3 ± 1.0	35.2 ± 1.0	34.1 ± 1.2	31.3 ± 0.6	28.6 ± 0.4**
Brain						
Absolute	0.456 ± 0.007	0.462 ± 0.010	0.461 ± 0.007	0.470 ± 0.005	0.472 ± 0.004	0.466 ± 0.006
Relative	13.94 ± 0.75	12.81 ± 0.42	13.18 ± 0.36	13.92 ± 0.47	15.13 ± 0.26	16.30 ± 0.21**
Heart						
Absolute	0.178 ± 0.008	0.171 ± 0.006	0.174 ± 0.010	0.183 ± 0.008	0.157 ± 0.004*	0.140 ± 0.004**
Relative	5.40 ± 0.25	4.75 ± 0.20	4.99 ± 0.31	5.36 ± 0.17	5.02 ± 0.16	4.90 ± 0.10
Right kidney						
Absolute	0.304 ± 0.013	0.291 ± 0.010	0.303 ± 0.004	0.291 ± 0.011	0.275 ± 0.007*	0.250 ± 0.007**
Relative	9.15 ± 0.29	8.03 ± 0.21**	8.67 ± 0.25	8.56 ± 0.25	8.81 ± 0.25	8.74 ± 0.21
Liver						
Absolute	1.762 ± 0.067	1.621 ± 0.044	1.995 ± 0.042	1.690 ± 0.038	1.552 ± 0.031**	1.612 ± 0.036**
Relative	53.19 ± 1.99	44.72 ± 0.74	56.96 ± 1.35	49.80 ± 0.96	49.67 ± 0.81	56.44 ± 1.49
Lungs						
Absolute	0.343 ± 0.020	0.387 ± 0.024	0.310 ± 0.013	0.371 ± 0.016	0.397 ± 0.011*	0.428 ± 0.013**
Relative	10.35 ± 0.63	10.72 ± 0.71	8.82 ± 0.32	10.93 ± 0.38	12.72 ± 0.36**	15.00 ± 0.56**
Spleen						
Absolute	0.081 ± 0.004	0.076 ± 0.004	0.075 ± 0.002	0.080 ± 0.004	0.084 ± 0.002	0.106 ± 0.005**
Relative	2.45 ± 0.13	2.10 ± 0.11	2.14 ± 0.08	2.36 ± 0.13	2.69 ± 0.08	3.70 ± 0.16**
Right testis						
Absolute	0.122 ± 0.003	0.123 ± 0.003	0.127 ± 0.003	0.127 ± 0.003	0.123 ± 0.005	0.118 ± 0.002
Relative	3.71 ± 0.19	3.42 ± 0.11	3.64 ± 0.11	3.75 ± 0.08	3.92 ± 0.17	4.14 ± 0.05*
Thymus						
Absolute	0.034 ± 0.002	0.036 ± 0.002	0.038 ± 0.003	0.038 ± 0.002	0.037 ± 0.001	0.033 ± 0.002
Relative	1.03 ± 0.07	1.00 ± 0.04	1.07 ± 0.06	1.12 ± 0.04	1.18 ± 0.03	1.15 ± 0.09
Left ventricle						
Absolute	0.071 ± 0.003	0.073 ± 0.002	0.056 ± 0.002	0.074 ± 0.002	0.066 ± 0.002	0.049 ± 0.002**
Relative	2.15 ± 0.10	2.02 ± 0.07	1.62 ± 0.09	2.18 ± 0.09	2.12 ± 0.09	1.71 ± 0.05**
Right ventricle						
Absolute	0.025 ± 0.001	0.025 ± 0.001	0.022 ± 0.001	0.023 ± 0.001	0.021 ± 0.001*	0.021 ± 0.001*
Relative	0.76 ± 0.03	0.70 ± 0.03	0.62 ± 0.02**	0.69 ± 0.03	0.68 ± 0.03	0.75 ± 0.04

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

2 n=3.

3 n=4.

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

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

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.33mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
MALE (continued)						
7-Week Recovery						
n	4	4	5	4	5	5
Necropsy body wt	45.1 ± 2.9	39.0 ± 1.5	42.2 ± 0.8	40.9 ± 2.3	40.6 ± 2.5	37.0 ± 1.4*
Brain						
Absolute	0.473 ± 0.006	0.473 ± 0.014	0.472 ± 0.011	0.480 ± 0.000	0.478 ± 0.007	0.488 ± 0.006
Relative	10.61 ± 0.68	12.14 ± 0.40	11.22 ± 0.41	11.87 ± 0.73	11.93 ± 0.72	13.29 ± 0.56**
Heart						
Absolute	0.190 ± 0.009	0.166 ± 0.004	0.167 ± 0.005	0.164 ± 0.007	0.161 ± 0.015*	0.150 ± 0.004**
Relative	4.23 ± 0.16	4.25 ± 0.12	3.96 ± 0.08	4.03 ± 0.07	4.00 ± 0.41	4.09 ± 0.19
Right kidney						
Absolute	0.405 ± 0.018	0.380 ± 0.016	0.388 ± 0.014	0.380 ± 0.014	0.342 ± 0.045	0.374 ± 0.012
Relative	9.05 ± 0.48	9.79 ± 0.56	9.22 ± 0.41	9.34 ± 0.26	8.43 ± 1.04	10.14 ± 0.28
Liver						
Absolute	2.430 ± 0.207	2.090 ± 0.083	2.332 ± 0.043	2.220 ± 0.126	2.098 ± 0.095	1.782 ± 0.095**
Relative	53.67 ± 1.63	53.61 ± 1.29	55.35 ± 1.00	54.36 ± 0.91	51.85 ± 1.29	48.14 ± 1.24**
Lungs						
Absolute	0.225 ± 0.009	0.208 ± 0.023	0.226 ± 0.009	0.218 ± 0.009	0.278 ± 0.029	0.288 ± 0.018*
Relative	5.06 ± 0.41	5.30 ± 0.50	5.36 ± 0.16	5.35 ± 0.17	6.91 ± 0.80*	7.90 ± 0.79**
Spleen						
Absolute	0.095 ± 0.005	0.093 ± 0.008	0.084 ± 0.002	0.100 ± 0.009	0.098 ± 0.007	0.110 ± 0.010
Relative	2.12 ± 0.10	2.38 ± 0.19	2.00 ± 0.07	2.44 ± 0.11	2.43 ± 0.20	2.99 ± 0.27**
Right testis						
Absolute	0.135 ± 0.001	0.121 ± 0.002*	0.129 ± 0.002	0.130 ± 0.003	0.134 ± 0.004	0.128 ± 0.002
Relative	3.03 ± 0.21	3.10 ± 0.14	3.07 ± 0.09	3.20 ± 0.12	3.32 ± 0.13	3.49 ± 0.20
Thymus						
Absolute	0.040 ± 0.001	0.039 ± 0.002	0.036 ± 0.002	0.035 ± 0.002	0.035 ± 0.001	0.037 ± 0.003
Relative	0.89 ± 0.03	0.98 ± 0.03	0.86 ± 0.05	0.86 ± 0.05	0.88 ± 0.06	1.01 ± 0.11
Left ventricle						
Absolute	0.081 ± 0.002	0.074 ± 0.005	0.074 ± 0.002	0.070 ± 0.004	0.075 ± 0.003	0.064 ± 0.004**
Relative	1.80 ± 0.10	1.89 ± 0.09	1.76 ± 0.07	1.71 ± 0.10	1.85 ± 0.06	1.75 ± 0.13
Right ventricle						
Absolute	0.032 ± 0.001	0.028 ± 0.001	0.026 ± 0.002	0.027 ± 0.002	0.028 ± 0.002	0.028 ± 0.004
Relative	0.73 ± 0.07	0.71 ± 0.04	0.61 ± 0.05	0.68 ± 0.07	0.68 ± 0.04	0.77 ± 0.12

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.33mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
MALE (continued)						
14-Week Recovery						
n	5	5	5	5	5	5
Necropsy body wt	45.5 ± 2.3	44.3 ± 2.1	43.4 ± 2.9	41.3 ± 2.8	35.6 ± 0.9**	33.9 ± 1.4**
Brain						
Absolute	0.488 ± 0.011	0.478 ± 0.009	0.484 ± 0.016	0.486 ± 0.004	0.488 ± 0.009	0.464 ± 0.010
Relative	10.86 ± 0.67	10.89 ± 0.57	11.42 ± 1.13	12.03 ± 0.92	13.77 ± 0.52*	13.78 ± 0.64*
Heart						
Absolute	0.184 ± 0.006	0.200 ± 0.007	0.193 ± 0.009	0.190 ± 0.014	0.189 ± 0.016	0.156 ± 0.004
Relative	4.08 ± 0.17	4.58 ± 0.33	4.50 ± 0.25	4.61 ± 0.15	5.32 ± 0.45*	4.64 ± 0.19*
Right kidney						
Absolute	0.406 ± 0.022	0.406 ± 0.009	0.400 ± 0.014	0.426 ± 0.021	0.398 ± 0.015	0.356 ± 0.011
Relative	8.94 ± 0.27	9.28 ± 0.62	9.36 ± 0.65	10.40 ± 0.38*	11.21 ± 0.42**	10.53 ± 0.25**
Liver						
Absolute	2.174 ± 0.203	2.246 ± 0.095	2.244 ± 0.150	2.120 ± 0.155	1.846 ± 0.042	1.660 ± 0.044*
Relative	47.50 ± 2.39	50.78 ± 0.75	51.69 ± 0.79	51.39 ± 1.12	52.06 ± 1.92	49.15 ± 1.42
Lungs						
Absolute	0.254 ± 0.011	0.272 ± 0.019	0.250 ± 0.008	0.250 ± 0.020	0.290 ± 0.031	0.263 ± 0.0232
Relative	5.59 ± 0.11	6.16 ± 0.40	5.82 ± 0.26	6.09 ± 0.43	8.17 ± 0.92**	7.50 ± 0.38**2
Spleen						
Absolute	0.092 ± 0.007	0.082 ± 0.004	0.096 ± 0.007	0.094 ± 0.002	0.092 ± 0.008	0.092 ± 0.010
Relative	2.04 ± 0.18	1.86 ± 0.08	2.21 ± 0.12	2.32 ± 0.18	2.60 ± 0.26	2.77 ± 0.40*
Right testis						
Absolute	0.137 ± 0.005	0.133 ± 0.002	0.133 ± 0.007	0.129 ± 0.003	0.126 ± 0.003	0.121 ± 0.001*
Relative	3.03 ± 0.09	3.04 ± 0.15	3.07 ± 0.13	3.18 ± 0.17	3.56 ± 0.08*	3.60 ± 0.17**
Thymus						
Absolute	0.043 ± 0.008	0.035 ± 0.003	0.045 ± 0.009	0.040 ± 0.004	0.034 ± 0.003	0.027 ± 0.004
Relative	0.92 ± 0.14	0.79 ± 0.04	1.01 ± 0.14	0.99 ± 0.10	0.95 ± 0.08	0.79 ± 0.11
Left ventricle						
Absolute	0.087 ± 0.007	0.080 ± 0.004	0.085 ± 0.004	0.074 ± 0.004	0.077 ± 0.005	0.067 ± 0.002**
Relative	1.91 ± 0.11	1.84 ± 0.19	1.99 ± 0.17	1.81 ± 0.11	2.17 ± 0.19	1.99 ± 0.06
Right ventricle						
Absolute	0.031 ± 0.002	0.035 ± 0.002	0.035 ± 0.003	0.034 ± 0.002	0.030 ± 0.001	0.025 ± 0.002
Relative	0.67 ± 0.02	0.79 ± 0.06	0.82 ± 0.09	0.83 ± 0.08	0.84 ± 0.03	0.75 ± 0.05

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.33mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
FEMALE						
13-Week Base Study						
n	8	10	10	10	10	9
Necropsy body wt	30.0 ± 0.8	27.7 ± 1.0	32.5 ± 0.8	30.0 ± 0.8	25.2 ± 0.4**	24.2 ± 0.3**
Brain						
Absolute	0.495 ± 0.006	0.493 ± 0.005	0.495 ± 0.005	0.489 ± 0.004	0.466 ± 0.005**	0.472 ± 0.005**
Relative	16.58 ± 0.40	17.94 ± 0.49	15.32 ± 0.42	16.38 ± 0.41	18.49 ± 0.29**	19.52 ± 0.30**
Heart						
Absolute	0.145 ± 0.007	0.138 ± 0.004	0.147 ± 0.004	0.143 ± 0.006	0.121 ± 0.004**	0.108 ± 0.005**
Relative	4.85 ± 0.23	5.02 ± 0.18	4.56 ± 0.17	4.78 ± 0.20	4.79 ± 0.13	4.46 ± 0.19
Right kidney						
Absolute	0.219 ± 0.008	0.195 ± 0.007	0.217 ± 0.005	0.212 ± 0.010	0.206 ± 0.018	0.188 ± 0.006
Relative	7.34 ± 0.34	7.05 ± 0.16	6.70 ± 0.14	7.09 ± 0.34	8.17 ± 0.73	7.75 ± 0.25
Liver						
Absolute	1.465 ± 0.029	1.300 ± 0.057	1.776 ± 0.044	1.468 ± 0.029	1.288 ± 0.027**	1.274 ± 0.031**
Relative	48.98 ± 0.90	46.92 ± 1.33	54.74 ± 1.02	49.02 ± 0.71	51.01 ± 0.51	52.62 ± 1.11*
Lungs						
Absolute	0.364 ± 0.008	0.334 ± 0.013	0.371 ± 0.009	0.362 ± 0.012	0.360 ± 0.011	0.374 ± 0.013
Relative	12.21 ± 0.50	12.12 ± 0.49	11.45 ± 0.30	12.14 ± 0.52	14.25 ± 0.29**	15.49 ± 0.63**
Spleen						
Absolute	0.105 ± 0.007	0.092 ± 0.004	0.106 ± 0.003	0.101 ± 0.005	0.106 ± 0.004	0.171 ± 0.026**
Relative	3.54 ± 0.29	3.33 ± 0.14	3.27 ± 0.09	3.38 ± 0.16	4.21 ± 0.18	7.04 ± 1.04**
Thymus						
Absolute	0.055 ± 0.004	0.055 ± 0.002	0.060 ± 0.003	0.058 ± 0.002	0.051 ± 0.002	0.041 ± 0.004**
Relative	1.85 ± 0.15	2.01 ± 0.10	1.85 ± 0.10	1.95 ± 0.07	2.03 ± 0.09	1.69 ± 0.18
Left ventricle						
Absolute	0.059 ± 0.002	0.056 ± 0.002	0.065 ± 0.002	0.058 ± 0.002	0.053 ± 0.002	0.046 ± 0.002**
Relative	1.98 ± 0.06	2.03 ± 0.10	2.02 ± 0.08	1.93 ± 0.05	2.11 ± 0.06	1.91 ± 0.08
Right ventricle						
Absolute	0.019 ± 0.002	0.020 ± 0.002	0.019 ± 0.001	0.018 ± 0.001	0.016 ± 0.001	0.015 ± 0.001
Relative	0.65 ± 0.06	0.74 ± 0.08	0.57 ± 0.04	0.59 ± 0.05	0.63 ± 0.04	0.62 ± 0.03

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.33mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
FEMALE (continued)						
7-Week Recovery						
n	3	4	4	5	5	4
Necropsy body wt	33.3 ± 2.6	38.0 ± 1.1	37.0 ± 2.8	36.0 ± 1.6	29.7 ± 0.2	27.5 ± 0.5*
Brain						
Absolute	0.490 ± 0.006	0.498 ± 0.011	0.495 ± 0.009	0.488 ± 0.0033	0.486 ± 0.002	0.485 ± 0.009
Relative	14.91 ± 1.36	13.10 ± 0.34	13.68 ± 1.29	13.64 ± 0.873	16.36 ± 0.17	17.65 ± 0.40*
Heart						
Absolute	0.171 ± 0.018	0.157 ± 0.012	0.158 ± 0.009	0.145 ± 0.007	0.134 ± 0.006*	0.123 ± 0.001**
Relative	5.11 ± 0.17	4.11 ± 0.27	4.40 ± 0.55	4.03 ± 0.18	4.51 ± 0.19	4.46 ± 0.11
Right kidney						
Absolute	0.240 ± 0.010	0.243 ± 0.018	0.245 ± 0.009	0.254 ± 0.017	0.238 ± 0.005	0.255 ± 0.006
Relative	7.28 ± 0.58	6.36 ± 0.38	6.72 ± 0.46	7.09 ± 0.53	8.01 ± 0.19	9.29 ± 0.33**
Liver						
Absolute	1.537 ± 0.148	1.733 ± 0.116	1.595 ± 0.095	1.562 ± 0.098	1.306 ± 0.010	1.243 ± 0.076
Relative	45.99 ± 1.79	45.55 ± 2.77	43.36 ± 1.44	43.42 ± 2.36	43.96 ± 0.56	45.09 ± 2.01
Lungs						
Absolute	0.207 ± 0.007	0.193 ± 0.014	0.188 ± 0.009	0.196 ± 0.009	0.218 ± 0.019	0.283 ± 0.022**
Relative	6.25 ± 0.37	5.06 ± 0.32	5.10 ± 0.16	5.44 ± 0.11	7.33 ± 0.64	10.24 ± 0.60**
Spleen						
Absolute	0.103 ± 0.009	0.098 ± 0.008	0.090 ± 0.004	0.112 ± 0.007	0.104 ± 0.007	0.163 ± 0.006**
Relative	3.12 ± 0.25	2.56 ± 0.15	2.46 ± 0.16	3.13 ± 0.22	3.50 ± 0.21	5.92 ± 0.29**
Thymus						
Absolute	0.050 ± 0.002	0.046 ± 0.0012	0.047 ± 0.004	0.051 ± 0.002	0.046 ± 0.003	0.039 ± 0.008
Relative	1.51 ± 0.12	1.19 ± 0.062	1.30 ± 0.14	1.41 ± 0.08	1.54 ± 0.10	1.43 ± 0.29
Left ventricle						
Absolute	0.055 ± 0.002	0.068 ± 0.008	0.060 ± 0.003	0.064 ± 0.005	0.058 ± 0.005	0.051 ± 0.002
Relative	1.67 ± 0.15	1.80 ± 0.21	1.65 ± 0.11	1.78 ± 0.09	1.94 ± 0.15	1.84 ± 0.09
Right ventricle						
Absolute	0.023 ± 0.002	0.026 ± 0.003	0.023 ± 0.001	0.025 ± 0.002	0.021 ± 0.001	0.022 ± 0.001
Relative	0.70 ± 0.01	0.67 ± 0.08	0.63 ± 0.06	0.70 ± 0.06	0.71 ± 0.04	0.78 ± 0.03

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.33mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
FEMALE (continued)						
14-Week Recovery						
n	4	4	5	5	5	4
Necropsy body wt	39.9 ± 3.4	37.5 ± 3.2	37.8 ± 2.2	36.8 ± 3.1	30.3 ± 2.1*	27.7 ± 0.6**
Brain						
Absolute	0.488 ± 0.013	0.508 ± 0.008	0.504 ± 0.006	0.492 ± 0.012	0.490 ± 0.016	0.490 ± 0.009
Relative	12.53 ± 1.25	13.83 ± 1.14	13.52 ± 0.76	13.74 ± 1.14	16.45 ± 1.20*	17.72 ± 0.58**
Heart						
Absolute	0.151 ± 0.006	0.162 ± 0.013	0.162 ± 0.003	0.156 ± 0.008	0.158 ± 0.008	0.143 ± 0.006
Relative	3.84 ± 0.30	4.36 ± 0.24	4.33 ± 0.25	4.37 ± 0.44	5.28 ± 0.40**	5.16 ± 0.20*
Right kidney						
Absolute	0.278 ± 0.023	0.270 ± 0.007	0.282 ± 0.012	0.270 ± 0.008	0.252 ± 0.015	0.245 ± 0.006
Relative	7.02 ± 0.52	7.36 ± 0.64	7.55 ± 0.46	7.55 ± 0.70	8.38 ± 0.43	8.86 ± 0.30*
Liver						
Absolute	1.818 ± 0.051	1.843 ± 0.117	1.986 ± 0.091	1.928 ± 0.100	1.612 ± 0.147	1.400 ± 0.049*
Relative	46.32 ± 3.31	49.77 ± 3.37	52.90 ± 2.17	53.38 ± 3.62	52.88 ± 2.40	50.51 ± 0.98
Lungs						
Absolute	0.263 ± 0.027	0.225 ± 0.013	0.230 ± 0.027	0.240 ± 0.019	0.248 ± 0.019	0.253 ± 0.018
Relative	6.64 ± 0.63	6.21 ± 0.89	6.14 ± 0.73	6.74 ± 0.83	8.23 ± 0.55	9.11 ± 0.59*
Spleen						
Absolute	0.148 ± 0.008	0.105 ± 0.003*	0.112 ± 0.007*	0.130 ± 0.011	0.104 ± 0.010*	0.173 ± 0.010
Relative	3.80 ± 0.42	2.86 ± 0.24	2.99 ± 0.23	3.70 ± 0.62	3.42 ± 0.19	6.22 ± 0.30**
Thymus						
Absolute	0.050 ± 0.002	0.044 ± 0.004	0.049 ± 0.005	0.040 ± 0.008	0.046 ± 0.004	0.040 ± 0.002
Relative	1.29 ± 0.14	1.18 ± 0.07	1.30 ± 0.10	1.05 ± 0.16	1.54 ± 0.16	1.45 ± 0.10
Left ventricle						
Absolute	0.064 ± 0.004	0.066 ± 0.003	0.067 ± 0.003	0.059 ± 0.003	0.057 ± 0.006	0.064 ± 0.016
Relative	1.60 ± 0.05	1.79 ± 0.11	1.81 ± 0.16	1.66 ± 0.15	1.89 ± 0.14	2.26 ± 0.51
Right ventricle						
Absolute	0.025 ± 0.002	0.024 ± 0.002	0.026 ± 0.001	0.025 ± 0.002	0.022 ± 0.001	0.021 ± 0.001
Relative	0.64 ± 0.06	0.64 ± 0.06	0.71 ± 0.04	0.68 ± 0.06	0.75 ± 0.05	0.75 ± 0.04

APPENDIX B

Hematology and Clinical Chemistry Results

TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

	Vehicle Control	0.1 mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
MALE						
n (Days 3, 14, and 30)	5	5	_[2]	5	_[2]	5
n (Day 93)	10	10	10	10	10	1
Hematology						
Hematocrit (%)						
Day 14	43.9 ± 0.6	43.4 ± 1.1	_	47.2 ± 0.9*	_	48.9 ± 1.8*
Day 30	48.9 ± 0.6	47.7 ± 0.9	_	49.6 ± 0.6	_	47.4 ± 1.1
Day 93	47.0 ± 0.5	49.1 ± 0.3**	46.8 ± 1.0	49.4 ± 0.3**	51.1 ± 0.6	50.0
Hemoglobin (g/dL)						
Day 14	15.6 ± 0.3	15.3 ± 1.1[3]	_	15.8 ± 0.2	_	16.9 ± 0.7
Day 30	17.4 ± 0.2	16.6 ± 0.2*	_	17.3 ± 0.2	_	16.2 ± 0.3**
Day 93	16.6 ± 0.1	16.9 ± 0.2*	16.5 ± 0.2	17.2 ± 0.1**	17.8 ± 0.2**	16.8
Erythrocytes (10^6/µL)						
Day 14	6.71 ± 0.10	6.80 ± 0.25	_	7.35 ± 0.12*	_	7.71 ± 0.36*
Day 30	8.22 ± 0.13	7.81 ± 0.14	_	7.99 ± 0.15	_	7.57 ± 0.23*
Day 93	9.08 ± 0.09	9.38 ± 0.06*	8.74 ± 0.11	9.22 ± 0.04	9.68 ± 0.09**	8.04
Reticulocytes (10^6/µL)						
Day 14	0.34 ± 0.03	0.40 ± 0.03	_	0.38 ± 0.04	_	0.57 ± 0.07**
Day 30	0.20 ± 0.02	0.17 ± 0.01	_	0.18 ± 0.01	_	0.42 ± 0.04*
Day 93	0.14 ± 0.02	0.16 ± 0.03	0.14 ± 0.03	0.18 ± 0.03	0.26 ± 0.04*	0.61
Mean cell volume (fL)						
Day 14	64.2 ± 0.7	62.8 ± 0.8	_	62.8 ± 0.7	_	62.4 ± 1.0
Day 30	58.4 ± 0.5	60.2 ± 0.5*	_	60.8 ± 0.6**	_	61.2 ± 1.0*
Day 93	50.7 ± 0.3	51.0 ± 0.2	51.6 ± 0.2*	52.3 ± 0.4**	51.5 ± 0.2*	62.0
Mean cell hemoglobin (pg)						
Day 14	23.0 ± 0.3	22.0 ± 0.6[3]	_	21.8 ± 0.5	_	22.0 ± 0.0*
Day 30	21.4 ± 0.2	21.2 ± 0.2	_	21.6 ± 0.2	_	21.6 ± 0.4
Day 93	18.1 ± 0.2	17.9 ± 0.2	19.0 ± 0.2*	18.4 ± 0.2	18.1 ± 0.1	21.0
Mean cell hemoglobin concentration (g/dL)						
Day 14	35.2 ± 0.4	34.7 ± 1.3[3]	_	33.6 ± 0.8	_	34.8 ± 0.4
Day 30	35.6 ± 0.2	34.6 ± 0.2*	_	34.6 ± 0.2*	_	34.2 ± 0.4**
Day 93	35.2 ± 0.4	34.8 ± 0.5	35.4 ± 0.7	34.7 ± 0.2	34.7 ± 0.2	34.0
Platelets (10^3/µL)						
Day 14	994.0 ± 22.8	998.0 ± 23.6	_	890.0 ± 95.3	_	243.6 ± 36.6**
Day 30	756.4 ± 114.9	905.2 ± 27.9	_	950.4 ± 9.2	_	79.6 ± 9.6
Day 93	685.1 ± 23.1	729.5 ± 16.8	676.9 ± 20.1	764.7 ± 14.3	598.6 ± 26.7[4]	48.0

1 Mean ± standard error. On days 3, 14, and 30 samples were collected from animals designated for the clinical pathology study. Day 93 and 94 analyses were conducted on samples obtained from rats in the 13-week core study.

2 No animals were designated for the clinical pathology study for this dose group.

3 n=3.

4 n=9.

5 n=4.

6 n=2.

7 n=1.

8 n=8.

9 n=7.

10 n=6.

11 n=5.

12 n=0.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1 mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
MALE (continued)						
Hematology (continued)						
Mean platelet volume (μm^3)						
Day 14	7.26 ± 0.07	7.10 ± 0.08	_	7.32 ± 0.11	_	8.10 ± 0.21*
Day 30	7.62 ± 0.31	7.16 ± 0.18	_	7.40 ± 0.13	_	10.96 ± 0.41*
Day 93	7.53 ± 0.10	7.60 ± 0.07	7.69 ± 0.09	7.75 ± 0.11	7.93 ± 0.10**	9.50
Leukocytes ($10^3/\mu L$)						
Day 14	8.85 ± 0.28[5]	8.92 ± 0.75	_	8.54 ± 0.97	±	10.28 ± 0.66
Day 30	10.02 ± 0.71	10.42 ± 0.62	_	11.48 ± 1.01	_	11.40 ± 1.38
Day 93	8.91 ± 0.35	9.73 ± 0.30*	9.65 ± 0.31	11.45 ± 0.18**	10.38 ± 0.19**	13.60
Segmented neutrophils ($10^3/\mu L$)						
Day 14	1.09 ± 0.23[5]	0.93 ± 0.20	_	0.74 ± 0.16	_	1.29 ± 0.20
Day 30	0.74 ± 0.19	0.90 ± 0.12	_	1.17 ± 0.27	_	1.09 ± 0.18
Day 93	1.17 ± 0.07	1.51 ± 0.18	1.51 ± 0.14	1.50 ± 0.15	1.27 ± 0.11	4.35
Lymphocytes ($10^3/\mu L$)						
Day 14	7.64 ± 0.43[5]	7.81 ± 0.61	_	7.57 ± 0.87	±	8.27 ± 0.59
Day 30	9.13 ± 0.87	9.44 ± 0.57	_	10.10 ± 0.80	_	9.72 ± 1.12
Day 93	7.40 ± 0.34	7.82 ± 0.23	7.58 ± 0.19	9.42 ± 0.26**	8.43 ± 0.21**	8.02
Monocytes ($10^3/\mu L$)						
Day 14	0.05 ± 0.03[5]	0.12 ± 0.06	_	0.21 ± 0.04*	_	0.61 ± 0.04**
Day 30	0.02 ± 0.02	0.02 ± 0.02	_	0.06 ± 0.04	_	0.23 ± 0.10
Day 93	0.29 ± 0.05	0.31 ± 0.06	0.47 ± 0.09	0.43 ± 0.07	0.42 ± 0.08	0.27
Eosinophils ($10^3/\mu L$)						
Day 14	0.02 ± 0.02[5]	0.00 ± 0.00	_	0.02 ± 0.02	_	0.06 ± 0.03
Day 30	0.06 ± 0.04	0.02 ± 0.02	_	0.09 ± 0.04	_	0.16 ± 0.05
Day 93	0.04 ± 0.02	0.09 ± 0.04	0.07 ± 0.04	0.09 ± 0.03	0.09 ± 0.03	0.27
Clinical Chemistry						
Alkaline phosphatase (IU/L)						
Day 3	758 ± 32	893 ± 73	_	854 ± 68	_	969 ± 31*
Day 14	653 ± 17	688 ± 28	_	836 ± 67*	_	1321 ± 43**
Day 30	522 ± 15	475 ± 14	_	557 ± 43	_	1288 ± 90*[5]
Day 93	246 ± 10	261 ± 7	286 ± 22*	268 ± 10	374 ± 29**	302
Alanine aminotransferase (IU/L)						
Day 3	34 ± 1	34 ± 1	_	37 ± 2	_	39 ± 2
Day 14	26 ± 4	33 ± 7	_	34 ± 3	_	109 ± 21**
Day 30	35 ± 3	29 ± 4	_	42 ± 3	_	136 ± 13**
Day 93	96 ± 10	60 ± 7*[4]	87 ± 12	61 ± 4*[4]	94 ± 10	283
Creatine phosphokinase (IU/L)						
Day 3	318 ± 139[5]	181 ± 59[6]	_	572 ± 218[6]	_	255 ± 68[5]
Day 14	139 ± 13[5]	280 ± 98	_	391 ± 86*	_	511 ± 215[3]
Day 30	1103 ± 499[6]	913 ± 339[5]	_	1258 ± 380[6]	_	194 ± 72[5]
Day 93	76 ± 5	177 ± 33**	103 ± 10	191 ± 43**	97 ± 11[4]	61
Creatine phosphokinase MB (IU/L)						
Day 3	72 ± 18[3]	45 ± 10[6]	_	71[7]	_	6[7]
Day 93	61 ± 6	74 ± 11	77 ± 7[8]	83 ± 15[9]	61 ± 10[8]	37[7]
Creatine phosphokinase MM (IU/L)						
Day 3	280 ± 163[6]	61[7]	_	392 ± 168[6]	_	128 ± 74[5]
Day 30	788 ± 487[6]	598 ± 216[5]	_	863 ± 331[6]	_	91 ± 46[5]
Day 93	18 ± 6[6]	92 ± 31[10]	55 ± 19[6]	232 ± 61[3]	19 ± 5[11]	_[12]

TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1 mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
MALE (continued)						
Clinical Chemistry (continued)						
Creatine phosphokinase BB (IU/L)						
Day 3	123 ± 16[5]	106 ± 19[6]	_	142 ± 12[6]	_	123 ± 19[5]
Day 14	105 ± 14[5]	188 ± 35*	_	280 ± 32**	_	268 ± 107*[3]
Day 30	276 ± 15[6]	264 ± 61[3]	_	280 ± 64[6]	_	99 ± 27*[5]
Glutamyl dehydrogenase (IU/L)						
Day 3	5 ± 1	5 ± 0	_	4 ± 0	_	5 ± 0
Day 14	5 ± 1	6 ± 1	_	6 ± 0	_	25 ± 6*
Day 30	9 ± 2[5]	15 ± 1*[5]	_	17 ± 2**	_	49 ± 7**[5]
Day 93	39 ± 11[4]	19 ± 2[4]	28 ± 7[9]	13 ± 1*[4]	54 ± 9[4]	106
Sorbitol dehydrogenase (IU/L)						
Day 3	16 ± 1	14 ± 1[5]	_	17 ± 1	_	16 ± 1
Day 14	19 ± 2	15 ± 0	_	19 ± 1	_	66 ± 12*
Day 30	20 ± 1	18 ± 2	_	22 ± 2	_	63 ± 3**
Day 93	39 ± 4[4]	33 ± 6[4]	42 ± 2	34 ± 3	41 ± 6[4]	66
FEMALE						
n (Days 3, 14, and 30)	5	5	—[2]	5	—[2]	5
n (Day 94)	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 14	43.9 ± 0.6	43.8 ± 0.9	_	48.5 ± 0.7*	_	52.6 ± 1.7**
Day 30	47.2 ± 1.2[5]	49.3 ± 0.5	_	46.0 ± 1.7	_	40.7 ± 3.2
Day 94	48.0 ± 0.6	43.9 ± 0.7	45.8 ± 0.4	45.4 ± 0.5	46.5 ± 0.9	51.5 ± 0.6
Hemoglobin (g/dL)						
Day 14	15.8 ± 0.1	16.3 ± 0.4[5]	_	16.9 ± 0.2**[5]	_	18.7 ± 0.6**
Day 30	17.6 ± 0.5[5]	17.0 ± 0.1	_	16.4 ± 0.5	_	15.4 ± 1.1
Day 94	17.0 ± 0.2	16.7 ± 0.3	16.5 ± 0.1	17.1 ± 0.2	17.2 ± 0.3	18.1 ± 0.2*
Erythrocytes (10^6/µL)						
Day 14	6.71 ± 0.11	6.70 ± 0.19	_	7.24 ± 0.13	_	8.22 ± 0.27**
Day 30	7.73 ± 0.21[5]	7.58 ± 0.09	_	7.25 ± 0.30	_	6.69 ± 0.53
Day 94	8.19 ± 0.08	8.09 ± 0.11	7.98 ± 0.05	8.24 ± 0.10	8.41 ± 0.15	8.88 ± 0.12**
Reticulocytes (10^6/µL)						
Day 14	0.23 ± 0.03	0.30 ± 0.03	_	0.30 ± 0.02	_	0.22 ± 0.02
Day 30	0.13 ± 0.03[5]	0.13 ± 0.02	_	0.12 ± 0.01	_	0.13 ± 0.02[5]
Day 94	0.09 ± 0.01	0.20 ± 0.02**	0.12 ± 0.02*	0.12 ± 0.01*	0.13 ± 0.02	0.49 ± 0.10**
Mean cell volume (fL)						
Day 14	64.6 ± 0.4	64.6 ± 0.8	_	65.6 ± 0.5	_	63.4 ± 0.4
Day 30	60.0 ± 0.0[5]	64.2 ± 0.8	_	62.4 ± 1.0	_	59.4 ± 0.4
Day 94	57.3 ± 0.4	53.3 ± 0.3**	55.8 ± 0.5	53.8 ± 0.1**	54.1 ± 0.5**	56.9 ± 0.5
Mean cell hemoglobin (pg)						
Day 14	23.4 ± 0.2	24.5 ± 1.2[5]	_	23.6 ± 0.2	_	23.3 ± 0.3[5]
Day 30	22.8 ± 0.3[5]	22.2 ± 0.2	_	22.8 ± 0.2	_	23.2 ± 0.4
Day 94	20.7 ± 0.2	20.5 ± 0.2	20.5 ± 0.2	20.8 ± 0.2	20.3 ± 0.2	20.4 ± 0.2
Mean cell hemoglobin concentration (g/dL)						
Day 14	36.0 ± 0.3	37.5 ± 1.5[5]	_	34.8 ± 0.5	_	35.5 ± 0.3[5]
Day 30	37.3 ± 0.3[5]	34.6 ± 0.2	_	35.6 ± 0.7	_	38.2 ± 0.8
Day 94	35.5 ± 0.3	38.1 ± 0.2**	36.0 ± 0.2	37.4 ± 0.2**	37.1 ± 0.3*	35.0 _ 0.2

TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1 mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
FEMALE (continued)						
Hematology (continued)						
Platelets (10^3/μL)						
Day 14	945.2 ± 34.6	987.2 ± 15.2	_	1020.0 ± 41.1	_	920.0 ± 38.3
Day 30	798.5 ± 27.5[5]	844.8 ± 11.5	_	840.0 ± 16.6	_	700.0 ± 51.3
Day 94	813.6 ± 27.9[4]	843.6 ± 86.6	765.8 ± 24.1	828.8 ± 17.0	767.3 ± 16.0[4]	150.7 ± 8.5**
Mean platelet volume (μm^3)						
Day 14	7.40 ± 0.07	7.08 ± 0.07	_	7.52 ± 0.15	_	7.50 ± 0.08
Day 30	7.30 ± 0.06[5]	6.92 ± 0.07	_	7.26 ± 0.17	_	7.84 ± 0.17
Day 94	7.06 ± 0.29	7.25 ± 0.10	7.18 ± 0.08	7.26 ± 0.07	7.45 ± 0.13	8.95 ± 0.12**
Leukocytes (10^3/μL)						
Day 14	7.90 ± 0.90	9.00 ± 0.95	_	8.56 ± 0.93	_	12.96 ± 1.11*
Day 30	9.05 ± 0.73[5]	10.52 ± 0.48	_	16.02 ± 5.15	_	14.00 ± 2.49
Day 94	7.28 ± 0.44	8.34 ± 0.74	7.04 ± 0.22	7.96 ± 0.33	8.42 ± 0.40	11.52 ± 0.25**[4]
Segmented neutrophils (10^3/μL)						
Day 14	0.50 ± 0.13	1.18 ± 0.04*	_	0.72 ± 0.17	_	0.91 ± 0.24
Day 30	0.94 ± 0.17[5]	0.85 ± 0.21	_	1.64 ± 0.48	_	1.54 ± 0.42
Day 94	0.71 ± 0.09	1.32 ± 0.06**[4]	1.03 ± 0.08**	1.02 ± 0.09*	1.21 ± 0.08**	1.71 ± 0.21**
Lymphocytes (10^3/μL)						
Day 14	6.91 ± 0.81	7.60 ± 0.97	_	7.60 ± 0.83	_	11.20 ± 0.88**
Day 30	7.95 ± 0.77[5]	9.56 ± 0.51	_	14.29 ± 4.69	_	12.20 ± 2.06
Day 94	6.21 ± 0.37	6.30 ± 0.36	5.82 ± 0.20	6.76 ± 0.29	6.86 ± 0.39	7.77 ± 0.74*
Monocytes (10^3/μL)						
Day 14	0.10 ± 0.07	0.13 ± 0.02	_	0.14 ± 0.03	_	0.56 ± 0.09**
Day 30	0.16 ± 0.02[5]	0.09 ± 0.02	_	0.14 ± 0.06	_	0.08 ± 0.03[5]
Day 94	0.31 ± 0.07	0.27 ± 0.07	0.21 ± 0.06	0.15 ± 0.05	0.31 ± 0.07	0.40 ± 0.11
Eosinophils (10^3/μL)						
Day 14	0.04 ± 0.04[5]	0.04 ± 0.04	_	0.06 ± 0.03	_	0.00 ± 0.00
Day 30	0.00 ± 0.00[5]	0.00 ± 0.00	_	0.05 ± 0.03	_	0.07 ± 0.04
Day 94	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.14 ± 0.04**
Clinical Chemistry						
Alanine aminotransferase (IU/L)						
Day 3	30 ± 1	33 ± 1	_	29 ± 2	_	37 ± 4
Day 14	22 ± 2	25 ± 1	_	25 ± 7	_	48 ± 15
Day 30	25 ± 6	28 ± 2	_	40 ± 6*	_	74 ± 11**
Day 94	42 ± 3	37 ± 3	48 ± 5[4]	39 ± 2	60 ± 7*	152 ± 6**
Alkaline phosphatase (IU/L)						
Day 3	577 ± 73	664 ± 32	_	740 ± 72	_	676 ± 24
Day 14	644 ± 35	485 ± 19	_	566 ± 35	_	884 ± 35[5]
Day 30	449 ± 9	346 ± 32	_	419 ± 18	_	676 ± 46
Day 94	190 ± 9	205 ± 4[4]	188 ± 9	195 ± 7	247 ± 11**	334 ± 35**[11]
Creatine phosphokinase (IU/L)						
Day 3	450 ± 134[6]	_[12]	_	116 ± 15[3]	_	_[12]
Day 14	205 ± 35	183 ± 35	_	198 ± 93[6]	_	121 ± 31[6]
Day 30	1279 ± 318[3]	1070 ± 408[3]	_	1389 ± 367[6]	_	435 ± 293[3]
Day 94	113 ± 13	182 ± 28	128 ± 22	111 ± 15	168 ± 24	107 ± 11
Creatine phosphokinase MB (IU/L)						
Day 94	46 ± 17[3]	85 ± 12[4]	96 ± 21[9]	78 ± 13[8]	97 ± 5[8]	_[12]
Creatine phosphokinase MM (IU/L)						
Day 3	196 ± 104[6]	_[12]	_	14 ± 7[6]	_	_[12]
Day 30	996 ± 246[3]	728 ± 283[3]	_	974 ± 100[6]	_	236 ± 200[3]

TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of Riddelliine (continued)

	Vehicle Control	0.1 mg/kg	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg
FEMALE (continued)						
Clinical Chemistry (continued)						
Creatine phosphokinase BB (IU/L)						
Day 3	228 ± 7[6]	_[12]	_	103 ± 8[3]	_	_[12]
Day 14	160 ± 28	177 ± 34	_	160 ± 76[6]	_	99 ± 18[6]
Day 30	259 ± 58[3]	287 ± 106[3]	_	229 ± 81[6]	_	162 ± 76[3]
Glutamyl dehydrogenase (IU/L)						
Day 3	4 ± 1	4 ± 0	_	4 ± 0	_	3 ± 1
Day 14	5 ± 0[3]	5 ± 0	_	5 ± 1[5]	_	10 ± 3[5]
Day 30	14 ± 3	18 ± 2	_	18 ± 2	_	25 ± 4*
Day 94	36 ± 6	29 ± 7	22 ± 3	17 ± 4*[4]	19 ± 3[4]	48 ± 6
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 0	14 ± 0	_	15 ± 1*	_	18 ± 2**
Day 14	20 ± 1	15 ± 0	_	32 ± 7	_	72 ± 18*
Day 30	30 ± 6	21 ± 1	_	22 ± 4	_	39 ± 2
Day 94	15 ± 1[10]	23 ± 2**[4]	22 ± 2**[8]	21 ± 2*	22 ± 3*[10]	53 ± 5**[9]

TABLE B2
Hematology Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine[1]

	Vehicle Control	0.33 mg/kg	1.0 mg/kg	3.3 mg/kg	10 mg/kg	25 mg/kg
MALE						
n	10	9	10	10	9	9
Hematocrit (%)	55.5 ± 0.9	59.4 ± 1.0*	54.9 ± 0.7	56.0 ± 1.1	62.9 ± 1.7**	66.1 ± 1.2**
Hemoglobin (g/dL)	18.0 ± 0.3	19.3 ± 0.4**	18.2 ± 0.2	18.0 ± 0.4	19.8 ± 0.6**	21.1 ± 0.5**
Erythrocytes (10^6/µL)	10.81 ± 0.20	11.76 ± 0.17**	10.82 ± 0.16	10.90 ± 0.25	11.90 ± 0.30**	12.37 ± 0.30**
Reticulocytes (10^6/µL)	0.20 ± 0.02	0.25 ± 0.04	0.25 ± 0.02	0.21 ± 0.02	0.21 ± 0.02	0.27 ± 0.03
Mean cell volume (fL)	50.2 ± 0.4	49.4 ± 0.3	49.8 ± 0.3	50.3 ± 0.4	51.7 ± 0.2**	52.4 ± 0.3**
Mean cell hemoglobin (pg)	16.5 ± 0.3	16.3 ± 0.2	16.8 ± 0.1	16.4 ± 0.2	16.7 ± 0.2	17.1 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	32.3 ± 0.3	32.7 ± 0.2	33.2 ± 0.3	32.0 ± 0.2	31.4 ± 0.2*	31.8 ± 0.3
Platelets (10^3/µL)	1070.7 ± 25.8	1131.8 ± 63.9[2]	1139.8 ± 69.7	1091.8 ± 32.2	673.8 ± 58.9**[2]	394.7 ± 29.6**
Mean platelet volume (µm^3)	5.63 ± 0.04	6.62 ± 0.25**[2]	5.80 ± 0.09	5.67 ± 0.03	5.83 ± 0.15[2]	6.06 ± 0.11**
Leukocytes (10^3/µL)	5.02 ± 0.34	3.11 ± 0.20**	5.14 ± 0.32	3.80 ± 0.37	4.41 ± 0.30	5.22 ± 0.42
Segmented neutrophils (10^3/µL)	0.56 ± 0.08[3]	0.24 ± 0.04*	0.56 ± 0.07	0.38 ± 0.07	0.34 ± 0.05	0.71 ± 0.07[4]
Lymphocytes (10^3/µL)	4.19 ± 0.33[3]	2.73 ± 0.19*	4.41 ± 0.24	3.17 ± 0.26	3.94 ± 0.29	4.18 ± 0.42[4]
Monocytes (10^3/µL)	0.36 ± 0.10[3]	0.13 ± 0.03	0.10 ± 0.03	0.21 ± 0.06	0.10 ± 0.02	0.43 ± 0.10[4]
Eosinophils (10^3/µL)	0.03 ± 0.02[3]	0.02 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.01[4]
FEMALE						
n	8	10	10	10	10	8
Hematocrit (%)	62.4 ± 1.4	61.6 ± 1.1	58.6 ± 1.5	63.3 ± 1.6	65.6 ± 1.3	63.9 ± 2.7
Hemoglobin (g/dL)	19.7 ± 0.4	19.5 ± 0.3	19.5 ± 0.5	20.2 ± 0.4	20.8 ± 0.4	19.9 ± 0.8
Erythrocytes (10^6/µL)	11.68 ± 0.23	11.45 ± 0.20	11.42 ± 0.29	11.93 ± 0.29	12.28 ± 0.24	11.45 ± 0.53
Reticulocytes (10^6/µL)	0.16 ± 0.02[5]	0.22 ± 0.02	0.26 ± 0.05	0.24 ± 0.03	0.28 ± 0.03*[3]	0.23 ± 0.02*[5]
Mean cell volume (fL)	52.3 ± 0.5	52.3 ± 0.3	50.3 ± 0.2	52.0 ± 0.3	52.3 ± 0.3	54.8 ± 0.4**
Mean cell hemoglobin (pg)	17.0 ± 0.0	17.1 ± 0.1	17.0 ± 0.0	17.0 ± 0.0	17.0 ± 0.0	17.4 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	31.4 ± 0.3	31.6 ± 0.2	33.0 ± 0.3*	32.0 ± 0.3	31.7 ± 0.2	31.0 ± 0.2
Platelets (10^3/µL)	944.6 ± 27.4	833.6 ± 26.7*	917.4 ± 35.7	706.2 ± 34.4**	546.5 ± 61.4**	323.5 ± 44.8**[2]
Mean platelet volume (µm^3)	5.80 ± 0.08	5.56 ± 0.05	5.85 ± 0.07	5.80 ± 0.04	6.00 ± 0.12	6.53 ± 0.11**[2]
Leukocytes (10^3/µL)	5.01 ± 0.25	4.16 ± 0.31	4.69 ± 0.20	4.63 ± 0.44	4.09 ± 0.23	5.19 ± 0.86
Segmented neutrophils (10^3/µL)	0.33 ± 0.05	0.25 ± 0.04	0.36 ± 0.05	0.35 ± 0.11	0.26 ± 0.03	0.66 ± 0.21
Lymphocytes (10^3/µL)	4.43 ± 0.24	3.77 ± 0.32	4.04 ± 0.18	4.19 ± 0.36	3.70 ± 0.24	4.19 ± 0.67
Monocytes (10^3/µL)	0.25 ± 0.04	0.09 ± 0.02*[2]	0.24 ± 0.05	0.06 ± 0.02**	0.12 ± 0.04	0.24 ± 0.07
Eosinophils (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.10 ± 0.03**

[1] Mean ± standard error.
[2] n=10.
[3] n=9.
[4] n=8.
[5] n=7.
* Significantly different (P_0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P_0.01) from the control group by Dunn's or Shirley's test.

APPENDIX C

Reproductive Tissue Evaluations and Results of Mating Trials

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

Study Parameters	Vehicle Control	0.1 mg/kg	1.0 mg/kg	3.3 mg/kg
Weights (g)				
Necropsy body weight	365 ± 5	351 ± 7	336 ± 5**	310 ± 8**
Right epididymis	0.450 ± 0.010	0.419 ± 0.007*	0.410 ± 0.004**	0.385 ± 0.012**
Right cauda epididymis	0.125 ± 0.003	0.122 ± 0.005	0.121 ± 0.004	0.118 ± 0.006
Right testis	1.519 ± 0.018	1.478 ± 0.027	1.466 ± 0.023*	1.404 ± 0.040*
Epididymal spermatozoal measurements				
Motility (%)	74.60 ± 3.58	65.00 ± 4.07	68.20 ± 2.87	68.80 ± 3.62
Concentration (10^6/g cauda epididymal tissue)	771.1 ± 44.6	718.7 ± 60.8	801.8 ± 45.5	849.0 ± 35.1
Abnormal sperm (%)	0.900 ± 0.100	0.800 ± 0.107	1.060 ± 0.085	0.920 ± 0.085

1 Data presented as mean ± standard error; n=10. Differences from the control group for cauda epididymal weights and spermatozoal measurements are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Shirley's (right epididymal weight) or Williams' test (right testicular weight).
** Significantly different (P≤0.01) from the control group by Shirley's (right epididymal weight) or Williams' test (necropsy body weight).

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Gavage Study of Riddelliine[1]

Study Parameters	Vehicle Control	0.1 mg/kg	1.0 mg/kg	10 mg/kg
Necropsy body weight	211 ± 5	207 ± 4	199 ± 4*	193 ± 3**
Estrous cycle length (days)	4.70 ± 0.15	4.75 ± 0.25[2]	4.40 ± 0.22	±[3]
Estrous stages (% of cycle)				
Diestrus	24.3	31.4	22.9	11.4
Proestrus	18.6	17.1	18.6	1.4
Estrus	35.7	32.9	31.4	81.4
Metestrus	21.4	18.6	24.3	5.7
Uncertain diagnoses	0.0	0.0	2.9	0.0

1 Data presented as mean ± standard error, n=10. Differences from the control group for estrous cycle length are not significant by Dunn's test. Evidence by multivariate analysis of variance suggests that rats in the 10mg/kg group differed significantly (P≤0.01, Wilk's criterion) from controls in the relative length of time spent in estrous stages.
2 Estrous cycle longer than seven days or unclear in 2 of 10 animals.
3 Estrous cycle longer than seven days or unclear in 10 of 10 animals.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine[1]

Study Parameters	Vehicle Control	0.33 mg/kg	3.3 mg/kg	25 mg/kg
Weights (g)				
Necropsy body weight	33.6 ± 1.9	36.3 ± 1.0	34.1 ± 1.2	28.6 ± 0.4**
Right epididymis	0.049 ± 0.001	0.047 ± 0.001	0.048 ± 0.001	0.043 ± 0.001**
Right cauda epididymis	0.013 ± 0.001	0.015 ± 0.001	0.015 ± 0.001[2]	0.012 ± 0.001
Right testis	0.122 ± 0.003	0.123 ± 0.003	0.127 ± 0.003	0.118 ± 0.002
Epididymal spermatozoal measurements				
Motility (%)	81.60 ± 2.19	76.40 ± 3.86	81.78 ± 1.93[2]	83.80 ± 1.41
Concentration (10^6/g cauda epididymal tissue)	1399 ± 93	1152 ± 93	1499 ± 71[2]	1643 ± 86
Abnormal sperm (%)	1.320 ± 0.127	1.120 ± 0.095	0.960 ± 0.160	1.360 ± 0.133

[1] Data presented as mean ± standard error; n=10. Differences from the control group for testicular weights are not significant by Dunnett's test; epididymal tail weights are not significant by Dunn's test; spermatozoal measurements are not significant by Dunn's or Shirley's test.

[2] n=9.

** Significantly different (P≤0.01) from the control group by Shirley's (right epididymal weight) or Williams' test (necropsy body weight).

TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of Riddelliine

Study Parameters[1]	Vehicle Control	0.33 mg/kg	3.3 mg/kg	25 mg/kg
Necropsy body weight	30.0 ± 0.8	27.7 ± 1.0	30.0 ± 0.8	24.2 ± 0.3**
Estrous cycle length (days)	4.83 ± 0.31[2]	4.38 ± 0.18[3]	4.20 ± 0.13	5.00 ± 0.00[4]
Estrous stages (% of cycle)				
Diestrus	25.0	28.6	24.3	40.0
Proestrus	21.4	18.6	22.9	8.6
Estrus	37.5	37.1	30.0	28.6
Metestrus	16.1	15.7	22.9	21.4
Uncertain diagnoses	0.0	0.0	0.0	1.4

[1] Data presented as mean ± standard error. For the 0.33, 3.3, and 25.0mg/kg dose groups, n=10. For the control group, n=8. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.

[2] Estrous cycle longer than seven days or unclear in 2 of 8 animals.

[3] Estrous cycle longer than seven days or unclear in 2 of 10 animals.

[4] Estrous cycle longer than seven days or unclear in 7 of 10 animals.

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

TABLE C5 Mean Body Weights and Length of Gestation for Female F344/N Rats in the Mating Trial of Riddelliine Administered by Gavage

	Vehicle Control	0.1 mg/kg	1.0 mg/kg
Dam Weight During Gestation[1]			
Day 0 weight	223 ± 3	209 ± 2**	208 ± 3**
Day 19 weight	278 ± 5	268 ± 3	264 ± 5*
Length of Gestation[1] (days)	22.11 ± 0.24	22.30 ± 0.13	22.26 ± 0.12
Dam Weight During Lactation[1] (g)			
Lactation day 0	240 ± 3	230 ± 2*	228 ± 4*
Lactation day 5	236 ± 2	223 ± 3**	218 ± 3**
Lactation day 14	261 ± 3	245 ± 3**	237 ± 4**
Lactation day 21	258 ± 4	241 ± 4**	241 ± 3**

[1] Data presented as mean ± standard deviation. All data were analyzed based on the number of males mated with at least one fertile female.

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

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

TABLE C6 Survival and Mean Body Weights of F344/N Rat Pups in the Mating Trial of Riddelliine Administered by Gavage

	Vehicle Control	0.1 mg/kg	1.0 mg/kg
Day 0			
No. of litters[1]	25	26	27
Live litter size[2]	7.0	8.0	8.1
Pups born alive[3] (%)	98.4 ± 0.9	98.0 ± 1.1	97.7 ± 1.1
Male pup weight	5.60 ± 0.09	5.45 ± 0.08	5.55 ± 0.07
Female pup weight	5.23 ± 0.07	5.08 ± 0.08	5.19 ± 0.06
Day 5			
Live litter size	6.2	7.5	7.9
Male survival (%)	90.2 ± 8.8	94.7 ± 4.0	100 ± 9
Female survival (%)	84.6 ± 7.0	92.4 ± 7.3	90.8 ± 4.6
Male and female survival (%)	85.0 ± 7.5	94.6 ± 1.7	92.5 ± 5.6
Male pup weight	9.97 ± 0.41	9.92 ± 0.27	9.60 ± 0.22
Female pup weight	9.79 ± 0.37	9.50 ± 0.30	9.30 ± 0.21
Day 14			
Live litter size	6.1	7.5	7.9
Male survival (%)	84.6 ± 8.3	95.3 ± 4.1	100 ± 9
Female survival (%)	86.1 ± 7.0	91.8 ± 7.3	90.8 ± 4.6
Male and female survival (%)	83.9 ± 7.5	94.6 ± 1.7	92.5 ± 5.6
Male pup weight	23.95 ± 0.80	23.17 ± 0.62	22.43 ± 0.43
Female pup weight	23.69 ± 0.62	22.19 ± 0.56	21.70 ± 0.39*
Day 21			
Live litter size	6.1	7.5	7.9
Male survival (%)	84.1 ± 8.2	95.3 ± 4.1	100 ± 9
Female survival (%)	86.8 ± 7.0	91.8 ± 7.3	90.8 ± 4.6
Male and female survival (%)	83.9 ± 7.5	94.6 ± 1.7	92.5 ± 5.6
Male pup weight	37.85 ± 1.34	36.41 ± 0.86	34.75 ± 0.76
Female pup weight	36.86 ± 1.25	34.39 ± 0.97	33.65 ± 0.64*

1 Number of litters delivered.

2 Mean live litter size.

3 Data presented as mean ± standard deviation. Pup weights, proportion of pups born alive, and pup survival were analyzed based on the number of treated males mated with at least one fertile female. Increases in survival over time indicate missexing of pups. Differences from the control group for percent pups born alive and percent pup survival are not significant by Dunn's test.

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

TABLE C7 Mean Body Weights and Length of Gestation for Female B6C3F$_1$ Mice in the Mating Trial of Riddelliine Administered by Gavage

	Vehicle Control	0.33 mg/kg	3.3 mg/kg	25mg/kg
Dam Weight During Gestation[1]				
Day 0 weight	30.3 ± 0.9	29.1 ± 0.4	28.7 ± 0.4	24.8 ± 0.7**
Day 18 weight	49.2 ± 1.0	48.8 ± 0.6	48.5 ± 1.2	50.3 ± 2.6
Length of Gestation[1] (days)	17.67 ± 0.67	18.75 ± 0.09	18.53 ± 0.14	17.91 ± 0.61
Dam Weight During Lactation[1] (g)				
Lactation day 0	33.6 ± 0.3	33.6 ± 0.4	33.3 ± 0.4	31.1 ± 0.7**
Lactation day 5	36.9 ± 0.4	36.4 ± 0.5	36.2 ± 0.3	31.9 ± 0.8**
Lactation day 14	38.4 ± 1.1	39.4 ± 0.4	39.0 ± 0.3	32.9 ± 0.7**
Lactation day 21	35.3 ± 0.5	35.2 ± 0.5	33.5 ± 0.3**	30.8 ± 0.5**

[1] Data presented as mean ± standard deviation. All data were analyzed based on the number of males mated with at least one fertile female.

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

TABLE C8 Survival and Mean Body Weights of B6C3F$_1$ Mouse Pups in the Mating Trial of Riddelliine Administered by Gavage

	Vehicle Control	0.33 mg/kg	3.3 mg/kg	25mg/kg
Day 0				
Number of litters[1]	35	36	38	30
Litter size[2]	9.7	9.5	9.6	5.1
Pups born alive[3] (%)	95.9 ± 2.7	98.1 ± 0.8	97.7 ± 1.0	76.6 ± 5.8**
Male pup weight	1.42 ± 0.02	1.41 ± 0.02	1.46 ± 0.02	1.17 ± 0.04**
Female pup weight	1.38 ± 0.02	1.38 ± 0.02	1.42 ± 0.02	1.15 ± 0.03**
Day 5				
Live litter size	9.7	9.3	9.4	4.4
Male survival (%)	98.1 ± 2.8	96.0 ± 3.0	94.0 ± 1.7	84.6 ± 4.0**
Female survival (%)	102 ± 3	101 ± 3	103 ± 2	96.2 ± 5.5
Male and female survival (%)	99.8 ± 0.4	97.6 ± 1.5	98.3 ± 0.3	88.3 ± 3.6**
Male pup weight	3.68 ± 0.09	3.68 ± 0.09	3.84 ± 0.07	2.84 ± 0.06**
Female pup weight	3.67 ± 0.08	3.66 ± 0.09	3.76 ± 0.05	2.91 ± 0.07**
Day 14				
Live litter size	9.3	9.0	9.4	4.1
Male survival (%)	94.9 ± 4.8	93.4 ± 2.7	94.0 ± 1.7	88.4 ± 4.9
Female survival (%)	95.3 ± 4.2	98.2 ± 3.0	103 ± 2	85.5 ± 8.6
Male and female survival (%)	94.7 ± 3.2	95.6 ± 2.3	98.3 ± 0.9	84.7 ± 4.4*
Male pup weight	7.28 ± 0.14	7.72 ± 0.16	7.92 ± 0.12*	6.06 ± 0.19**
Female pup weight	7.44 ± 0.13	7.71 ± 0.15	7.95 ± 0.10	6.10 ± 0.20**
Day 21				
Live litter size	9.2	8.9	9.4	4.1
Male survival (%)	91.4 ± 4.8	92.2 ± 2.9	94.0 ± 1.7	89.5 ± 4.7
Female survival (%)	97.0 ± 4.8	97.7 ± 2.6	103 ± 2	84.3 ± 8.5
Male and female survival (%)	94.0 ± 3.6	94.8 ± 2.4	98.3 ± 0.9	84.7 ± 4.4
Male pup weight	11.34 ± 0.19	11.81 ± 0.26	12.23 ± 0.15	9.25 ± 0.27**
Female pup weight	10.90 ± 0.18	11.14 ± 0.25	11.52 ± 0.10	9.35 ± 0.30**

[1] Number of litters delivered.

[2] Mean live litter size.

[3] Data presented as mean ± standard deviation. Pup weights, proportion of pups born alive, and pup survival were analyzed based on the number of males mated with at least one fertile female.

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

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

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of Riddelliine in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+ hamster S9 10%	+ hamster S9 30% Trial 1	+ hamster S9 30% Trial 2	+ rat S9 10%	+ rat S9 30% Trial 1	+ rat S9 30% Trial 2
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
	1000	136 ± 4.4	129 ± 4.2	153 ± 4.0	209 ± 13.5	218 ± 11.1	186 ± 7.2	331 ± 15.5	326 ± 12.8
	3333	147 ± 12.7	176 ± 6.7	166 ± 9.8	291 ± 13.5	315 ± 12.7	186 ± 3.5	355 ± 14.9[3]	370 ± 6.2
	5000	150 ± 3.0	188 ± 7.8	196 ± 4.6	342 ± 15.1	305 ± 26.4	185 ± 7.4	363 ± 2.1[3]	381 ± 7.3
Trial summary		Negative	Negative	Negative	Positive	Positive	Negative	Positive	Positive
Positive control[4]		1499 ± 63.7	1484 ± 24.8	2108 ± 55.9	1009 ± 33.8	889 ± 9.2	2373 ± 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	
	1000	34 ± 6.2	29 ± 5.0	20 ± 2.4	13 ± 1.5		20 ± 1.7	8 ± 2.0	
	3333	30 ± 3.6	27 ± 2.2	19 ± 1.0	7 ± 0.7		16 ± 0.9	6 ± 0.6[3]	
	5000	32 ± 3.8	23 ± 3.5	23 ± 3.8	5 ± 1.2[3]		17 ± 1.7	8 ± 1.2[3]	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative	
Positive control		1379 ± 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[3]
	333	106 ± 6.7	85 ± 0.9	139 ± 3.5	160 ± 11.0		133 ± 5.8	184 ± 6.4	184 ± 11.5[3]
	1000	110 ± 5.6	5 ± 1.5	152 ± 7.2	159 ± 5.0		132 ± 5.8	212 ± 4.9	208 ± 20.8[3]
	3333	103 ± 2.6	8 ± 0.3	140 ± 1.5	163 ± 2.9		120 ± 7.9	232 ± 13.3[3]	177 ± 17.9[3]
	5000	97 ± 3.7	10 ± 1.0[3]	141 ± 4.7	168 ± 10.5[3]		147 ± 4.9	248 ± 20.3[3]	209 ± 3.5[3]
Trial summary		Negative	Negative	Negative	Negative		Negative	Equivocal	Equivocal
Positive control		1009 ±80.5	698 ±36.0	1395 ± 54.8	573 ±19.4		1636 ± 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	
	1000	20 ± 4.3	22 ± 2.3	32 ± 2.1	29 ± 1.3		30 ± 2.1	27 ± 1.9	
	3333	16 ± 3.1	20 ± 5.5	29 ± 2.7	31 ± 4.3		26 ± 0.9	26 ± 2.8[3]	
	5000	16 ± 0.7	18 ± 6.1	31 ± 2.0	24 ± 1.8		32 ± 4.7	20 ± 5.2[3]	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative	
Positive control		1913 ± 86.1	1392 ± 19.9	1076 ± 37.1	715 ± 22.8		1731 ± 26.0	419 ± 5.0	

1 Study performed at Microbiological Associates, Inc. The detailed protocol and these data are presented in Zeiger *et al.* (1988).

2 Revertants are presented as mean ± standard error from three plates; 0 ±g/plate is the solvent control.

3 Slight toxicity.

4 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 D2 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Riddelliine[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent[2] (%)
-S9								
Summary: Positive								
Dimethylsulfoxide		50	1031	432	0.41	8.6	25.5	
Mitomycin-C	0.001	50	1022	671	0.65	13.4	25.5	56.69
	0.01	5	105	240	2.28	48.0	25.5	445.50
Riddelliine	30	50	1028	538	0.52	10.8	25.5	24.90*
	100	50	1040	702	0.67	14.0	25.5	61.09*
	300	50	1036	1224	1.18	24.5	25.5	181.96*
								P<0.001[3]
+S9								
Summary: Positive								
Dimethylsulfoxide		50	1018	432	0.42	8.6	26.0	
Cyclophosphamide	0.3	50	1009	538	0.53	10.8	26.0	25.65
	2	5	101	141	1.39	28.2	26.0	228.97
Riddelliine	3	50	1017	1537	1.51	30.7	26.0	256.14*
	10	20	408	1539	3.77	77.0	26.0	788.88*
	30	3	63	441	7.00	147.0	26.0	1549.54*
	100	0					35.0[4]	
								P<0.001

1 Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the protocol and these data are presented in Galloway *et al.* (1987).

2 Percentage increase in SCEs/chromosome of cells exposed to riddelliine relative to cells exposed to solvent.

3 Significance was tested by the linear regression trend test versus log of the dose.

4 Because riddelliine induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis. No suitable cells were found.

* Positive (>20% increase over solvent control).

TABLE D3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Riddelliine[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time: 10.5 hours					Harvest time: 20.5 hours[2]				
Summary: Negative					Summary: Positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	1	0.01	1.0		100	3	0.03	3.0
Mytomycin-C					Cyclophosphamide				
1	50	14	0.28	18.0	15	50	9	0.18	16.0
Riddelliine					Riddelliine				
402	100	0	0.00	0.0	300	25	73	2.92	92.0*
498	100	1	0.01	1.0	400	25	64	2.56	76.0*
600	100	2	0.02	2.0	498	25	73	2.92	96.0*
					600[3]	0			
				P=0.186[4]				P<0.001	

1 Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are found in Galloway *et al.* (1987). The high dose, in the absence of S9, was limited by solubility of riddelliine.

2 Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.

3 Not scored due to poor chromosome morphology.

4 Significance of percent cells with aberrations was tested by the linear regression trend test versus log of the dose.

* Positive (P≤0.05).

TABLE D4 Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes Following Treatment with Riddelliine by Gavage for 4 Weeks[1]

Dose (mg/kg)	Micronucleated NCEs/ 1000 NCEs	PCEs (%)	Number of Animals
MALE			
0	1.44 ± 0.08	1.62 ± 0.06	5
3.3	1.95 ± 0.28	2.14 ± 0.33	4
10.0	1.91 ± 0.19	1.71 ± 0.41	4
25.0	1.45 ± 0.30	2.10 ± 0.14	4
Trend test[2]	P=0.912		
ANOVA[3]		P=0.269	
FEMALE			
0	1.69 ± 0.36	2.25 ± 0.47	4
3.3	1.15 ± 0.20	2.36 ± 0.20	5
10.0	1.01 ± 0.27	2.19 ± 0.22	5
25.0	1.46 ± 0.36	2.05 ± 0.09	4
Trend test	P=0.480		
ANOVA		P=0.413	

1 Study performed at Western Regional Research Center, United States Department of Agriculture, Albany, CA. PCEs=polychromatic erythrocytes; NCEs=normochromatic erythrocytes. Data presented as mean ± standard error of the group.

2 Analysis of variance using the SAS GLM procedure.

3 Analysis of variance on ranks.

TABLE D5 Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes Following Treatment with Riddelliine by Gavage for 13 Weeks[1]

Dose (mg/kg)	Micronucleated Cells/1000 Cells			Number of Animals
	PCEs	NCEs	PCEs (%)	
MALE				
0	2.24 ± 0.50	1.33 ± 0.10	2.33 ± 0.13	10
10	2.42 ± 0.42	1.58 ± 0.13	2.46 ± 0.08	10
25	1.42 ± 0.15	1.55 ± 0.09	2.13 ± 0.14	9
Trend test[2]	P=0.910	P=0.087		
ANOVA[3]			P=0.226	
FEMALE				
0	1.09 ± 0.13	1.14 ± 0.08	1.98 ± 0.11	8
10	1.04 ± 0.10	1.15 ± 0.06	2.14 ± 0.13	10
25	1.88 ± 0.35	1.28 ± 0.07	2.69 ± 0.55	10
Trend test	P=0.043	P=0.179		
ANOVA			P=0.457	

1 PCEs=polychromatic erythrocytes; NCEs=normochromatic erythrocytes. Smears were prepared from peripheral blood samples from the animals used in the 13-week toxicity study. Data presented as mean ± standard error of the group. Two thousand PCEs and 10,000NCEs were scored per animal.

2 Cochran-Armitage linear regression of proportions for PCEs or analysis of variance using the SAS GLM procedure for NCEs.

3 Analysis of variance on ranks.

TABLE D6 Frequency of Micronucleated Polychromatic Erythrocytes in Peripheral Blood and Bone Marrow of Male B6C3F$_1$ Mice Administered a Single Treatment of Riddelliine by Gavage[1]

Dose (mg/kg)	Micronucleated PCEs/ 1000 PCEs	PCE (%)	Number of Animals
48-HOUR BLOOD SAMPLE			
Urethane[2]			
200	14.93 ± 1.53	2.71 ± 0.32	
Riddelliine			
0	1.65 ± 0.41	3.55 ± 0.30	8
75	2.40 ± 0.49	3.17 ± 0.34	8
150	3.62 ± 0.75**	2.38 ± 0.42*	8
300			0
Trend test[3]	P=0.005		
ANOVA[4]		P=0.105	
24-HOUR BONE MARROW SAMPLE			
Urethane			
200	16.38 ± 0.60	54.88 ± 1.44	
Riddelliine			
0	1.88 ± 0.30	59.50 ± 1.24	8
75	3.00 ± 0.42	56.63 ± 2.08	8
150	2.00 ± 0.38	51.38 ± 2.72**	8
270	3.43 ± 0.57*	48.14 ± 2.43**	8
300	4.00	61.00	1
Trend test	P=0.071		
ANOVA		P=0.006	

1 Study performed at Western Regional Research Center, United States Department of Agriculture, Albany, CA. Data are presented as mean ± standard error.
2 Positive control.
3 Cochran-Armitage linear regression of proportions.
4 Analysis of variance on ranks.
* P<0.05.
** P<0.01.

TABLE D7 Induction of Unscheduled DNA Synthesis and S-Phase DNA Synthesis by Riddelliine in the Hepatocytes of F344/N Rats Following 5 Days or 30 Days of Treatment[1]

Dose (mg/kg)	Cells (%)		Number of Animals
	Unscheduled DNA Synthesis	S-Phase Synthesis	
FOLLOWING 5 DAYS OF TREATMENT			
Male			
0	1.10 ± 0.49	0.99 ± 0.09	5
0.33	2.66 ± 1.35	6.08 ± 0.82**	5
1.0	4.18 ± 1.25*[2]	2.90 ± 0.47	5
3.3	2.20 ± 0.60	2.33 ± 0.29	5
Female			
0	0.66 ± 0.27	0.49 ± 0.04	5
0.33	1.98 ± 0.54	2.25 ± 0.68**	5
1.0	4.22 ± 0.83**	4.77 ± 1.22**	5
3.3	7.56 ± 3.50**	9.06 ± 1.56**	5
FOLLOWING 30 DAYS OF TREATMENT			
Male			
0	0.22 ± 0.22	0.52 ± 0.09	5
0.33	0.66 ± 0.27	1.84 ± 0.21**	5
1.0	0.66 ± 0.44	2.42 ± 0.49**	5
3.3	2.20 ± 0.64*	1.24 ± 0.29*	4
Female			
0	0.22 ± 0.22	0.52 ± 0.12	5
0.33	0.22 ± 0.22	0.82 ± 0.27	5
1.0	0.88 ± 0.41	1.86 ± 0.30**	5
3.3	1.10 ± 0.70	1.15 ± 0.15*	5

1 Study performed at SRI International. Data presented as mean ± standard error.

2 n=3.

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

** Significantly different from the control group (P≤0.01) by Dunn's test or Shirley's test.

TABLE D8 Induction of Unscheduled DNA Synthesis and S-Phase DNA Synthesis by Riddelliine in the Hepatocytes of B6C3F$_1$ Mice Following 5 Days or 30 Days of Treatment[1]

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

[1] Study performed at SRI International. Data presented as mean ± standard error.

* Significantly different from the control group (P≤0.05) by Dunn's or Shirley's test.

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