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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of 5-(Hydroxymethyl)-2-Furfural (CAS No. 67-47-0) in F344/N Rats and B6C3F1 Mice (Gavage Studies)

NTP TR 554

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 5-(HYDROXYMETHYL)-2-FURFURAL

(CAS NO. 67-47-0)

IN F344/N RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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FOREWORD

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

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

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

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CONTENTS

SUMMARY

Background

5-(Hydroxymethyl)-2-furfural occurs when sugars are heated in the presence of amino acids. Thus, it occurs naturally in many foods, including dried fruits, caramel products, certain fruit juices, and instant coffee. We studied the effects of 5-(hydroxymethyl)-2-furfural on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We deposited solutions containing 5-(hydroxymethyl)-2-furfural in deionized water directly into the stomach through a tube to groups of 50 male and female rats and mice for 2 years. Exposed animals received either 188, 375, or 750 milligrams (mg) of 5-(hydroxymethyl)-2-furfural per kilogram (kg) of body weight. Control animals received deionized water with no chemical added by the same method. At the end of the study, tissues from more than 40 sites were examined for every animal.

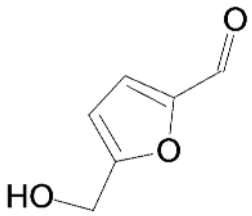
Results

There were increased incidences of lesions (degeneration and metaplasia) of the olfactory and respiratory epithelium of the nose in male and female rats and male and female mice that received 5-(hydroxymethyl)-2-furfural. Many of the male and female mice receiving 750 mg/kg died before the end of the study, and some exhibited seizures or other signs of neurological response. In the other two groups of female mice receiving 5-(hydroxymethyl)-2-furfural, there were increased incidences of hepatocellular adenoma of the liver.

Conclusions

We conclude that 5-(hydroxymethyl)-2-furfural caused liver cancer in female mice but did not cause cancer in male or female rats or male mice. In addition, 5-(hydroxymethyl)-2-furfural was associated with increased lesions of the olfactory and respiratory epithelium of the nose in male and female rats and mice.

ABSTRACT



5-(HYDROXYMETHYL)-2-FURFURAL

CAS No. 67-47-0

Chemical Formula: $C_6H_6O_3$ Molecular Weight: 126.11

Synonyms: 5-Hydroxymethyl-2-formylfuran; 5-(hydroxymethyl)-2-furaldehyde; 5-(hydroxymethyl)-2-furancarbonal; 5-(hydroxymethyl)-2-furancarboxaldehyde (9CI); hydroxymethyl furfuraldehyde; 5-oxymethylfurfurole

5-(Hydroxymethyl)-2-furfural is formed when reducing sugars such as fructose and glucose are heated in the presence of amino acids. 5-(Hydroxymethyl)-2-furfural is ubiquitous in the human diet and occurs at concentrations greater than 1 g/kg in dried fruits, caramel products, and certain types of fruit juices and at up to 6.2 g/kg in instant coffee. 5-(Hydroxymethyl)-2-furfural also occurs naturally and has been identified in honey, apple juice, citrus juices, beer, brandy, milk, breakfast cereal, baked foods, tomato products, and home cooking of sugar and carbohydrates. Industrially, 5-(hydroxy-methyl)-2-furfural is used in the synthesis of dialdehydes, glycols, ethers, aminoalcohols, acetals, and phenol/furfural novolak-type resins. 5-(Hydroxymethyl)-2-furfural was nominated by the National Institute of Environmental Health Sciences for study because of extensive human exposure and the lack of adequate data characterizing its toxicity and carcinogenicity. Male and female F344/N rats and B6C3F1 mice were administered 5-(hydroxymethyl)-2-furfural (at least 99% pure) by gavage in deionized water for 3 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli* and mouse peripheral blood erythrocytes.

3-Week Study in Rats

Core study groups of five male and five female rats were administered 0, 94, 188, 375, 750, or 1,500 mg 5-(hydroxymethyl)-2-furfural/kg body weight in deionized water by gavage for a total of 13 doses over a 22-day period. Special study groups of five male and five female rats designated for neuropathology were administered 0 or 1,500 mg/kg on the same schedule. Except for one 1,500 mg/kg core study male rat, all rats survived to the end of the study. The final mean body weight of 1,500 mg/kg males was significantly less than that of the vehicle control group. No chemical-related histopathologic lesions were observed in core or special study animals.

3-Week Study in Mice

Groups of five male and five female mice were administered 0, 94, 188, 375, 750, or 1,500 mg 5-(hydroxymethyl)-2-furfural/kg body weight in deionized water by gavage for a total of 13 doses over a 22-day period. Three male and three female mice administered 1,500 mg/kg died before the end of the study. Mean body weights of 1,500 mg/kg males were significantly

less than those of the vehicle control group. Heart weights of 1,500 mg/kg females were significantly greater than those of the vehicle controls. No chemical-related lesions were observed.

3-Month Study in Rats

Core groups and special study groups (for clinical pathology and neuropathologic evaluation) of 10 male and 10 female rats were administered 0, 94, 188, 375, 750, or 1,500 mg 5-(hydroxymethyl)-2-furfural/kg body weight in deionized water by gavage for 3 months. One male and three female rats administered 1,500 mg/kg died before the end of the study; the male died as a result of gavage trauma. Mean body weights of 750 and 1,500 mg/kg males were significantly less than those of the vehicle control group. Female rats had elongated estrous cycles; fewer 750 and 1,500 mg/kg females had regular cycles, and 375, 750, and 1,500 mg/kg females had a significantly increased probability of extended diestrus. No chemical-related lesions were observed in core or special study animals.

3-Month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 47, 94, 188, 375, or 750 mg 5-(hydroxymethyl)-2-furfural/kg body weight in deionized water by gavage for 3 months. One 750 mg/kg male and one 375 mg/kg female died before the end of the study; the death of the female was attributed to ovarian teratoma. The final mean body weight of 750 mg/kg males and body weight gains of 750 mg/kg males and females were significantly less than those of the vehicle controls. The incidences of minimal to mild cytoplasmic alteration of the kidney were significantly increased in males administered 188 mg/kg or greater.

2-Year Study in Rats

Groups of 50 male and 50 female rats were administered 0, 188, 375, or 750 mg 5-(hydroxymethyl)-2-furfural/kg body weight in deionized water by gavage for 104 weeks. Survival of 188 and 750 mg/kg males was greater than that of the vehicle control group. Mean body weights of dosed groups of males and females were generally similar to those of the vehicle controls throughout the study.

Incidences of olfactory epithelium degeneration were significantly increased in 750 mg/kg males and 188 and 375 mg/kg females. Incidences of olfactory epithelium respiratory metaplasia and respiratory epithelium squamous metaplasia were significantly increased in 750 mg/kg males and females. Incidences of suppurative inflammation of the nose and chronic active inflammation of the nasolacrimal duct were significantly increased in 750 mg/kg females.

2-Year Study in Mice

Groups of 50 male and 50 female mice were administered 0, 188, 375, or 750 mg 5-(hydroxymethyl)-2-furfural/kg body weight in deionized water by gavage for 104 weeks. Survival of 750 mg/kg males and females was significantly less than that of the vehicle control groups. Mean body weights of 750 mg/kg males were 14% less than those of the vehicle controls after week 26. Mean body weights of 375 and 750 mg/kg females were 9% and 30% less, respectively, than those of the vehicle controls after week 36. Beginning in month 8 and continuing until the end of the study, 750 mg/kg males and females exhibited clinical signs indicative of neurological effects of 5-(hydroxymethyl)-2-furfural administration. These signs included decreased exploratory behavior, piloerection, salivation, Straub tail, catatonia, excitation, dyspnea, clonic-tonic seizures, and unconsciousness. Because of the reduced survival of this group and the presence of the treatment-related clinical signs, groups of mice that received 750 mg/kg were not included in the evaluation of carcinogenic potential.

The incidences of hepatocellular adenoma were significantly increased in 188 and 375 mg/kg females.

In the nose, the incidences of olfactory epithelium metaplasia, degeneration, and hyaline droplet accumulation; chronic active inflammation; respiratory epithelium hyaline droplet accumulation; and hyperplasia, dilatation, and chronic active inflammation of the glands were significantly increased in 375 and 750 mg/kg males and females. Incidences of olfactory epithelium hyperplasia were significantly increased in 375 and 750 mg/kg females.

Genetic Toxicology

5-(Hydroxymethyl)-2-furfural was tested in two independent bacterial mutagenicity assays. In the first study, the chemical was weakly mutagenic in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation; no mutagenic activity was detected in TA100 with activation or in strains

TA97, TA98, TA102, or TA1535, with or without activation. In the second study, no mutagenicity was detected, with or without activation, in TA98 or TA100 or *Escherichia coli* WP2 *uvrA*/pKM101. No increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female mice administered 5-(hydroxymethyl)-2-furfural by gavage for 3 months.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of 5-(hydroxymethyl)-2-furfural in male or female F344/N rats administered 188, 375, or 750 mg/kg. There was *no evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in male B6C3F1 mice administered 188 or 375 mg/kg. There was *some evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in female B6C3F1 mice based on increased incidences of hepatocellular adenoma in the 188 and 375 mg/kg groups.

Administration of 5-(hydroxymethyl)-2-furfural was associated with increased incidences of lesions of the olfactory and respiratory epithelium of the nose in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 5-(Hydroxymethyl)-2-furfural

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in deionized water administered by gavage	0, 188, 375, or 750 mg/kg	0, 188, 375, or 750 mg/kg	0, 188, 375, or 750 mg/kg	0, 188, 375, or 750 mg/kg
Body weights	Dosed groups similar to vehicle control group	Dosed groups similar to vehicle control group	750 mg/kg group was 14% less than vehicle control group after week 26	375 mg/kg group 9% less and 750 mg/kg group 30% less than vehicle control group after week 36
Survival rates	22/50, 34/50, 31/50, 35/50	31/50, 32/50, 27/50, 30/50	40/50, 35/50, 43/50, 15/49	39/50, 42/50, 32/50, 22/50
Nonneoplastic effects	Nose: olfactory epithelium, degeneration (18/50, 22/49, 26/48, 29/49); olfactory epithelium, metaplasia, respiratory (2/50, 5/49, 3/48, 11/49); respiratory epithelium, metaplasia, squamous (0/50, 2/49, 1/48, 16/49)	Nose: olfactory epithelium, degeneration (21/50, 35/49, 36/49, 28/49); olfactory epithelium, metaplasia, respiratory (1/50, 1/49, 0/49, 11/49); respiratory epithelium, metaplasia, squamous (1/50, 1/49, 0/49, 24/49); inflammation, suppurative (0/50, 0/49, 0/49, 8/49); nasolacrimal duct, inflammation, chronic (2/50, 2/49, 3/49, 12/49)	Nose: olfactory epithelium, metaplasia (1/50, 7/50, 38/50, 43/47); inflammation, chronic active (0/50, 6/50, 18/50, 45/47); glands, inflammation, chronic active (4/50, 12/50, 34/50, 43/47); glands, hyperplasia (3/50, 7/50, 45/50, 45/47); glands, dilatation (16/50, 22/50, 47/50, 45/47); olfactory epithelium, degeneration (4/50, 2/50, 17/50, 39/47); olfactory epithelium, accumulation, hyaline droplet (13/50, 17/50, 29/50, 27/47); respiratory epithelium, accumulation, hyaline droplet (14/50, 17/50, 23/50, 31/47)	Nose: olfactory epithelium, metaplasia (1/49, 5/49, 30/50, 40/50); inflammation, chronic active (0/49, 1/49, 14/50, 41/50); glands, inflammation, chronic active (1/49, 6/49, 21/50, 38/50); glands, hyperplasia (0/49, 7/49, 42/50, 43/50); glands, dilatation (12/49, 36/49, 48/50, 47/50); olfactory epithelium, degeneration (2/49, 1/49, 34/50, 24/50); olfactory epithelium, accumulation, hyaline droplet (1/49, 1/49, 27/50, 25/50); respiratory epithelium, accumulation, hyaline droplet (4/49, 4/49, 36/50, 27/50); olfactory epithelium, hyperplasia (0/49, 0/49, 8/50, 24/50)
Neoplastic effects	None	None	None	Liver: hepatocellular adenoma (14/50, 26/49, 26/50, 6/50)
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	Some evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:		Weakly positive in strain TA100 without S9; negative in TA100 with S9; negative in strains TA97, TA98, TA102, and TA1535 and in *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative in males and females		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

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

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 5-(hydroxymethyl)-2-furfural on February 28, 2008, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 28, 2008, the draft Technical Report on the toxicology and carcinogenesis studies of 5-(hydroxymethyl)-2-furfural received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of 5-(hydroxymethyl)-2-furfural by describing its ubiquitous occurrence in foods, reviewing the background literature relating to its metabolic activation to a potential carcinogen, and describing the design and results of the short- and long-term NTP studies. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in male or female F344/N rats, *no evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in male B6C3F1 mice, and *some evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in female B6C3F1 mice.

Dr. Mirsalis, the first principal reviewer, thought the study was well performed and written. He inquired if some overall human exposure level could be estimated.

Dr. Soper, the second principal reviewer, also agreed with the proposed conclusions. He suggested that a trend test should not include a group that exceeded a maximum tolerated dose that was not used for consideration of carcinogenic response.

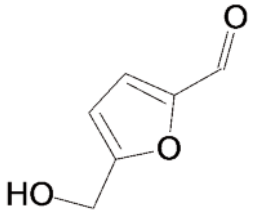
Dr. Pino, the third principal reviewer, inquired about thyroid gland C-cell adenomas and liver foci that were mentioned in the results but not in the summary. Dr. Irwin replied that certain increases in lesion incidences in particular groups are mentioned for completeness even if they are not considered treatment related.

Dr. P.C. Howard, National Center for Toxicological Research, said that inclusion of estimates of human consumption compared with doses administered would be quite difficult to calculate given the wide variety of diets, and inclusion in the discussion would enter the domain of risk assessment.

Dr. Crump also thought the occurrence of the thyroid gland tumors seemed noteworthy. Dr. Irwin noted that the control incidence in this study was unusually low.

Dr. Mirsalis moved, and Dr. Soper seconded, that the conclusions be accepted as written. The motion was approved unanimously with eight votes.

INTRODUCTION



5-(HYDROXYMETHYL)-2-FURFURAL

CAS No. 67-47-0

Chemical Formula: $C_6H_6O_3$ Molecular Weight: 126.11

Synonyms: 5-Hydroxymethyl-2-formylfuran; 5-(hydroxymethyl)-2-furaldehyde; 5-(hydroxymethyl)-2-furancarbonal; 5-(hydroxymethyl)-2-furancarboxaldehyde (9CI); hydroxymethyl furfuraldehyde; 5-oxymethylfurfurole

Chemical and Physical Properties

5-(Hydroxymethyl)-2-furfural occurs as a solid (needles) or a dark yellow liquid or powder with the odor of chamomile flowers (*Merck*, 1989; MSDS, 1994; Lewis, 1997). 5-(Hydroxymethyl)-2-furfural has a boiling point of 110° C at 0.02 mm mercury and 114° to 116° C at 1 mm mercury, a melting point of 31.5° to 35° C, a density of 1.2062, a refractive index of 1.5627 at 18° C, and a flash point of 79° C; it is freely soluble in water, methanol, ethanol, acetone, ethyl acetate, and dimethylformamide; soluble in ether, benzene, and chloroform; less soluble in carbon tetrachloride; and sparingly soluble in petroleum ether (*Merck*, 1989; MSDS, 1994).

Production, Use, and Human Exposure

5-(Hydroxymethyl)-2-furfural is a major product of the Maillard reaction, a sequence of nonenzymatic browning reactions that occur when reducing sugars such as fructose and glucose are heated in the presence of amino acids (Antal *et al.*, 1990). A recent examination of approximately 500 different food samples revealed 5-(hydroxymethyl)-2-furfural concentrations greater than 1 g/kg in dried fruits, caramel products, and certain types of fruit juices; up to 3.5 g/kg in dried pears (Bachmann *et al.*, 1997); and up to 6.2 g/kg in instant coffee (Schultheiss *et al.*, 2000). 5-(Hydroxymethyl)-2-furfural also has been identified in honey, apple juice, citrus juices, beer, brandy, milk, breakfast cereal baked foods, tomato products, and home cooking of sugar and carbohydrates (Jeuring and Kuppers, 1980; Li *et al.*, 1988; Blanco Gomis *et al.*, 1991; Porretta and Sandei, 1991; García-Villanova *et al.*, 1993). Therefore, 5-(hydroxymethyl)-2-furfural is ubiquitous in the human diet.

Ulbricht *et al.* (1984) reported the results of several studies that determined the concentrations of 5-(hydroxymethyl)-2-furfural in parenteral solutions. In sterile glucose solutions, 5-(hydroxymethyl)-2-furfural concen-trations of approximately 1 to 90 mg/L of solution have been reported. Parenteral solutions containing inver-tose or glucose have been reported to have 5-(hydroxy-methyl)-2-furfural concentrations ranging from 3 to 56 mg/L and 1 to 4 mg/L, respectively. 5-(Hydroxy-methyl)-2-furfural concentration correlated positively with high acidity (pH less than 4), high sterilization temperature (greater than 110° C), and a long sterilization time (30 minutes). 5-(Hydroxymethyl)-2-furfural has been identified in solutions containing fructose for intravenous injection (Jellum *et al.*, 1973). It appears to form during sterilization whenever a fructose-containing solution with a pH lower than 3.5 to 4.0 is heated to 110° to 130° C. 5-(Hydroxymethyl)-2-furfural has been detected in caramel, a widely used coloring agent in food and pharmaceutical syrups (Hewala *et al.*, 1993). While the reported levels of 5-(hydroxymethyl)-

2-furfural in pharmaceutical syrups is very low, the possibility of interaction between it and active drugs containing an amino group appears to be of some concern. 5-(Hydroxy-methyl)-2-furfural forms in milk at temperatures higher than 120° C, and its formation in milk is attributed to the presence of the reducing sugar lactose (Morales *et al.*, 1992).

5-(Hydroxymethyl)-2-furfural is used in the synthesis of dialdehydes, glycols, ethers, aminoalcohols, and acetals (*Merck*, 1989). Generation of 5-(hydroxymethyl)-2-furfural from glucose in the presence of phenol is used to produce phenol/furfural novolak-type resins (Brode, 1982). Acid-catalyzed dehydration of 5-(hydroxymethyl)-2-furfural yields 5,5′-oxydimethylenebis (2-furfural), an intermediate in the synthesis of several crown ethers (Larousse *et al.*, 1992). Due to its various functionalities, it has been proposed that 5-(hydroxymethyl)-2-furfural could be utilized to produce a wide range of products such as polymers, surfactants, solvents, pharmaceuticals, and plant protection agents (Kunz, 1993).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Ulbricht *et al.* (1984) reviewed earlier pharmacokinetic studies of 5-(hydroxymethyl)-2-furfural. No *in vivo* radiolabelling studies were reported. Species tested included rabbits, dogs, chickens, rats, and mice. In rabbits, dogs, and chickens, hydroxymethyl furoic acid was found in the urine at levels of 44% to 67% of the 5-(hydroxymethyl)-2-furfural administered (dose, route, and species differences were not reported) (Ulbricht *et al.*, 1984). Lang *et al.* (1970) found 12 mg of 5-(hydroxymethyl)-2-furfural in the urine of rabbits administered 1 g of the chemical in feed; no other metabolites were found. Rats and mice given 100 mg 5-(hydroxymethyl)-2-furfural/kg, either orally or intravenously, showed only trace amounts in urine, blood plasma, and bile samples (Czok, 1970). No information was provided pertaining to dosing regimen (single or repeated), time between dosing and sampling, or metabolite(s) found.

The disposition and metabolism of 5-(hydroxymethyl)-2-furfural has been examined in male Sprague-Dawley rats using uniformly labeled ^{14}C-5-(hydroxymethyl)-2-furfural (Germond *et al.*, 1987). Nearly all (95% to 98%) of the administered radioactivity was eliminated in the urine within 24 hours; less than 1% was eliminated as CO_2, and less than 1% remained in the carcass. The urine contained two metabolites, 5-(hydroxymethyl)-2-furoic acid and 5-(hydroxymethyl)-2-furoylglycine, which together accounted for all the radioactivity present in the urine. Urinary elimination was rapid with 85% of the administered dose eliminated during the first 8 hours after administration at all dose levels examined. At low doses (0.08 to 1.3 mg/kg), 5-(hydroxymethyl)-2-furoylglycine predominated; however, at doses greater than 13 mg/kg, 5-(hydroxymethyl)-2-furoic acid predominated. At the highest dose administered (330 mg/kg), the initial concentration of the furoic acid metabolite was 20- to 30-fold greater than the glycine conjugate; however, administration of 3 g of glycine prior to administration of 330 mg/kg 5-(hydroxymethyl)-2-furfural resulted in an initial concentration ratio of only 3-fold, suggesting that formation of the glycine conjugate was limited by glycine availability.

The disposition and metabolism of ^{14}C-5-(hydroxymethyl)-2-furfural has also been examined in F344 rats and B6C3F1 mice (Godfrey *et al.*, 1999). Following oral administration of 5, 10, 100, or 500 mg/kg, tissue distribution results indicated that absorption of 5-(hydroxymethyl)-2-furfural is rapid in male rats and mice and that tissue concentrations in male mice at the earliest time point are not linearly proportional to dose. Excretion was primarily via the urine in both rats and mice with 60% to 80% of the administered dose excreted in the urine within 48 hours after administration. The major urinary metabolite in both rats and mice, accounting for 80% of the administered radioactivity, was 5-(hydroxymethyl)-2-furoic acid. Two other metabolites identified in the urine were N-(5-hydroxymethyl)-2-furoylglycine and furandicarboxylic acid, suggesting the metabolic scheme shown in Figure 1. None of the metabolites were sulfate conjugates nor likely to be formed from sulfate conjugates. There were relatively low levels of nonextractable radioactivity in liver, kidney, and intestines.

Humans

No studies of the absorption, distribution, metabolism, and excretion of 5-(hydroxymethyl)-2-furfural in humans were found in the literature.

Toxicity

No studies of the toxicity of 5-(hydroxymethyl)-2-furfural in experimental animals or humans were found in the literature.

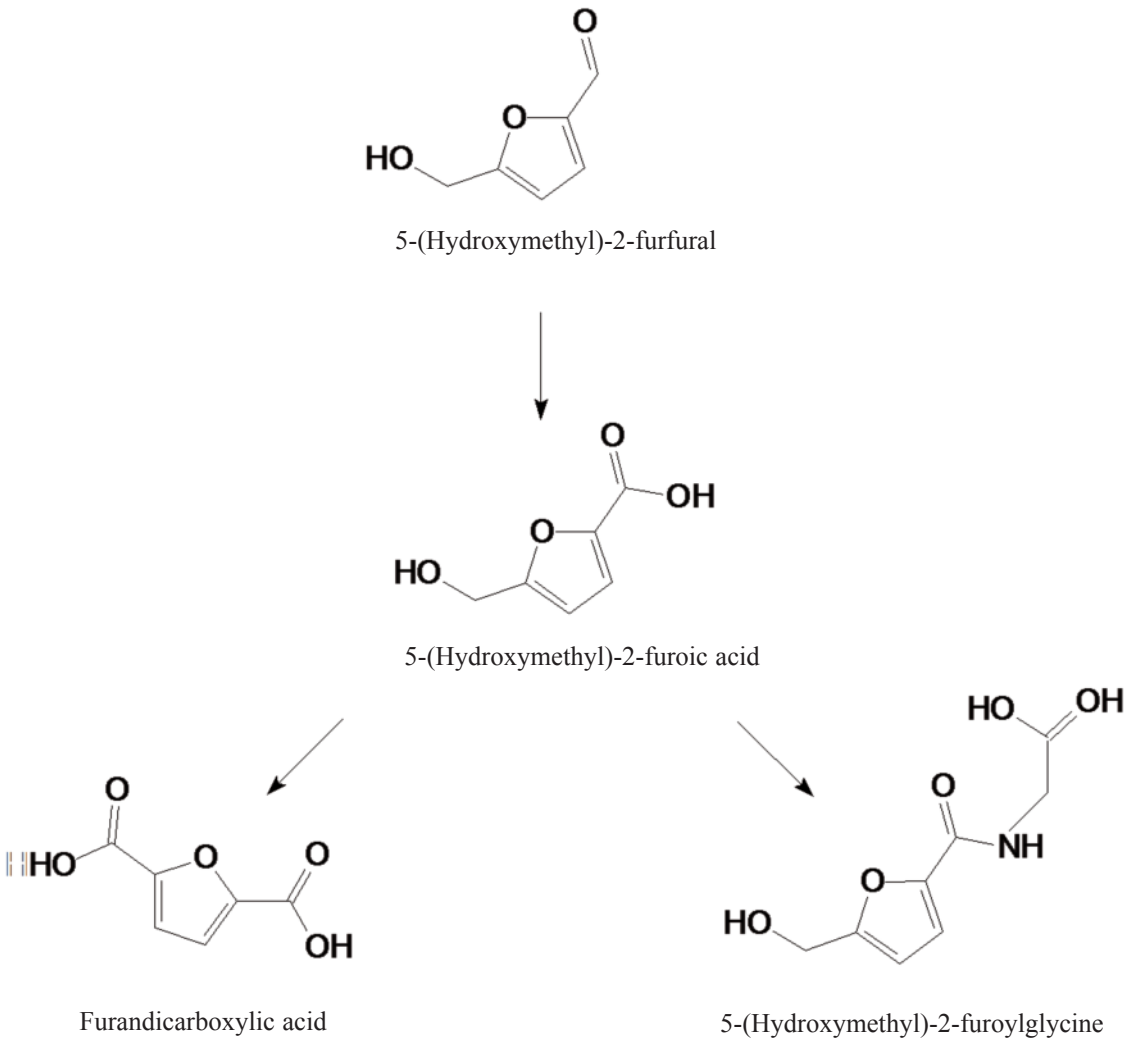


FIGURE 1
Suggested Metabolic Scheme for 5-(Hydroxymethyl)-2-furfural (Godfrey *et al.*, 1999)

Reproductive and Developmental Toxicity

No studies of the reproductive and developmental toxicity of 5-(hydroxymethyl)-2-furfural in experimental animals or humans were found in the literature.

Carcinogenicity

Experimental Animals

Zhang *et al.* (1992) gave groups of rats and mice a single treatment of azoxymethane followed by diets containing 20% thermolyzed sucrose or 20% unheated sucrose. After 100 days, the colons of rats and mice that received diets containing thermolyzed sucrose had significantly more and larger aberrant crypt foci than animals that received diets containing untreated sucrose. High-performance liquid chromatography analysis of the thermolyzed sucrose revealed that it contained 1% 5-(hydroxymethyl)-2-furfural.

To examine whether 5-(hydroxymethyl)-2-furfural was responsible for the apparent promoting effect of thermolyzed sucrose on aberrant crypt foci, a second series of experiments was conducted (Zhang *et al.*, 1993) in which rats were given a single initiating dose of azoxymethane and then randomized into four treatment groups which received, respectively, control diet, a diet containing 20% thermolyzed sucrose, a diet containing 20% sucrose extracted with butanol to remove 5-(hydroxymethyl)-2-furfural, or a diet containing 1% 5-(hydroxymethyl)-2-furfural. Groups that received diets containing thermolyzed sucrose or 5-(hydroxymethyl)-2-furfural had larger aberrant crypt foci both with regard to number and size than groups that received control diet or diet containing sucrose extracted with butanol, suggesting that the promoting ability of thermolyzed sucrose was due to 5-(hydroxymethyl)-2-furfural. To examine whether 5-(hydroxymethyl)-2-furfural was capable of initiating aberrant crypt foci in the colon, groups of female rats were administered doses of 5-(hydroxymethyl)-2-furfural (300 mg/kg) or azoxymethane (5 mg/kg) by gavage, and the colons were examined 30 days later (Zhang *et al.*, 1993). Compared to controls, groups that received 5-(hydroxymethyl)-2-furfural exhibited dose-dependent increases in the total number of aberrant crypt foci, although the increases were much smaller than those observed in groups that received azoxymethane.

Surh and Tannenbaum (1994) examined the possibility that 5-(hydroxymethyl)-2-furfural could undergo metabolic activation to a more reactive species as a possible explanation for its reported mutagenicity and to explain the results reported by Zhang *et al.* on the initiation and promotion of aberrant crypt foci in the colon of rats and mice. One potential activating pathway they considered was sulfation of the 5-allylic hydroxyl group via sulfotransferase to produce a sulfate ester; subsequent loss of the sulfate would leave a reactive electrophilic allylic carbocation. Surh and Tannenbaum synthesized 5-[(sulfooxy)methyl]-furfural and found that it was a direct mutagen in both human lymphoblastoid cells and in bacteria.

In an extension of this work, Surh *et al.* (1994) found that 5-[(sulfooxy)methyl]-furfural and 5-chloromethylfurfural, the product of the reaction of the sulfooxymethyl compound with chloride, were both better initiators of mouse skin than 5-(hydroxymethyl)-2-furfural. In addition, Surh *et al.* injected 12-day-old B6C3F1 mice intraperitoneally with 5-chloromethylfurfural or 5-(hydroxymethyl)-2-furfural and determined the tumor response 10 months later. Groups that received the chloromethyl compound developed a significantly higher incidence of liver tumors (described as hepatomas) compared to animals that received 5-(hydroxymethyl)-2-furfural or dimethyl sulfoxide, the solvent used for injection.

Humans

No epidemiology studies of 5-(hydroxymethyl)-2-furfural were found in the literature.

Structure Activity Relationships

The NTP has conducted in-depth evaluations of three compounds structurally related to 5-(hydroxymethyl)-2-furfural: furan, furfural, and furfuryl alcohol. Furan administered by gavage was a potent carcinogen in both rats and mice, causing significantly increased incidences of cholangiocarcinoma in rats and hepatocellular neoplasms in rats and mice (NTP, 1993). Furfural administered by gavage caused a significant increase of hepatocellular neoplasms in male mice (NTP, 1990). Furfuryl alcohol administered by inhalation caused slight increases in the incidences of renal tubule neoplasms in female rats and male mice (NTP, 1999).

In F344 rats, furfuryl alcohol is oxidized to furfural, which undergoes further oxidation to furoic acid and conjugation with glycine (Nomeir *et al.*, 1992; Parkash and Caldwell, 1994) similar to the metabolism of 5-(hydroxymethyl)-2-furfural. However, there are also some differences between furfural and 5-(hydroxymethyl)-2-furfural. Nomeir *et al.* (1992) report the conversion of approximately 7% of administered furfural to CO_2, whereas Germond *et al.* (1987) and Godfrey *et al.* (1999) report little conversion of administered 5-(hydroxymethyl)-2-furfural to CO_2. In addition, furanacrylic acid and furanacryloylglycine are urinary metabolites of furfural, whereas neither was detected as 5-(hydroxymethyl)-2-furfural metabolites (Nomeir *et al.*, 1992; Parkash and Caldwell, 1994; Godfrey *et al.* 1999). Furoic acid, furoylglycine, and furanacryloylglycine have been identified as urinary metabolites of furfural in humans (Flek and Sedivec, 1978), suggesting that the metabolism of these compounds in humans and rodents is similar.

Genetic Toxicity

5-(Hydroxymethyl)-2-furfural has been tested for mutagenicity in bacterial and mammalian test systems; in general, the few reports available indicate a mix of results. It appears that high concentrations of 5-(hydroxymethyl)-2-furfural can result in weak genotoxic effects. As part of a large screening study of 239 compounds, 5-(hydroxymethyl)-2-furfural (3 µM/plate) dissolved in ethanol was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 using the spot test, a qualitative measure of mutagenicity, with and without rat liver S9 (Florin *et al.*, 1980); no mutagenic activity was observed. Kim *et al.* (1987) also reported a lack of mutagenicity in *S. typhimurium* TA100 treated with 5-(hydroxymethyl)-2-furfural (4.4 µM/plate) in the absence of S9, using a preincubation protocol. Janzowski *et al.* (2000) reported increased levels of SOS repair in *S. typhimurium* strain TA1535 in the *umu* DNA repair assay at 5-(hydroxymethyl)-2-furfural concentrations of 12 mM and greater. In a study designed to evaluate the potential antimutagenic activity of several α-hydroxycarbonyl compounds, including 5-(hydroxymethyl)-2-furfural, Kim *et al.* (1987) autoclaved each of five known mutagenic heterocyclic amine compounds with 5-(hydroxymethyl)-2-furfural for 20 minutes at 121° C and compared the mutagenic activity of the reaction mixtures with that of the heterocyclic amine compounds alone in *S. typhimurium* strain TA98 with S9 and strain TA100 without S9; the mutagenicity of each of the reaction mixtures was significantly reduced in both tester strains. No induction of DNA damage was seen *in vitro* in several mammalian cell types (V79 cells, Caco-2 cells, primary rat hepatocytes, and primary human colon cells) treated with 5-(hydroxymethyl)-2-furfural concentrations up to 80 or 120 mM, but increased frequencies of HPRT mutations were observed in V79 cells treated with moderately cytotoxic concentrations of 120 to 140 mM 5-(hydroxymethyl)-2-furfural (Janzowski *et al.*, 2000). Nishi *et al.* (1989) treated cultured Chinese hamster V79 cells with 2000 µg/mL 5-(hydroxymethyl)-2-furfural for 3 hours and reported a significant increase in chromosomally aberrant cells (chromatid breaks and exchanges) examined 24 hours after chemical treatment; no significant increases in aberrations were observed at lower doses of 5-(hydroxymethyl)-2-furfural.

Omura *et al.* (1983) examined the mutagenicity of solutions of glucose heated in the presence of different amino acids and found that mutagens were formed in several of the reaction mixtures. 5-(Hydroxymethyl)-2-furfural was identified as a product of the reaction between glucose and lysine and was subsequently shown to be mutagenic in bacteria and clastogenic in Chinese hamster ovary cells (Stich *et al.*, 1981a,b).

Study Rationale

5-(Hydroxymethyl)-2-furfural was nominated for study by the NIEHS because of extensive human exposure and the lack of adequate data characterizing its toxicity and carcinogenicity. Humans ingest 5-(hydroxymethyl)-2-furfural as part of their daily food intake, and therefore, administration in the diet was initially selected for this study. However, 5-(hydroxymethyl)-2-furfural was not stable in NTP-2000 rat and mouse ration, so gavage was selected as the route of administration.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 5-(HYDROXYMETHYL)-2-FURFURAL

5-(Hydroxymethyl)-2-furfural was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), in five lots (11129CQ-MRI796, 14901MQ, 34266-63, 34266-72, and 34266-76). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory, Battelle Columbus Operations (Columbus, OH) (Appendix J). Karl Fischer titration was performed by the analytical chemistry laboratory, Galbraith Laboratories, Inc. (Knoxville, TN), and Prevalere Life Sciences, Inc. (Whitesboro, NY). Reports on analyses performed in support of the 5-(hydroxymethyl)-2-furfural studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a yellow powder or light orange crystalline solid, was identified as 5-(hydroxymethyl)-2-furfural by infrared, ultraviolet/visible, and ^{1}H or proton nuclear magnetic resonance spectroscopy and by liquid chromatography/mass spectrometry and melting point determination. The purity of lot 34266-72 was determined by thin-layer chromatography (TLC), and the purities of all five lots were determined by high-performance liquid chromatography (HPLC).

Karl Fischer titration of lots 34266-63, 34266-72, and 34266-76 indicated water content ranging from 0.1% to 2.9%. TLC detected a single major spot and no impurities. For lot 11129CQ-MRI796, HPLC by one system indicated one major peak and one impurity. For lot 14901MQ, HPLC by a second system indicated one major peak and one impurity. HPLC by a third system indicated one major peak and three impurities for lots 34266-63, 34266-72, and 34266-76. The overall purity of all lots was determined to be 99% or greater.

Stability studies of the bulk chemical were performed using HPLC. These studies indicated that 5-(hydroxymethyl)-2-furfural was stable as a bulk chemical for 2 weeks when stored protected from light under a nitrogen headspace at temperatures up to 25° C. To ensure stability, the bulk chemical for the 3-week and 3-month studies was stored at refrigerated temperatures (approximately 5° C), protected from light in the original shipping containers. For the 2-year studies, the bulk chemical was stored at less than or equal to –20° C in amber glass bottles under an argon headspace.

Periodic reanalyses of the bulk chemical were performed during the 3-week, 3-month, and 2-year studies using HPLC; no degradation of the bulk chemical was detected. No excessive moisture levels were detected in the bulk chemical during the 2-year studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing 5-(hydroxymethyl)-2-furfural with deionized water to give the required concentrations (Table J2). Because all dose formulations in the studies were determined to be solutions, no homogeneity studies were required. Stability studies of 9.4 and 300 mg/mL formulations of a lot not used in the animal studies were performed by the analytical chemistry laboratory using HPLC. Stability was confirmed for at least 35 days for dose formulations stored in glass vials at refrigerated and ambient temperatures and for at least 3 hours under simulated animal room conditions. The dose formulations were stored at approximately 5° C in amber glass bottles with Teflon®-lined lids for up to 35 days.

Periodic analyses of the dose formulations of 5-(hydroxymethyl)-2-furfural were conducted by the study laboratory using HPLC. During the 3-week studies, the dose formulations were analyzed once; all six dose formulations for rats and all five for mice were within 10% of the target concentrations (Table J3). During the 3-month studies, the dose formulations were

analyzed at the beginning, midpoint, and end of the studies; of the dose formulations analyzed and used in the studies, 13 of 15 for rats and all 15 for mice were within 10% of the target concentrations; all were within 12% (Table J4). During the 2-year studies, the dose formulations were analyzed approximately every 3 months; all 27 dose formulations used for rats and all 30 used for mice were within 10% of the target concentrations (Table J5).

3-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 13 (rats) or 14 (mice) days and were approximately 6 weeks old on the first day of the studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Core study groups of five male and five female rats and mice were administered 5-(hydroxymethyl)-2-furfural in deionized water by gavage at doses of 94, 188, 375, 750, or 1,500 mg/kg for a total of 13 doses over a 22-day period. Vehicle control rats and mice were administered deionized water alone. Special study groups of five male and five female rats designated for neuropathology were administered deionized water or 1,500 mg/kg 5-(hydroxymethyl)-2-furfural in deionized water by gavage on the same schedule. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. The animals were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded on dosing days and at necropsy for rats and mice. Details of the study design and animal maintenance are summarized in Table 1.

After dosing on study day 21, all surviving core study animals were placed in metabolism cages, and urine was collected over ice for 24 hours. During collection, feed and water were available *ad libitum*. After collection, the total volume and creatinine of the samples were determined; the concentrations of 5-(hydroxymethyl)-2-furoic acid and 5-(hydroxymethyl)-2-furoylglycine concentrations were determined using HPLC.

Necropsies were performed on all core study rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin. Histopathologic examinations were performed on the colon, liver, and stomach of all core study animals. For special study rats, tissues were perfused with fixative (10% neutral buffered formalin followed by 4% glutaraldehyde) using a transcardial technique. The brain and spinal cord sections at the levels of C1, C2, T1, T2, L1, and L2 were collected and placed in glutaraldehyde for approximately 24 hours before being transferred to 10% neutral buffered formalin. One sagittal section through the cerebrum and cerebellum, slightly off-center from the midline, three coronal sections of cerebrum, and all spinal cord sections were trimmed and embedded in glycomethacrylate. All sections were cut to a thickness of approximately 1 µm and stained with hematoxylin and eosin, together with toluidine blue.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to 5-(hydroxymethyl)-2-furfural and to determine the approximate doses to be used in the 2-year studies. Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 days (male rats), 12 days (female rats and mice), or 13 days (male mice) and were approximately 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Serologic analyses were performed on five male and five female sentinel rats and mice 1 month after the studies began and at the end of the studies using the protocols of the NTP Sentinel Animal Program (Appendix L).

Core study groups of 10 male and 10 female rats and mice and special study groups of 10 male and 10 female rats designated for clinical pathology and neuropathology were administered 5-(hydroxymethyl)-2-furfural in deionized water by gavage at doses of 47 (mice only), 94, 188, 375, 750, or 1,500 (rats only) mg/kg, 5 days per week for 3 months. Vehicle control rats and mice were administered deionized water alone. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Core study animals were weighed initially, weekly, and at necropsy; special study animals were weighed ini-

tially and weekly. Clinical findings were recorded weekly and at necropsy for core study rats and mice. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with a CO_2/O_2 mixture, and blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats only) analyses. Blood samples for hematology analyses were placed in microcollection tubes containing potassium EDTA (Sarstedt, Inc., Nümbrecht, Germany). Erythrocyte, leukocyte, and platelet counts, hematocrit value, hemoglobin concentration, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were determined using a Cell-Dyn® automated cell counter (Abbott Laboratories, Abbott Park, IL). Leukocyte differentials and nucleated erythrocyte counts were determined by light microscopic examination of blood films stained with a modified Wright-Giemsa stain using an Ames Hema-Tek® slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Reticulocytes were stained with new methylene blue and counted using the Miller disc method. Blood samples for clinical chemistry analyses were placed in microcollection serum separator tubes containing separator gel (Sarstedt, Inc.) and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Hitachi 911® automated analyzer (Roche Diagnostics Corp., Indianapolis, IN) using reagents provided by the instrument manufacturer or Sigma Chemical Co. (St. Louis, MO). Table 1 lists the parameters measured.

After dosing on days 16, 44, 92 (mice), and 93 (rats), all surviving core study animals were placed in metabolism cages, and urine was collected over ice for 24 hours. During collection, feed and water were available *ad libitum*. After collection, the total volume was measured, and creatinine concentration was analyzed using a Roche Hitachi 911® system (Roche Diagnostics Corp.); urine concentrations of 5-(hydroxymethyl)-2-furoic acid and 5-(hydroxymethyl)-2-furoylglycine were determined using HPLC.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on rats and mice exposed to 0, 188 (mice only), 375, 750, or 1,500 (rats only) mg/kg per day. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. To quantify spermatogenesis, the testicular head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethylsulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all core study vehicle control and 750 (mice) and 1,500 (rats) mg/kg groups. In addition, histopathology was performed on brain and spinal cord tissues collected from all special study rats not used in the neuropathology study (see below). Table 1 lists the tissues and organs routinely examined.

At the end of the study, five randomly selected special study rats per dose group were used for a neuropathology assessment. The anesthetized animals were perfused with fixative (10% neutral buffered formalin followed by 4% glutaraldehyde) using a transcardial technique. For perfused and nonperfused special study rats, the brain and spinal cord sections at the levels of C1, C2, T1, T2, L1, and L2 were collected and placed in 4% glutaraldehyde for approximately 24 hours before being transferred to 10% neutral buffered formalin. The brain was sectioned along one sagittal plane that was made from the olfactory bulb to the brain stem and three coronal planes

of the cerebrum. Brain sections were embedded in paraffin, and spinal cord sections were embedded in glycomethacrylate. Brain and spinal cord sections were sectioned to a thickness of approximately 1 µm and stained with hematoxylin and eosin, together with toluidine blue.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were administered 5-(hydroxymethyl)-2-furfural in deionized water by gavage at doses of 188, 375, or 750 mg/kg, 5 days per week for 104 weeks. Vehicle control rats and mice were administered deionized water alone.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). Animals were quarantined for 13 days (male rats and female mice) or 14 days (female rats and male mice) and were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Rats were housed two or three (males) or five (females) per cage, and mice were housed individually (males) or five (females) per cage. Feed and water were available *ad libitum*. Cages were changed once (male mice) or twice weekly, and racks were changed and rotated every 2 weeks; cages were rotated at the time of rack change out. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded on day 1, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the studies. Clinical findings were recorded on day 29, every 4 weeks thereafter, and at the end of the studies.

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

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

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

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 5-(Hydroxymethyl)-2-furfural

3-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
Rats: 13 days Mice: 14 days	Rats: 11 days (males) or 12 days (females) Mice: 13 days (males) or 12 days (females)	Rats: 13 days (males) or 14 days (females) Mice: 14 days (males) or 13 days (females)
Average Age When Studies Began		
6 weeks	6 weeks	6 to 7 weeks
Date of First Dose		
Rats: December 18, 1996 Mice: December 19, 1996	Rats: April 7 (males) or 8 (females), 1997 Mice: April 15 (females) or 16 (males), 1997	Rats: March 6 (males) or 7 (females), 2002 Mice: August 8 (females) or 9 (males), 2001
Duration of Dosing		
13 doses over a 22-day period	5 days/week for 14 weeks	5 days/week for 104 weeks
Date of Last Dose		
Rats: January 8, 1997 Mice: January 9, 1997	Rats: July 8 (males) or 9 (females), 1997 Mice: July 16 (females) or 17 (males), 1997	Rats: March 2 (males) or 4 (females), 2004 Mice: August 5 (females) or 7 (males), 2003
Necropsy Dates		
Rats: January 8, 1997 Mice: January 9, 1997	Rats: July 9 (males) or 10 (females), 1997 Mice: July 17 (females) or 18 (males), 1997	Rats: March 3 (males) or 5 (females), 2004 Mice: August 6 (females) or 8 (males), 2003
Average Age at Necropsy		
9 weeks	19 weeks	109 to 111 weeks
Size of Study Groups		
Rats: 5 males and 5 females (core study) 5 males and 5 females (special study) Mice: 5 males and 5 females	Rats: 10 males and 10 females (core study) 10 males and 10 females (special study) Mice: 10 males and 10 females	50 males and 50 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-week studies	Same as 3-week studies
Animals per Cage		
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 2 or 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 5-(Hydroxymethyl)-2-furfural

3-Week Studies	3-Month Studies	2-Year Studies
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*; changed weekly	Same as 3-week studies	Same as 3-week studies, except wafer form
Water		
Tap water (Columbus, OH, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 3-week studies	Same as 3-week studies
Cages		
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed weekly (male mice) or twice weekly, rotated every 2 weeks	Same as 3-week studies	Same as 3-week studies
Bedding		
Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (male mice) or twice weekly	Same as 3-week studies	Same as 3-week studies
Cage Filters		
Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-week studies	Same as 3-week studies
Racks		
Stainless steel (Lab Products, Inc., Maywood, NJ), changed and rotated every 2 weeks	Same as 3-week studies	Same as 3-week studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses		
0, 94, 188, 375, 750, or 1,500 mg/kg in deionized water by gavage (core study) 0 or 1,500 mg/kg in deionized water by gavage (special study) Dosing volume = 5 mL/kg body weight for rats or 10 mL/kg for mice	Rats: 0, 94, 188, 375, 750, or 1,500 mg/kg in deionized water by gavage Mice: 0, 47, 94, 188, 375, or 750 mg/kg in deionized water by gavage Dosing volume = 5 mL/kg for rats or 10 mL/kg for mice	0, 188, 375, or 750 mg/kg in deionized water by gavage Dosing volume = 5 mL/kg for rats or 10 mL/kg for mice
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, weekly, and at necropsy; clinical findings were recorded on dosing days and at necropsy.	Observed twice daily; core study animals were weighed initially, weekly, and at necropsy; special study rats were weighed initially and weekly; clinical findings were recorded weekly and at necropsy for core study animals.	Observed twice daily; animals were weighed initially, weekly for the first 13 weeks, every 4 weeks thereafter, and at necropsy; clinical findings were recorded on day 29, every 4 weeks thereafter, and at necropsy.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 5-(Hydroxymethyl)-2-furfural

3-Week Studies	3-Month Studies	2-Year Studies
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 3-week studies	Same as 3-week studies
Necropsy		
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats at the end of the studies for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology		
Histopathology was performed on all core study rats and mice. In addition to gross lesions and tissue masses, the colon, liver, and stomach were examined.	Complete histopathology was performed on core study vehicle control and 750 (mice) and 1,500 (rats) mg/kg groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 5-(Hydroxymethyl)-2-furfural

3-Week Studies	3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from core study males in the 0, 188 (mice), 375, 750, and 1,500 (rats) mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for 12 consecutive days prior to the end of the studies from females in the 0, 188 (mice), 375, 750, and 1,500 (rats) mg/kg groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None
Urinalysis and Metabolites		
Core study animals were placed in metabolism cages for 24-hour urine collection on day 21. Urine samples were analyzed for total volume, creatinine, and 5-(hydroxymethyl)-2-furoic acid and 5-(hydroxymethyl)-2-furoylglycine concentrations.	Core study animals were placed in metabolism cages for 24-hour urine collection on days 16, 44, 92 (mice), and 93 (rats). Urine samples were analyzed for total volume, creatinine, and 5-(hydroxymethyl)-2-furoic acid and 5-(hydroxymethyl)-2-furoylglycine concentrations.	None
Neuropathology		
At the end of the study, the brain and spinal cord sections at the levels of C1, C2, T1, T2, L1, and L2 were collected from special study rats that were perfused using a transcardial technique.	At the end of the study, the brain and spinal cord sections at the levels of C1, C2, T1, T2, L1, and L2 were collected from five special study rats in each dose group that were perfused using a transcardial technique.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, B1, B3, C1, C3, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., Harderian gland, intestine, mammary gland, and skin) before microscopic evaluation or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

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

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

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess

the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regularly cycling females in each dose group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

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

Quality Assurance Methods

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

Genetic Toxicology

The genetic toxicity of 5-(hydroxymethyl)-2-furfural was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However,

it should be noted that not all cancers arise through genotoxic mechanisms.

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

The predictivity for carcinogenicity of a positive response in acute *in vivo* micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS
3-WEEK STUDY

One 1,500 mg/kg male rat died on day 6 of the study (Table 2). The final mean body weight of 1,500 mg/kg males was significantly less than that of the vehicle control group. There were no clinical findings related to 5-(hydroxymethyl)-2-furfural administration.

There were no biologically significant differences in organ weights between dosed and vehicle control groups of rats (Table H1). No chemical-related histopathologic lesions were observed in any core study animals, and no treatment-related lesions were observed in the brains or spinal cords of the special study neuropathology animals.

Dose Selection Rationale: Based on minimal effects on survival, body weights, and organ weights, 5-(hydroxymethyl)-2-furfural doses selected for the 3-month gavage study in rats were 94, 188, 375, 750, and 1,500 mg/kg.

TABLE 2
Survival and Body Weights of Rats in the 3-Week Gavage Study of 5-(Hydroxymethyl)-2-furfural

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	111 ± 4	199 ± 3	88 ± 2	
94	5/5	112 ± 3	207 ± 3	95 ± 2	104
188	5/5	111 ± 3	198 ± 4	87 ± 3	99
375	5/5	112 ± 3	200 ± 6	88 ± 3	100
750	5/5	111 ± 3	207 ± 3	95 ± 2	104
1,500	4/5[c]	111 ± 4	181 ± 8*	75 ± 7	91
Female					
0	5/5	101 ± 2	139 ± 2	38 ± 2	
94	5/5	99 ± 2	140 ± 2	41 ± 1	101
188	5/5	100 ± 2	140 ± 2	40 ± 3	100
375	5/5	100 ± 2	137 ± 4	37 ± 3	99
750	5/5	102 ± 2	139 ± 3	37 ± 3	100
1,500	5/5	100 ± 2	134 ± 3	34 ± 2	97

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 3 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Day of death: 6

3-Month Study

One 1,500 mg/kg male and three 1,500 mg/kg female rats died before the end of the study; the male died due to gavage trauma, but the deaths of the females could not be explained (Table 3). Final mean body weights and body weight gains of 750 and 1,500 mg/kg males were significantly less than those of the vehicle control group. There were no clinical findings related to 5-(hydroxymethyl)-2-furfural administration.

There were no changes in hematology or clinical chemistry variables in rats that were considered attributable to 5-(hydroxymethyl)-2-furfural administration (Table F1). Slight differences were observed in the amount of the furoic acid metabolite and glycine conjugate formed at day 17 compared to day 94; however, there was no indication of glycine depletion with continuous dosing (Table G2). No biologically significant organ weight changes were observed (Table H2). No chemical-related lesions were observed in core or special study animals.

The 1,500 mg/kg group of males had a significantly increased relative epididymis weight (data not shown), but 5-(hydroxymethyl)-2-furfural did not cause any significant changes in the sperm parameters (Table I1). Female rats had elongated estrous cycles; fewer 750 and 1,500 mg/kg females had regular cycles, and the difference from the vehicle controls was significant at 1,500 mg/kg (Table I2). Females in the 375, 750, and 1,500 mg/kg groups had a significantly increased probability of extended diestrus ($P<0.004$, $P<0.001$, $P<0.001$, respectively). There were no significant changes in the histopathology of the reproductive organs for male or female rats.

Dose Selection Rationale: The administration of 5-(hydroxymethyl)-2-furfural for 3 months produced minimal toxicity in rats except for the deaths of three 1,500 mg/kg females. Therefore, doses of 188, 375, and 750 mg/kg per day were selected for the 2-year gavage study in rats.

Table 3
Survival and Body Weights of Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	89 ± 5	336 ± 8	247 ± 6	
94	10/10	89 ± 4	330 ± 5	242 ± 5	98
188	10/10	89 ± 4	324 ± 7	236 ± 4	97
375	10/10	90 ± 4	332 ± 4	242 ± 5	99
750	10/10	89 ± 4	309 ± 6**	220 ± 4**	92
1,500	9/10[c]	89 ± 4	315 ± 7*	224 ± 5**	94
Female					
0	10/10	90 ± 3	196 ± 4	106 ± 3	
94	10/10	89 ± 3	189 ± 3	99 ± 4	96
188	10/10	89 ± 3	189 ± 4	100 ± 3	96
375	10/10	91 ± 3	194 ± 3	103 ± 2	99
750	10/10	90 ± 3	190 ± 3	100 ± 3	97
1,500	7/10[d]	89 ± 4	187 ± 2	98 ± 4	95

* Significantly different ($P \le 0.05$) from the vehicle control group by Williams' test
** $P \le 0.01$
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 3
[d] Weeks of death: 1, 5, 14

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 2). Survival of 188 and 750 mg/kg males was greater than that of the vehicle control group; survival of the other dosed groups of rats was similar to that of the vehicle control groups.

Body Weights and Clinical Findings

Mean body weights of dosed groups of males and females were generally similar to those of the vehicle controls throughout the study (Figure 3; Tables 5 and 6). An increased incidence of nasal/eye discharge was observed in 750 mg/kg females.

Table 4
Survival of Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	1	1
Moribund	20	9	9	10
Natural deaths	8	7	9	4
Animals surviving to study termination	22	34	31	35
Percent probability of survival at end of study[b]	44	68	63	72
Mean survival (days)[c]	673	696	682	692
Survival analysis[d]	P=0.019N	P=0.018N	P=0.085N	P=0.007N
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	0	0	2
Moribund	14	7	13	7
Natural deaths	5	11	10	11
Animals surviving to study termination	31	32	27	30[e]
Percent probability of survival at end of study	62	64	54	63
Mean survival (days)	679	682	632	673
Survival analysis	P=1.000N	P=1.000N	P=0.320	P=1.000N

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

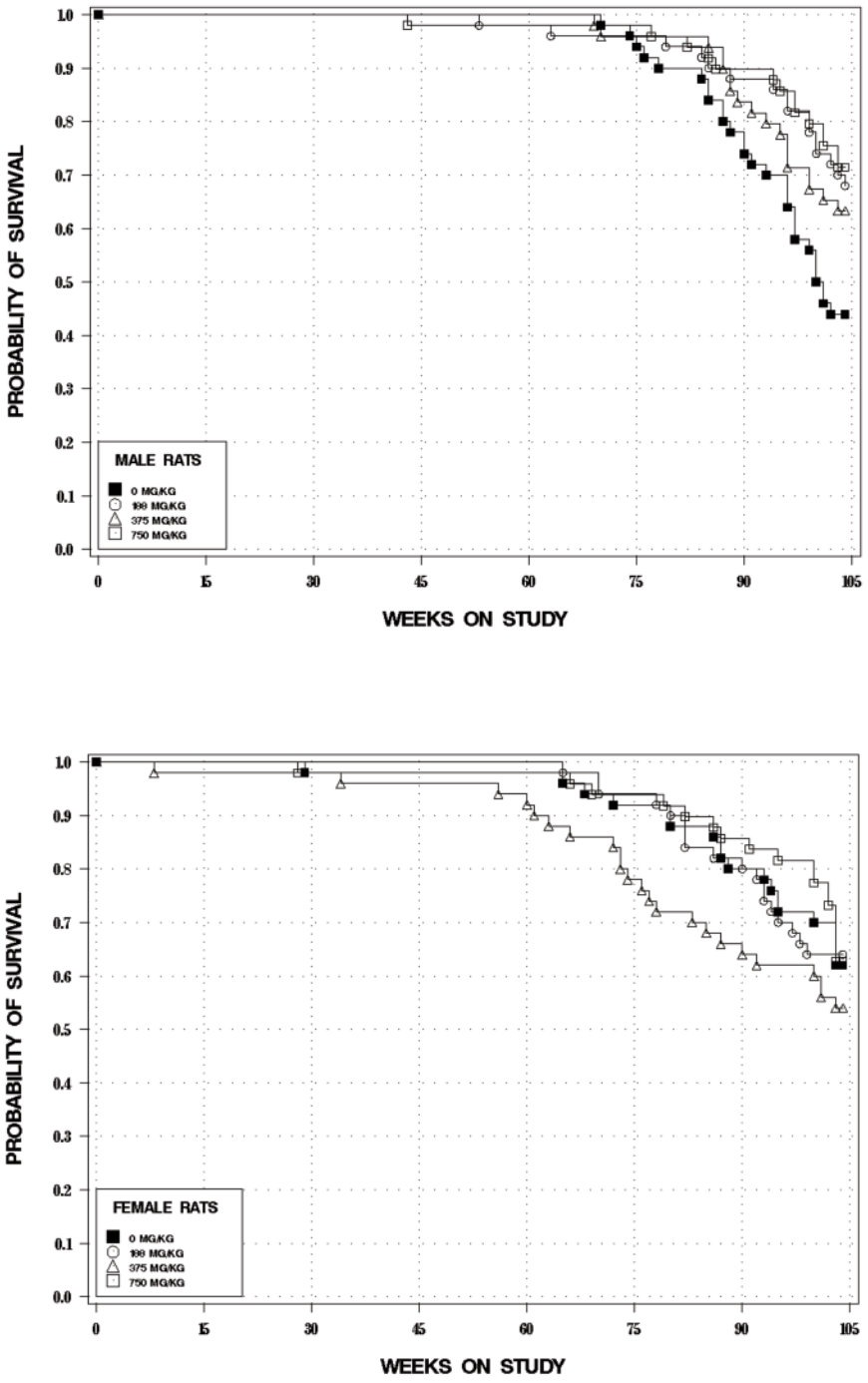
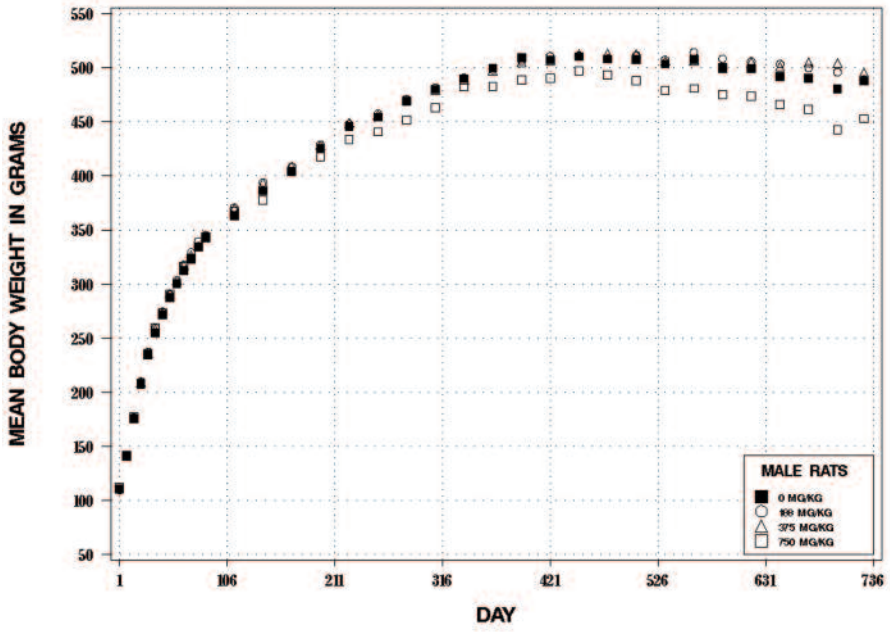


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Administered 5-(Hydroxymethyl)-2-furfural by Gavage for 2 Years



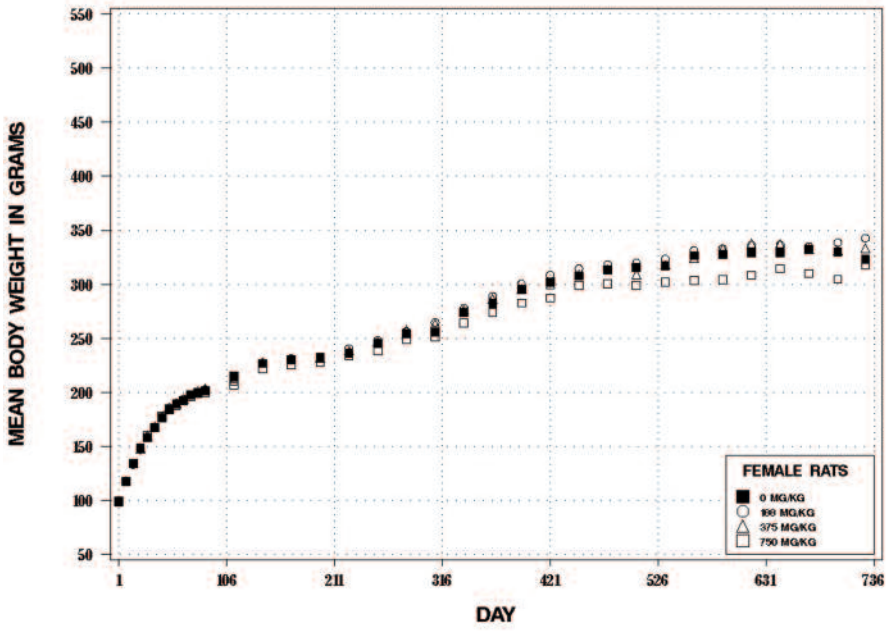


FIGURE 3
Growth Curves for Male and Female Rats Administered 5-(Hydroxymethyl)-2-furfural by Gavage for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

Days on Study	Vehicle Control		188 mg/kg			375 mg/kg			750 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	111	50	110	99	50	111	100	50	112	102	50
8	141	50	142	101	50	142	101	50	142	101	50
15	176	50	177	101	50	177	101	50	177	101	50
22	208	50	210	101	50	210	101	50	208	100	50
29	235	50	237	101	50	236	101	50	236	100	50
36	255	50	259	102	50	259	101	50	260	102	50
43	272	50	274	101	50	275	101	50	273	101	50
50	288	50	290	101	50	291	101	50	291	101	50
57	300	50	304	101	50	304	101	50	301	100	50
64	312	50	318	102	50	317	102	50	316	101	50
71	324	50	329	102	50	327	101	50	323	100	50
78	335	50	340	102	50	335	100	50	335	100	50
85	343	50	345	101	50	344	100	50	344	100	50
113	364	50	371	102	50	371	102	50	367	101	50
141	386	50	394	102	50	394	102	50	378	98	50
169	404	50	409	101	50	409	101	50	405	100	50
197	426	50	429	101	50	428	101	49	417	98	50
225	446	50	448	101	50	449	101	49	434	97	50
253	454	50	457	101	50	456	100	49	441	97	50
281	469	50	471	100	50	470	100	49	452	96	50
309	480	50	482	100	50	479	100	49	463	97	49
337	490	50	491	100	50	489	100	49	483	99	49
365	500	50	498	100	50	497	100	49	483	97	48
393	509	50	506	99	49	505	99	49	489	96	48
421	507	50	511	101	49	507	100	49	490	97	48
449	510	50	511	100	48	512	100	49	497	97	48
477	508	50	508	100	48	513	101	49	493	97	48
505	508	49	511	101	48	513	101	47	488	96	48
533	504	46	507	101	48	506	101	47	479	95	47
561	508	45	514	101	47	507	100	47	481	95	47
589	500	43	508	102	45	500	100	47	475	95	46
617	499	39	506	101	44	506	101	42	474	95	44
645	492	36	503	102	44	501	102	40	466	95	44
673	490	32	500	102	41	505	103	35	465	95	41
701	484	24	496	103	37	504	104	33	446	92	38
Mean for weeks											
1-13	254		257	101		256	101		255	101	
14-52	435		439	101		438	101		427	98	
53-101	501		506	101		506	101		479	96	

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

Days on Study	Vehicle Control		188 mg/kg			375 mg/kg			750 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	99	50	99	100	50	99	100	50	99	101	50
8	118	50	118	100	50	118	100	50	118	100	50
15	134	50	133	99	50	134	100	50	134	100	49
22	148	50	147	100	50	148	100	50	149	101	49
29	158	50	159	101	50	160	101	50	160	101	49
36	167	50	167	100	50	169	101	50	167	100	49
43	177	50	177	100	50	178	101	50	178	101	49
50	184	50	185	100	50	186	101	50	185	100	49
57	189	50	190	101	50	190	100	49	188	99	49
64	193	50	194	101	50	194	101	49	193	100	49
71	198	50	198	100	50	198	100	49	196	99	49
78	200	50	200	100	50	201	101	49	199	100	49
85	202	50	203	100	50	203	101	49	200	99	49
113	215	50	212	99	50	211	98	49	207	96	49
141	227	50	227	100	50	228	101	49	222	98	49
169	230	50	231	101	50	230	100	49	226	98	49
197	233	50	233	100	50	232	100	49	228	98	48
225	236	49	241	102	50	238	101	49	234	99	48
253	246	49	248	101	50	247	101	48	238	97	48
281	254	49	257	101	50	258	101	48	249	98	48
309	256	49	265	104	50	264	103	48	251	98	48
337	274	49	278	101	50	276	101	48	264	97	48
365	283	49	289	102	50	287	101	48	274	97	48
393	295	49	300	102	50	296	100	47	283	96	48
421	302	49	309	102	50	300	99	46	287	95	48
449	308	49	314	102	49	309	101	44	299	97	48
477	314	47	318	102	49	314	100	43	301	96	47
505	316	46	320	101	47	311	98	41	299	95	46
533	317	46	323	102	47	318	100	38	302	95	46
561	327	44	331	101	45	324	99	36	303	93	45
589	328	44	333	102	42	333	102	34	305	93	44
617	329	40	336	102	41	338	103	33	309	94	42
645	331	39	336	102	39	337	102	31	315	95	40
673	332	36	335	101	35	333	100	31	310	93	39
701	330	35	339	103	32	331	100	30	305	92	37
Mean for weeks											
1-13	167		167	100		168	101		167	100	
14-52	241		244	101		243	101		235	98	
53-101	316		322	102		318	100		299	95	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the nose, liver, and thyroid gland. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Nose: A spectrum of nonneoplastic changes was observed in olfactory and respiratory epithelia of males and females (Tables 7, A3, and B3). Incidences of olfactory epithelium degeneration were increased in all dosed groups of male and female rats and were significantly increased in 750 mg/kg males and 188 and 375 mg/kg females. Degeneration of the olfactory epithelium was a minimal to mild change that consisted of vacuolization, disorganization, and variable loss of epithelial cells in segments of the olfactory epithelium with concomitant reduction in the number of epithelial cell layers and decrease in the thickness of the epithelium (Plates 1 and 2).

Incidences of olfactory epithelium respiratory metaplasia were significantly increased in 750 mg/kg males and females. Olfactory epithelium respiratory metaplasia occurred primarily in the dorsal meatus in Level II and consisted of replacement of the pseudostratified olfactory epithelium by a single layer of tall columnar epithelial cells (Plate 3).

Incidences of mild respiratory epithelium squamous metaplasia were significantly increased in 750 mg/kg males and females. Squamous metaplasia of the respiratory epithelium occurred primarily along the nasal septum and was characterized by replacement of the ciliated cuboidal to columnar epithelium that normally lines the septum by nonkeratinized, stratified squamous epithelium (Plate 4). The incidence of respiratory epithelium hyperplasia was significantly decreased in 375 mg/kg males.

Incidences of suppurative inflammation of the nose and chronic inflammation of the nasolacrimal duct were significantly increased in 750 mg/kg females; the incidence of chronic active inflammation was also increased in this group, but the increase was not statistically significant.

Incidences of hyaline droplet accumulation in the olfactory epithelium were significantly decreased in all dosed groups of males and females. Incidences of hyaline droplet accumulation in the respiratory epithelium were significantly decreased in all dosed groups of males and in 750 mg/kg females.

Liver: The incidence of clear cell focus was significantly increased in 750 mg/kg males (vehicle control, 4/50; 188 mg/kg, 6/50; 375 mg/kg, 11/50; 750 mg/kg, 20/50; Table A3). Incidences of minimal chronic active inflammation were increased in all dosed groups of males, and the increase in 750 mg/kg males was significant (25/50, 34/50, 30/50, 38/50). Two hepatocellular adenomas occurred in 375 mg/kg males (Table A1).

Thyroid gland: Incidences of C-cell adenoma in 375 mg/kg males (vehicle control, 1/50; 188 mg/kg, 6/49; 375 mg/kg, 12/48; 750 mg/kg, 4/48) and C-cell adenoma or carcinoma (combined) in 188 and 375 mg/kg males (1/50, 7/49, 14/48, 6/48) were significantly increased (Table A2).

TABLE 7
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control		188 mg/kg		375 mg/kg		750 mg/kg	
Male								
Number Examined Microscopically	50		49		48		49	
Olfactory Epithelium, Degeneration[a]	18	(1.1)[b]	22	(1.1)	26	(1.2)	29*	(1.5)
Olfactory Epithelium, Metaplasia, Respiratory	2	(2.0)	5	(1.4)	3	(1.3)	11*	(1.4)
Olfactory Epithelium, Accumulation, Hyaline Droplet	6	(1.3)	0*		0*		0*	
Respiratory Epithelium, Metaplasia, Squamous	0		2	(1.5)	1	(1.0)	16**	(2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	7	(1.6)	0*		0*		0*	
Respiratory Epithelium, Hyperplasia	28	(1.9)	24	(1.7)	18*	(1.6)	23	(1.9)
Female								
Number Examined Microscopically	50		49		49		49	
Olfactory Epithelium, Degeneration	21	(1.2)	35**	(1.2)	36**	(1.2)	28	(1.3)
Olfactory Epithelium, Metaplasia, Respiratory	1	(1.0)	1	(2.0)	0		11**	(1.8)
Olfactory Epithelium, Accumulation, Hyaline Droplet	34	(1.9)	15**	(1.3)	22*	(1.5)	0**	
Respiratory Epithelium, Metaplasia, Squamous	1	(2.0)	1	(2.0)	0		24**	(2.1)
Respiratory Epithelium, Accumulation, Hyaline Droplet	9	(1.8)	3	(1.3)	4	(1.3)	0**	
Inflammation, Suppurative	0		0		0		8**	(1.5)
Inflammation, Chronic Active	4	(1.8)	3	(1.3)	2	(1.0)	7	(1.4)
Nasolacrimal Duct, Inflammation, Chronic	2	(2.0)	2	(2.0)	3	(1.7)	12**	(2.0)

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

MICE

3-WEEK STUDY

Three 1,500 mg/kg male and three 1,500 mg/kg female mice died before the end of the study (Table 8). The final mean body weight and body weight gain of 1,500 mg/kg males were significantly less than those of the vehicle control group. There were no clinical findings related to 5-(hydroxymethyl)-2-furfural administration.

Heart weights of 1,500 mg/kg females were significantly greater than those of the vehicle controls (Table H3). No chemical-related lesions were observed.

Dose Selection Rationale: Based on mortality observed in the 1,500 mg/kg groups, doses of 47, 94, 188, 375, and 750 mg/kg were selected for the 3-month gavage study in mice.

TABLE 8
Survival and Body Weights of Mice in the 3-Week Gavage Study of 5-(Hydroxymethyl)-2-furfural

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	20.3 ± 0.6	23.3 ± 0.6	2.9 ± 0.2	
94	5/5	20.3 ± 0.4	23.3 ± 0.3	2.9 ± 0.4	100
188	5/5	20.2 ± 0.4	22.3 ± 0.5	2.1 ± 0.5	96
375	5/5	20.1 ± 0.3	22.7 ± 0.3	2.6 ± 0.3	98
750	5/5	20.3 ± 0.4	22.2 ± 0.3	1.9 ± 0.1	95
1,500	2/5[c]	20.0 ± 0.4	21.2 ± 0.8*	1.5 ± 0.4**	91
Female					
0	5/5	18.0 ± 0.5	19.4 ± 0.5	1.5 ± 0.4	
94	5/5	18.2 ± 0.4	19.9 ± 0.3	1.7 ± 0.2	102
188	5/5	18.0 ± 0.4	19.1 ± 0.3	1.2 ± 0.2	99
375	5/5	17.8 ± 0.7	19.7 ± 0.6	1.9 ± 0.1	102
750	5/5	17.5 ± 0.2	19.6 ± 0.2	2.0 ± 0.1	101
1,500	2/5[d]	18.4 ± 0.3	19.4 ± 0.8	1.4 ± 0.3	100

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 3 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Days of death: 2, 5, 9
[d] Days of death: 8, 8, 9

3-Month Study

One 750 mg/kg male and one 375 mg/kg female died before the end of the study. The death of the 750 mg/kg male could not be attributed to chemical exposure; the death of the female was attributed to ovarian teratoma (Table 9). The final mean body weight of 750 mg/kg males and body weight gains of 750 mg/kg males and females were significantly less than those of the vehicle controls. There were no clinical findings related to 5-(hydroxymethyl)-2-furfural administration.

There were no hematological effects in mice administered 5-(hydroxymethyl)-2-furfural (Table F2). In mice, continuous exposure to 5-(hydroxymethyl)-2-furfural appeared to induce the enzymes involved in oxidation to the furoic acid metabolite, as well as glycine conjugation, because considerably more of each metabolite was excreted in the urine of mice at day 94 compared to the amount present at day 17 (Table G4). The absolute kidney weights of 188 mg/kg or greater males and the relative kidney weight of 375 mg/kg males were significantly less than those of the vehicle controls (Table H4). The absolute weights of the heart and liver were significantly decreased in 750 mg/kg males, and the relative testis weight of 750 mg/kg males and relative lung weight of 375 mg/kg females were significantly increased. None of these organ weight changes were considered chemical-related.

The relative epididymis weight of 750 mg/kg males was significantly increased (data not shown), but 5-(hydroxymethyl)-2-furfural did not cause any biologically significant changes in the sperm parameters (Table I3). There were no significant differences between dosed and vehicle control groups in vaginal

Table 9
Survival and Body Weights of Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.0 ± 0.3	37.7 ± 0.9	14.7 ± 0.9	
47	10/10	23.1 ± 0.5	38.0 ± 0.9	14.9 ± 0.9	101
94	10/10	23.0 ± 0.4	37.5 ± 1.1	14.5 ± 0.9	99
188	10/10	22.1 ± 0.3	35.5 ± 0.8	13.4 ± 0.6	94
375	10/10	22.4 ± 0.2	36.6 ± 0.5	14.3 ± 0.5	97
750	9/10[c]	22.6 ± 0.3	32.0 ± 0.5**	9.4 ± 0.5**	85
Female					
0	10/10	18.6 ± 0.3	29.8 ± 0.8	11.2 ± 0.7	
47	10/10	18.6 ± 0.2	28.8 ± 0.6	10.2 ± 0.5	97
94	10/10	18.5 ± 0.2	28.6 ± 0.6	10.1 ± 0.6	96
188	10/10	18.5 ± 0.3	29.7 ± 0.8	11.2 ± 0.6	100
375	9/10[d]	18.6 ± 0.2	28.4 ± 0.7	9.9 ± 0.6	95
750	10/10	18.8 ± 0.2	27.8 ± 0.8	9.0 ± 0.6*	93

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 14
[d] Week of death: 11

cytology parameters in females (Table I4). There were no significant changes in the histopathology of the reproductive organs for male or female mice.

The incidences of minimal to mild cytoplasmic alteration of the kidney were significantly increased in males administered 188 mg/kg or greater (vehicle control, 1/10; 47 mg/kg, 0/10; 94 mg/kg, 1/10; 188 mg/kg, 6/10; 375 mg/kg, 8/10; 750 mg/kg, 10/10). Cytoplasmic alteration was characterized by the variable depletion of large, clear cytoplasmic vacuoles in the proximal tubule epithelial cells that were readily observed in the proximal tubule epithelial cells of vehicle controls; depletion of cytoplasmic vacuoles was most pronounced in 750 mg/kg males. These cytoplasmic vacuoles are typical in the kidneys of B6C3F1 mice.

Dose Selection Rationale: The administration of 5-(hydroxymethyl)-2-furfural produced a mild toxic response in mice. Mean body weights of 750 mg/kg males were decreased compared to the vehicle control group. The only histologic lesion related to chemical administration was cytoplasmic alteration in the renal proximal tubule epithelium in 188 mg/kg or greater male mice. Therefore, doses selected for the 2-year gavage study in mice were 188, 375, and 750 mg/kg.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 4). Survival of 750 mg/kg males and females was significantly less than that of the vehicle control groups.

Table 10
Survival of Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	0	2
Missing[a]	0	0	0	1
Moribund	6	9	4	1
Natural deaths	4	6	3	31
Animals surviving to study termination	40	35	43	15[e]
Percent probability of survival at end of study[b]	80	70	86	31
Mean survival (days)[c]	702	688	704	547
Survival analysis[d]	P<0.001	P=0.327	P=0.595N	P<0.001
Female				
Animals initially in study	50	50	50	50
Missing[a]	0	1	0	0
Moribund	4	5	8	6
Natural deaths	7	2	10	22
Animals surviving to study termination	39	42	32	22
Percent probability of survival at end of study	78	86	64	44
Mean survival (days)	694	713	695	553
Survival analysis	P<0.001	P=0.433N	P=0.211	P<0.001

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

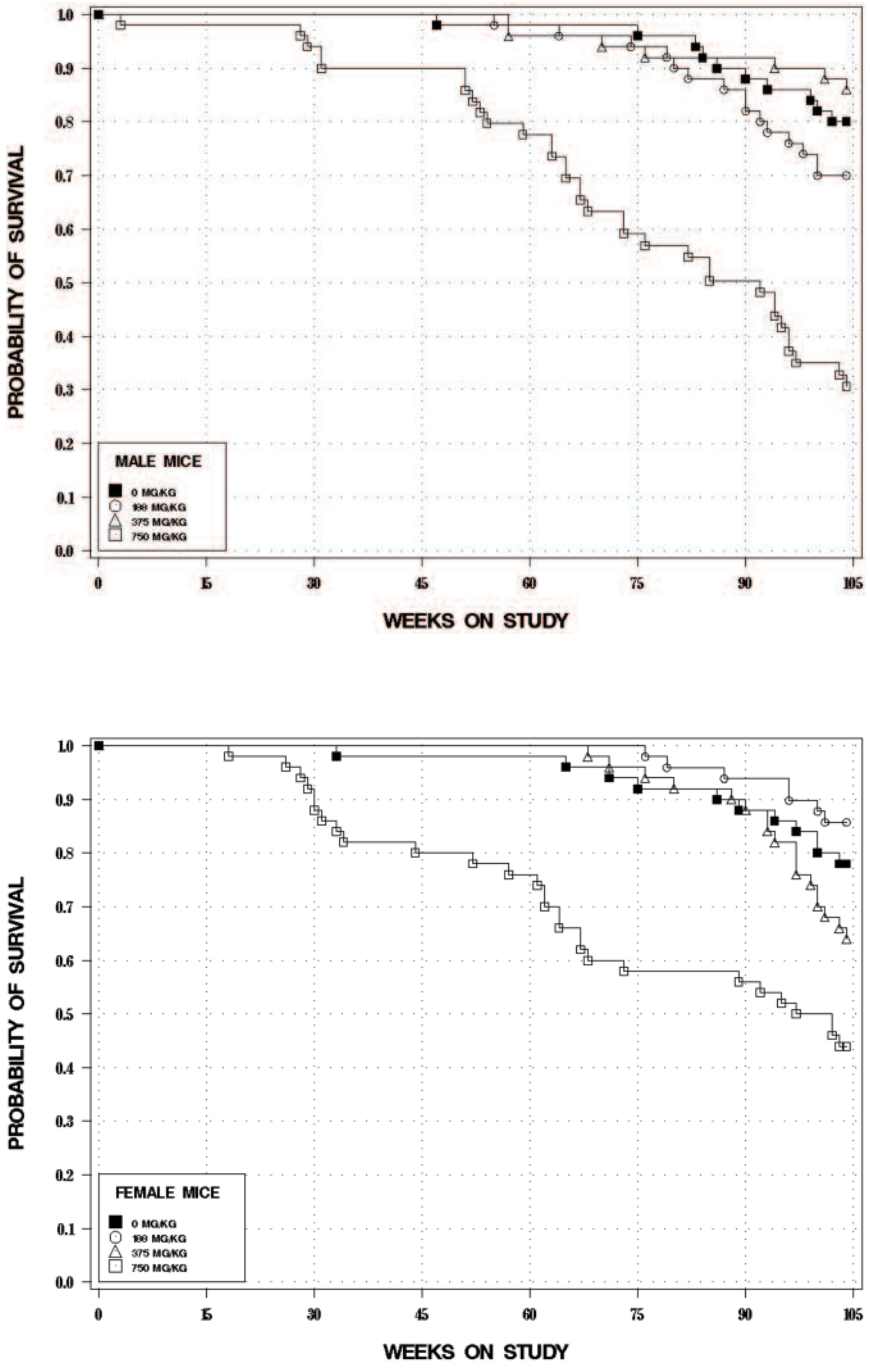


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered 5-(Hydroxymethyl)-2-furfural by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of 750 mg/kg males were significantly less than those of the vehicle controls after week 13 (Table 11 and Figure 5). Mean body weights of 375 and 750 mg/kg females were less than those of the vehicle controls after weeks 17 and 9, respectively (Table 12 and Figure 5).

Beginning during study month 8 and continuing until the end of the study, 750 mg/kg males and females showed clinical signs associated with neurological effects of 5-(hydroxymethyl)-2-furfural administration. These signs included decreased exploratory behavior, piloerection, salivation, Straub tail, catatonia, excitation, dyspnea, clonic-tonic seizures, and unconsciousness and in a few instances, death immediately following the seizures. The onset of these neurological signs was closely associated with the time of gavage administration and progressively increased in severity, although the duration of the toxicity lasted less than an hour. Seizures consisted of repeated contractions and extensions of the limbs followed by full limb extension and an arched back. Seizure duration was 30 to 60 seconds and was followed by apparent unconsciousness and depression of central nervous system function. In general, affected animals fully recovered within 30 minutes after dosing; however, in some instances, the animals did not recover and died shortly after. In general, there were no histopathological lesions in the brain that would explain the clinical neurological signs.

TABLE 11
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

Days on Study	Vehicle Control		188 mg/kg			375 mg/kg			750 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	21.4	50	21.5	101	50	21.5	101	50	21.4	100	50
8	22.9	50	23.0	101	50	23.2	101	50	22.9	100	50
15	24.0	50	24.2	101	50	24.2	101	50	24.2	101	50
22	25.0	50	25.0	100	50	25.1	101	50	24.9	100	49
29	26.0	50	26.4	102	50	26.3	101	50	25.7	99	49
36	27.0	50	27.3	101	50	27.1	101	50	26.7	99	49
43	28.0	50	28.1	100	50	28.0	100	50	27.2	97	49
50	28.5	50	28.6	101	50	28.6	100	50	28.0	98	49
57	29.7	50	30.0	101	50	29.7	100	50	28.4	95	49
64	30.3	50	30.8	102	50	30.6	101	50	29.3	97	49
71	31.1	50	31.1	100	50	31.2	100	50	29.6	95	49
78	31.9	50	32.0	100	50	32.2	101	50	30.5	96	49
85	32.7	50	32.9	101	50	32.9	101	50	31.1	95	49
113	37.3	50	37.2	100	50	37.1	100	50	34.5	92	49
141	40.3	50	40.4	100	50	40.5	101	50	35.7	89	49
169	43.6	50	43.2	99	50	43.2	99	50	37.6	86	49
197	45.1	50	45.1	100	50	44.6	99	50	37.7	84	47
225	46.2	50	46.4	101	50	46.4	100	50	38.4	83	44
253	48.1	50	47.9	99	50	47.5	99	50	40.0	83	44
281	49.1	50	48.5	99	50	48.4	99	50	40.7	83	44
309	50.1	50	49.6	99	50	49.3	98	50	42.0	84	44
337	51.0	49	50.7	100	50	51.1	100	50	43.6	86	44
365	51.1	49	50.2	98	50	50.6	99	50	44.5	87	41
393	51.3	49	50.4	98	49	50.5	98	50	44.8	87	39
421	51.4	49	50.5	98	49	50.9	99	48	45.7	89	38
449	50.4	49	49.5	98	48	50.1	99	48	44.0	87	35
477	50.7	49	50.1	99	48	50.5	100	48	44.3	88	31
505	51.1	49	50.1	98	48	51.1	100	47	44.6	87	30
533	51.3	48	50.8	99	47	51.3	100	46	45.8	89	26
561	51.6	48	51.6	100	45	51.7	100	46	45.9	89	26
589	52.2	46	50.7	97	44	51.7	99	46	45.1	86	23
617	52.3	45	50.9	97	43	52.6	101	46	45.1	86	23
645	52.2	44	51.5	99	40	51.7	99	46	45.7	88	22
673	51.8	43	51.0	99	38	51.3	99	45	44.9	87	17
701	52.0	41	51.1	98	35	50.8	98	45	44.6	86	16
Mean for weeks											
1-13	27.6		27.8	101		27.7	101		26.9	98	
14-52	45.6		45.4	100		45.3	99		38.9	85	
53-101	51.5		50.6	98		51.1	99		45.0	87	

TABLE 12
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

Days on Study	Vehicle Control		188 mg/kg			375 mg/kg			750 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	17.8	50	17.8	100	50	17.7	100	50	17.5	99	50
8	18.4	50	18.3	100	50	18.1	99	50	18.0	98	50
15	19.2	50	19.6	102	50	19.2	100	50	19.1	99	50
22	20.7	50	20.8	101	50	20.6	100	50	20.5	99	50
29	21.1	50	21.6	102	50	20.9	99	50	20.9	99	50
36	22.4	50	22.6	101	50	22.2	99	50	21.7	97	50
43	22.3	50	23.1	104	50	22.7	102	50	22.3	100	50
50	23.5	50	23.6	100	49	23.3	99	50	22.5	96	50
57	24.7	50	24.8	101	49	24.1	98	50	23.7	96	50
64	25.9	50	25.8	100	49	25.3	98	50	24.3	94	50
71	26.1	50	26.0	100	49	25.8	99	50	24.5	94	50
78	26.8	50	26.4	98	49	25.4	95	50	25.1	94	50
85	26.4	50	26.0	99	49	25.9	98	50	24.7	94	50
113	31.0	50	30.5	98	49	29.7	96	50	28.0	90	50
141	34.1	50	33.8	99	49	31.6	93	50	29.7	87	49
169	37.2	50	37.4	100	49	35.1	94	50	32.0	86	49
197	41.4	50	39.7	96	49	38.1	92	50	33.1	80	47
225	43.7	50	44.2	101	49	41.3	95	50	33.0	76	43
253	47.8	49	46.1	97	49	43.5	91	50	33.6	70	41
281	48.9	49	48.6	100	49	44.6	91	50	34.0	70	41
309	52.6	49	51.9	99	49	46.8	89	50	36.3	69	40
337	54.7	49	53.3	97	49	50.0	91	50	36.9	67	40
365	56.7	49	54.6	96	49	51.5	91	50	38.6	68	39
393	58.1	49	56.2	97	49	53.6	92	50	39.4	68	39
421	59.3	49	58.6	99	49	54.4	92	50	40.1	68	38
449	58.9	49	58.8	100	49	54.7	93	50	39.6	67	33
477	60.2	48	58.6	97	49	54.6	91	49	40.2	67	30
505	60.2	47	59.9	99	49	54.3	90	48	38.2	64	30
533	61.8	46	61.4	99	48	55.4	90	47	38.3	62	29
561	62.8	46	62.2	99	47	55.8	89	46	37.8	60	29
589	64.0	46	60.9	95	47	56.5	88	46	37.7	59	29
617	64.4	45	61.6	96	46	56.9	88	45	37.4	58	29
645	64.0	44	61.2	96	46	57.4	90	44	38.0	59	27
673	63.5	43	59.9	94	44	55.8	88	39	38.3	60	26
701	58.9	40	58.2	99	43	56.2	96	34	37.9	64	25
Mean for weeks											
1-13	22.7		22.8	100		22.4	99		21.9	97	
14-52	43.5		42.8	99		40.1	93		33.0	77	
53-101	61.0		59.4	97		55.2	91		38.6	63	

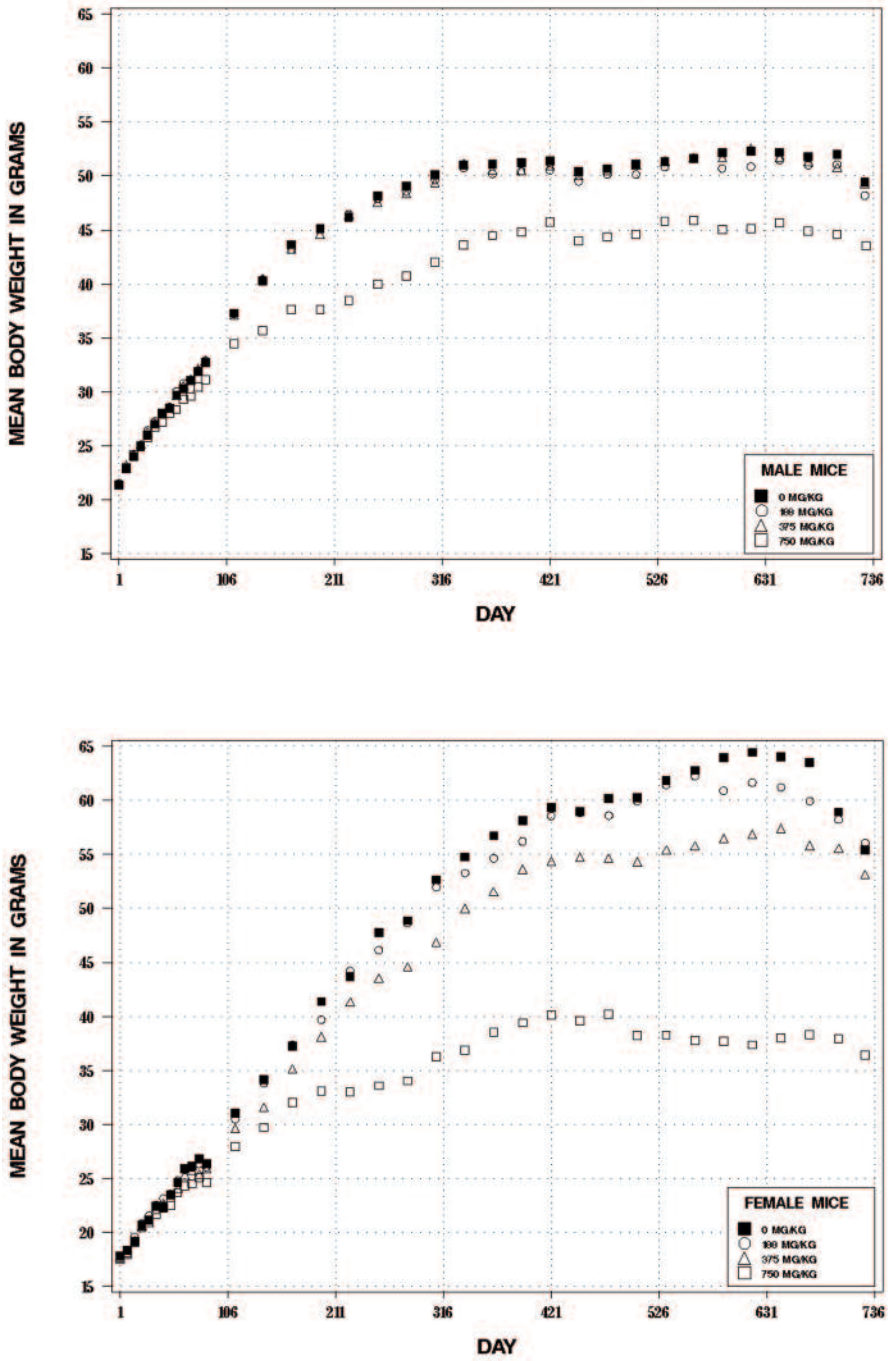


FIGURE 5
Growth Curves for Male and Female Mice Administered 5-(Hydroxymethyl)-2-furfural by Gavage for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, nose, and lung. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in 188 and 375 mg/kg female mice were significantly increased and exceeded the historical control ranges for water gavage studies; however, these incidences were within the historical ranges for all routes of administration (Tables 13, D2, and D3). The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly decreased in 750 mg/kg males (Table C2); these decreased incidences of age-related neoplasms were due to early and high mortality in this dosed group rather than a direct chemical effect.

Incidences of mononuclear cell infiltration and eosinophilic focus were significantly increased in 188 mg/kg females, and the incidences of basophilic foci were significantly increased in 188 and 375 mg/kg females (Tables 13 and D4).

Nose: A spectrum of nonneoplastic lesions considered to be related to administration of 5-(hydroxymethyl)-2-furfural occurred in the nose in both sexes (Tables 14, C3, and D4). The incidences of olfactory epithelium degeneration were significantly increased in 375 and 750 mg/kg males and females. The incidences of olfactory epithelium metaplasia and chronic active inflammation were significantly increased in all dosed groups of males and in 375 and 750 mg/kg females. The incidences of hyaline droplet accumulation in the olfactory and respiratory epithelia were significantly increased in 375 and 750 mg/kg males and females. The incidences of olfactory epithelium hyperplasia were significantly increased in 375 and 750 mg/kg females.

Olfactory epithelium degeneration consisted of segmental loss or dropout of olfactory epithelial cells with concomitant reduction in the number of epithelial cell layers and decrease in the thickness of the epithelium (Plate 5). Respiratory epithelial metaplasia of the olfactory epithelium occurred in the dorsal meatus and dorsal nasal septum of Level II and in the ethmoid turbinates of Level III of the nose and consisted of focal, multifocal, or diffuse replacement of the segments of the normal pseudostratified olfactory epithelium by tall, ciliated, columnar epithelium resembling normal respiratory epithelium (Plate 6). Chronic active inflammation consisted of mixed inflammatory cell infiltrates of macrophages, lymphocytes, and neutrophils in varying numbers in the lamina propria of the dorsal aspects of Levels II and III. Olfactory epithelium hyperplasia consisted of disorganized foci and/or clusters of round discrete cells adjacent to the basal epithelial and extending into the lamina propria (Plate 7).

Hyaline droplet accumulation observed in the olfactory and respiratory epithelia consisted of displacement of the cytoplasm of the mucosal epithelial cells by accumulations of homogenous, brightly eosinophilic droplets/globules (Plate 8). Hyaline droplet accumulation is frequently observed in aging mice, and the severity increases with age. After long-term inhalation exposure to a variety of chemicals, the incidence and severity are often increased in a dose-related manner and are considered a protective response to chronic irritation.

The incidences of hyperplasia and dilatation of the submucosal glands of the nose were significantly increased in 375 and 750 mg/kg males and in all dosed groups of females. The incidences of chronic active inflammation of the glands were significantly increased in all dosed groups of males and in 375 and 750 mg/kg females. The epithelial metaplasia observed in the olfactory mucosa extended into the submucosal Bowman's glands of the olfactory mucosa. Concomitantly, the Bowman's glands were tortuous and there were increased numbers of acinar profiles (glandular hyperplasia) within the lamina propria (Plate 6). The epithelial cells lining the acini were often hypertrophied, and focally, the epithelium was multilayered and disorganized. The lumens of the glands were often dilated and contained varying numbers of macrophages, lymphocytes, and neutrophils (chronic active inflammation) and degenerate cellular debris.

Lung: In males, the incidences of alveolar/bronchiolar carcinoma in the 188 and 375 mg/kg groups (vehicle control, 7/50; 188 mg/kg, 1/50; 375 mg/kg, 1/50; 750 mg/kg, 0/49) and alveolar/bronchiolar adenoma or carcinoma (combined) in the 188 and 750 mg/kg groups (16/50, 6/50, 11/50, 3/49) were significantly decreased

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Number Examined Microscopically	50	49	50	50
Infiltration Cellular, Mononuclear Cell[a]	32 (1.4)[b]	45** (1.1)	34 (1.2)	27 (1.2)
Basophilic Focus	0	6*	5*	0
Eosinophilic Focus	6	14**	6	3
Hepatocellular Adenoma, Multiple	4	10	7	1
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	14/50 (28%)	26/49 (53%)	26/50 (52%)	6/50 (12%)
Adjusted rate[e]	30.8%	55.4%	56.5%	19.7%
Terminal rate[f]	14/39 (36%)	24/42 (57%)	19/32 (59%)	6/22 (27%)
First incidence (days)	727 (T)	695	555	727 (T)
Poly-3 test[g]	P=0.354N	P=0.013	P=0.009	P=0.216N
Poly-3 test[h]	P<0.001			
Hepatocellular Carcinoma, Multiple	0	0	1	1
Hepatocellular Carcinoma (includes multiple)[i]	2	1	2	4
Hepatocellular Adenoma or Carcinoma[j]				
Overall rate	14/50 (28%)	26/49 (53%)	26/50 (52%)	10/50 (20%)
Adjusted rate	30.8%	55.4%	56.5%	31.5%
Terminal rate	14/39 (36%)	24/42 (57%)	19/32 (59%)	7/22 (32%)
First incidence (days)	727 (T)	695	555	507
Poly-3 test	P=0.408	P=0.013	P=0.009	P=0.572
Poly-3 test[h]	P<0.001			

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with water vehicle control groups (mean ± standard deviation): 20/100 (20.0% ± 11.3%), range 12%-28%; all routes: 345/1,245 (27.8% ± 17.0%), range 2%-62%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[h] Excluding 750 mg/kg group
[i] Historical incidence for water gavage studies: 6/100 (6.0% ± 2.8%), range 4%-8%; all routes: 131/1,245 (10.5% ± 7.7%), range 0%-28%
[j] Historical incidence for water gavage studies: 23/100 (23.0% ± 7.1%), range 18%-28%; all routes: 419/1,245 (33.7% ± 19.1%), range 8%-64%

TABLE 14
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Male				
Number Examined Microscopically	50	50	50	47
Olfactory Epithelium, Degeneration[a]	4 (2.0)[b]	2 (1.5)	17** (1.6)	39** (2.6)
Olfactory Epithelium, Metaplasia	1 (2.0)	7* (2.1)	38** (1.9)	43** (3.9)
Olfactory Epithelium, Accumulation, Hyaline Droplet	13 (1.7)	17 (1.8)	29** (1.7)	27** (1.4)
Inflammation, Chronic Active	0	6* (1.3)	18** (1.0)	45** (1.5)
Respiratory Epithelium, Accumulation, Hyaline Droplet	14 (1.6)	17 (1.5)	23* (1.8)	31** (1.7)
Glands, Hyperplasia	3 (1.7)	7 (2.0)	45** (1.8)	45** (2.9)
Glands, Dilatation	16 (1.9)	22 (1.9)	47** (2.0)	45** (2.0)
Glands, Inflammation, Chronic Active	4 (1.5)	12* (1.7)	34** (1.6)	43** (1.7)
Female				
Number Examined Microscopically	49	49	50	50
Olfactory Epithelium, Degeneration	2 (1.5)	1 (1.0)	34** (2.1)	24** (2.4)
Olfactory Epithelium, Metaplasia	1 (1.0)	5 (1.6)	30** (1.9)	40** (4.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	1 (1.0)	27** (1.6)	25** (1.8)
Olfactory Epithelium, Hyperplasia	0	0	8** (1.1)	24** (1.3)
Inflammation, Chronic Active	0	1 (1.0)	14** (1.0)	41** (1.7)
Respiratory Epithelium, Accumulation, Hyaline Droplet	4 (1.5)	4 (1.3)	36** (2.2)	27** (1.8)
Glands, Hyperplasia	0	7** (1.4)	42** (1.9)	43** (2.8)
Glands, Dilatation	12 (1.4)	36** (1.8)	48** (1.9)	47** (2.2)
Glands, Inflammation, Chronic Active	1 (1.0)	6 (1.0)	21** (1.3)	38** (1.7)

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

relative to the incidences in the vehicle control group (Table C2).

GENETIC TOXICOLOGY

5-(Hydroxymethyl)-2-furfural was tested in two independent bacterial mutagenicity assays (Table E1). In the study conducted at BioReliance Corporation, 5-(hydroxymethyl)-2-furfural was found to be weakly mutagenic in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation (S9) over a concentration range of 100 to 10,000 µg/plate; no mutagenic activity was detected in TA100 with S9 or in strains TA97, TA98, TA102, or TA1535, with or without S9. In the study conducted at SITEK Research Laboratories, no mutagenicity was detected over a concentration range of 1,500 to 10,000 µg/plate, with or without S9, in strains TA98 or TA100 or *Escherichia coli* WP2 *uvrA*/pKM101. No increases in the frequencies of micronucleated normochromatic erythrocytes, which are biomarkers of induced chromosomal damage, were observed in peripheral blood of male or female mice administered 5-(hydroxymethyl)-2-furfural by gavage for 3 months (Table E2); in addition, no significant dose-related changes in the percentage of immature polychromatic erythrocytes were observed in mice administered 5-(hydroxymethyl)-2-furfural, suggesting that the chemical did not cause bone marrow toxicity.

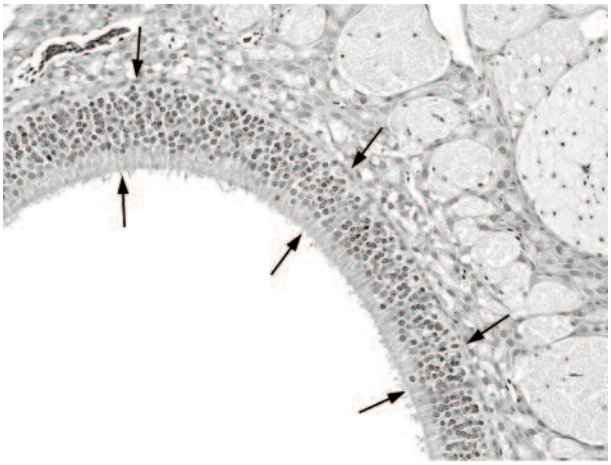


PLATE 1
Normal olfactory epithelium lining the ethmoid turbinates in a female F344/N vehicle control rat at 2 years in the gavage study of 5-(hydroxymethyl)-2-furfural. The epithelium is pseudostratified with multiple layers of cell nuclei (arrows). H&E

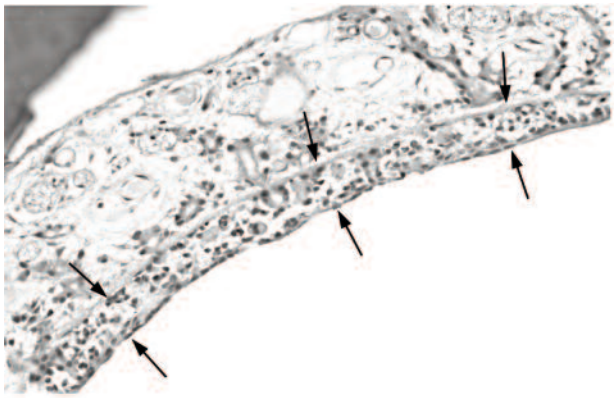


PLATE 2
Olfactory epithelium degeneration in a female F344/N rat administered 750 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. Note the vacuolization and loss of olfactory epithelial cells and decreased thickness of the epithelium (arrows) compared to the normal epithelium in Plate 1. H&E

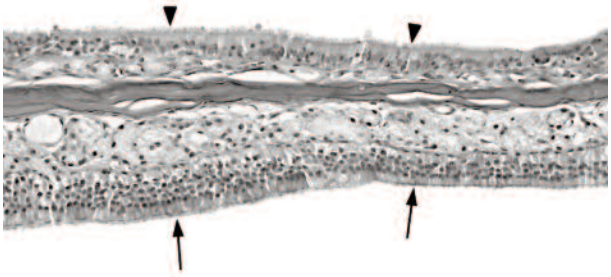


PLATE 3
Olfactory epithelium respiratory metaplasia in a female F344/N rat administered 750 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. In contrast to the normal pseudostratified olfactory epithelium lining one side of the nasal septum (arrows), the epithelium lining the opposite side is replaced by a single layer of tall columnar (metaplastic) epithelial cells (arrowheads). H&E

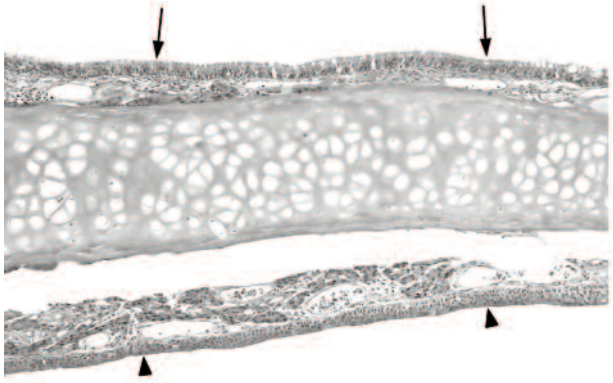


PLATE 4
Respiratory epithelium squamous metaplasia in a female F344/N rat administered 750 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. In contrast to the normal single layer of columnar epithelium lining one side of the nasal septum (arrows), the epithelium lining the opposite side is replaced by flattened, mature, stratified squamous epithelium (arrowheads). H&E

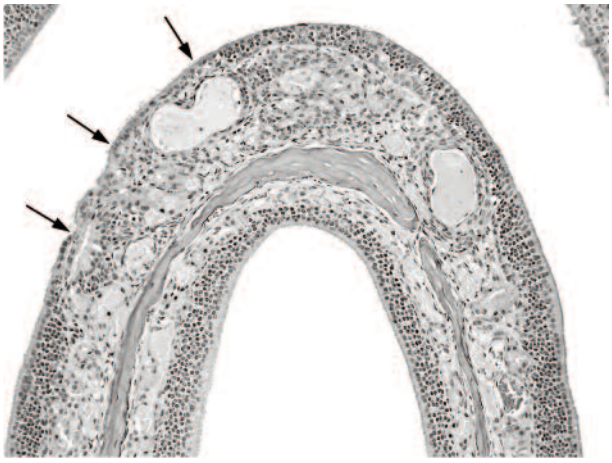


PLATE 5
Olfactory epithelium degeneration in a male B6C3F1 mouse administered 750 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. Note the focal loss of olfactory epithelial cells with reduction in the number of epithelial cell layers and decrease in the thickness of the epithelium (arrows) in contrast to the adjacent normal olfactory epithelium. H&E

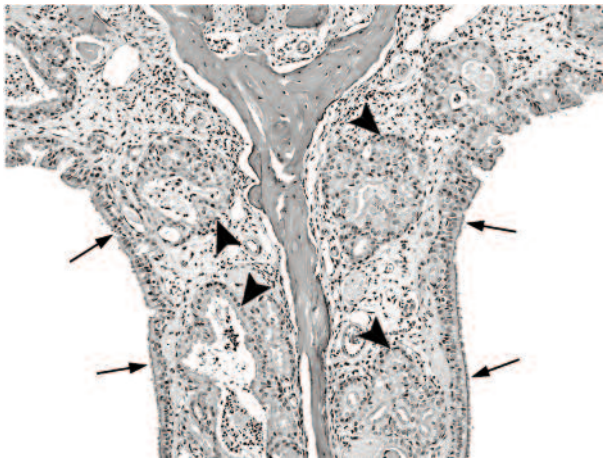


PLATE 6
Olfactory epithelium metaplasia in a male B6C3F1 mouse administered 750 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. The normal olfactory epithelium has been replaced by a single layer of tall columnar epithelial cells (arrows). Note also submucosal Bowman's gland hyperplasia, dilatation, and chronic active inflammation (arrowheads) characterized by large glands lined by tall columnar epithelial cells with focal disorganization and piling up of the cells (hyperplasia) and dilated lumens that contain inflammatory cells and cellular debris (chronic active inflammation). H&E

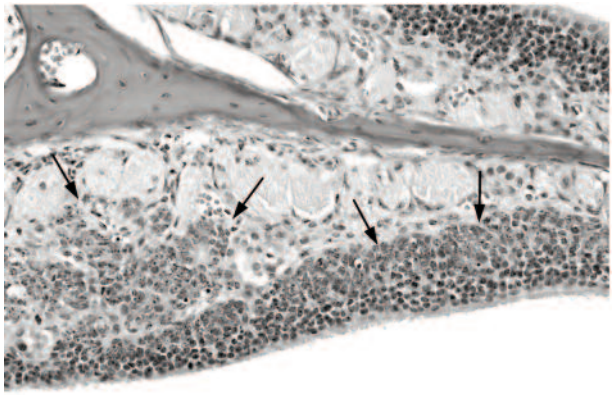


PLATE 7
Olfactory epithelium hyperplasia in a female B6C3F1 mouse administered 750 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. Note the proliferation of round discrete cells at the base of the epithelium and as a focal cluster within the lamina propria (arrows). H&E

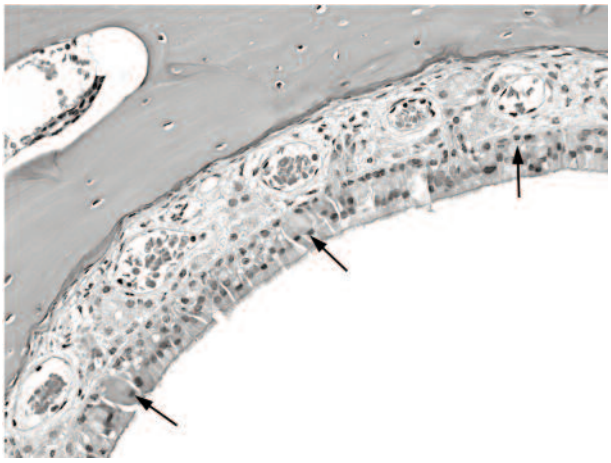


PLATE 8
Olfactory epithelium hyaline droplet accumulation in a female B6C3F1 mouse administered 375 mg/kg 5-(hydroxymethyl)-2-furfural by gavage for 2 years. Note the homogenous droplets/globules within the cytoplasm of the mucosal epithelium (arrows). H&E

DISCUSSION AND CONCLUSIONS

5-(Hydroxymethyl)-2-furfural, a major product of the Maillard reaction, is formed when reducing sugars such as fructose and glucose are heated at acid pH in the presence of amino acids (Antal *et al.*, 1990). 5-(Hydroxymethyl)-2-furfural is ubiquitous in the human diet, and a recent examination of approximately 500 different food samples revealed 5-(hydroxymethyl)-2-furfural concentrations greater than 1 g/kg in dried fruits, caramel products, and certain types of fruit juices; concentrations up to 3.5 g/kg in dried pears (Bachmann *et al.*, 1997); and concentrations up to 6.2 g/kg in instant coffee (Schultheiss *et al.*, 2000). Formation of 5-(hydroxy-methyl)-2-furfural in foods varies with processing, storage conditions, storage temperature, and pH. Because of the widespread human exposure to 5-(hydroxy-methyl)-2-furfural and the absence of adequate data on the toxicology and carcinogenic potential, 5-(hydroxymethyl)-2-furfural was selected for in-depth evaluation by the National Toxicology Program.

During the 3-week study, one 1,500 mg/kg male rat died on study day 6; no deaths occurred among female rats. During the 3-month study, three 1,500 mg/kg females died during weeks 1, 5, and 14. Mean body weights of 750 and 1,500 mg/kg male rats were slightly less than those of the vehicle controls, but no treatment-related microscopic lesions or other indicators of toxicity were observed. Based on these results, doses of 188, 375, and 750 mg/kg were selected for the 2-year study.

In the 3-month study, female rats had elongated estrous cycles; fewer 750 and 1,500 mg/kg females had regular cycles, and dosed females spent significantly more time in diestrus. Based on these data, 5-(hydroxymethyl)-2-furfural has the potential to produce adverse effects in studies of fertility and reproductive performance. Based on the SMVCE results, the reproductive organ weights, and histopathology of the reproductive system, there was no evidence of toxicity to the reproductive system of male rats in this 3-month study.

Oxidation to furoic acid and conjugation with glycine is one of the major routes of elimination of 5-(hydroxymethyl)-2-furfural in rats and mice. Previous studies of glycine conjugation capacity have shown that glycine availability is limiting for conjugation in rats (Gregus *et al.*, 1992, 1993). This was also found by Godfrey *et al.* (1999) and Germond *et al.* (1987) who observed that in rats the yield of 5-(hydroxymethyl)-2-furoylglycine in urine was inversely related to the dose of 5-(hydroxymethyl)-2-furfural administered. In addition, Godfrey *et al.* (1999) found that 5-(hydroxymethyl)-2-furfural crossed the blood-brain barrier. The effect of glycine availability on the toxic responses of rats to benzyl acetate was examined by Abdo *et al.* (1998). Conjugation with glycine and elimination in urine is the major path of excretion for benzyl acetate in rats. Rats fed diets containing only benzyl acetate exhibited increased mortality, reduced body weights, increased incidences of neurobehavioral signs such as ataxia and convulsions, as well as astrocyte hypertrophy and neuronal necrosis in the cerebellum, hippocampus, and pyriform cortex of the brain. The effects were reduced significantly by supplementation with glycine but not with L-alanine. These results suggest that neurodegeneration induced by benzyl acetate is mediated by depletion of the glycine pool. Therefore, to monitor for possible neurotoxicity associated with glycine depletion during prechronic studies, special study rats that received 0 or 1,500 mg/kg 5-(hydroxymethyl)-2-furfural in the 3-week study or the same doses as the core animal groups in the 3-month study underwent perfusion fixation and special processing and examination for microscopic lesions in the brain and central nervous system. In addition, urine was collected from core study rats and mice at the end of the 3-week studies and at three time points during the 3-month studies to determine whether the ratio of the furoic acid metabolite to the furoylglycine metabolite would change with continuous chemical exposure (Appendix G).

No microscopic lesions associated with chemical exposure or indicative of a toxic response were found in the brain or central nervous system of rats. Slight differences were observed in the amount of the furoic acid metabolite and glycine conjugate formed at day 17 compared to day 94 (Table G2; Figures G1 and G2); however, there was no indication of glycine depletion with

continuous dosing. In mice, continuous exposure to 5-(hydroxymethyl)-2-furfural appeared to induce the enzymes involved in oxidation to the furoic acid metabolite as well as glycine conjugation because considerably more of each metabolite was excreted in the urine of mice on day 94 compared to the amount present on day 17 (Table G4; Figures G3 and G4). However, as with rats, there was no evidence of glycine depletion with time of dosing.

During the 2-year rat study, survival and mean body weights of dosed groups of male and female rats were similar to those of the vehicle control groups. There were no neoplasms in rats associated with exposure to 5-(hydroxymethyl)-2-furfural. Treatment-related nonneoplastic lesions of the olfactory and respiratory epithelium of the nose were present in both male and female rats. The incidences and severities of these lesions were highest in the 750 mg/kg groups. The only other treatment-related nonneoplastic lesion was a significant increase in the incidence of clear cell foci in the liver of 750 mg/kg males.

Three male and three female 1,500 mg/kg mice died during the 3-week study. These deaths all occurred after approximately 1 week of dosing, which did not suggest a cumulative toxic effect. Based on these results, doses of 47, 94, 188, 375, and 750 mg/kg were selected for the 3-month study. Administration of 5-(hydroxymethyl)-2-furfural for up to 3 months produced few indications of toxicity in mice. The mean body weight of 750 mg/kg males was less than that of the vehicle controls, and mean body weight gains of 750 mg/kg males and females were less than those of the vehicle control groups. One 750 mg/kg male and one 375 mg/kg female died before the end of the study of unknown causes. Based on the SMVCE results, the reproductive organ weights, and histopathology of the reproductive system, there was no evidence of toxicity to the reproductive system of male or female mice in this 3-month study. The only treatment-related lesion was cytoplasmic alteration of the epithelium of the renal proximal tubules in 188 mg/kg or greater males. Based on these results, doses of 188, 375, and 750 mg/kg were selected for the 2-year study.

Survival and mean body weights of mice that received 188 or 375 mg/kg were comparable to those of the vehicle control groups during the 2-year study; however, survival of 750 mg/kg male and female mice was significantly less than that of the vehicle control groups beginning at approximately week 45 in males and week 30 in females. Starting at study month 8, both male and female mice exhibited clinical signs suggestive of some type of neurological response. Immediately (within the first minute) after dosing, animals exhibited decreased exploratory behavior, piloerection, salivation, Straub tail, catatonia, and overreactive response to auditory stimulus. Within 5 to 10 minutes after dosing, clonic-tonic seizures (30 to 60 seconds in duration) were frequently observed followed by apparent unconsciousness. By 30 minutes after dosing, the animals had recovered to predosing activity and exhibited normal behavior such as eating and grooming. However, on some occasions, the animals did not recover and died shortly after the seizure.

The disposition studies of Godfrey *et al.* (1999) found that 2 hours after administration 5-(hydroxymethyl)-2-furfural derived C^{14} was present in mouse brain, and the amount increased with increasing dose. However, the rapid onset of clinical signs immediately after dosing seems incompatible with the kinetics of oral absorption since it is unlikely that sufficient 5-(hydroxymethyl)-2-furfural could be absorbed through the gastrointestinal tract and produce blood levels high enough to cause the clinical signs within 1 to 10 minutes after gavage administration. In addition, the onset of clinical signs did not occur until study month 8, suggesting that some type of cumulative injury had to progress to a critical level before 5-(hydroxymethyl)-2-furfural could reach high enough concentrations in the brain to trigger a neurological response.

An alternative route of transfer of 5-(hydroxymethyl)-2-furfural into the brain is suggested by the presence of nasal lesions, primarily in the olfactory epithelium, which in mice exhibited dose-related increases in severity. Similar but less severe lesions were observed in male and female rats, clearly indicating that 5-(hydroxymethyl)-2-furfural was present in the nasal cavity of both species. The progression of inflammatory lesions of the olfactory and respiratory epithelium, which in mice were more severe in the 750 mg/kg groups, with repeated dosing, could explain why the clinical signs did not appear until study month 8, at which time the inflammation may have become severe enough to allow rapid transfer of 5-(hydroxymethyl)-2-furfural directly into the brain. For compounds readily absorbed through the olfactory epithelium into the brain, peak concentrations

are reached within minutes after intranasal administration (Einer-Jensen and Larsen, 2000), suggesting that 5-(hydroxymethyl)-2-furfural entering the brain through compromised olfactory epithelium might follow similar kinetics. This would be more consistent with the rapid time of onset of clinical signs. At present, however, the cause of death for mice receiving 750 mg/kg is unknown. Because of the reduced survival of this group and the presence of the treatment-related clinical signs, groups of mice that received 750 mg/kg were not included in the evaluation of carcinogenic potential.

Incidences of hepatocellular adenoma were significantly increased in female mice administered 188 or 375 mg/kg. Although incidences of hepatocellular carcinoma were not increased in the 188 or 375 mg/kg groups, the incidences of hepatocellular adenoma in these groups were nearly twice that in the vehicle controls and were considered to be associated with 5-(hydroxymethyl)-2-furfural administration. There were no other neoplasms in mice related to 5-(hydroxymethyl)-2-furfural administration.

It is interesting to compare the results of the present study of 5-(hydroxymethyl)-2-furfural to the 2-year studies of furfural and furan (NTP 1990, 1993). Both compounds were administered by gavage in corn oil. Furan was extremely hepatotoxic in prechronic studies, producing cytomegaly and degeneration of hepatocytes, bile duct hyperplasia, cholangiofibrosis, and nodular hyperplasia in rats and similar lesions in mice (NTP, 1993). Doses used in the 2-year furan study were 2, 4, and 8 mg/kg for rats and 8 and 15 mg/kg for mice. Exposure to furan at these doses caused significant dose-related increases in the incidences of hepatocellular neoplasms and of cholangiocarcinoma (approximately a 100% incidence rate in the 8 mg/kg groups) in rats of both sexes and of hepatocellular neoplasms in mice of both sexes. These results were judged to constitute clear evidence of carcinogenic activity of furan in male and female rats and mice.

Furfural was much less hepatotoxic than furan in prechronic studies and was administered at doses of 30 or 60 mg/kg to rats and 50, 100, or 175 mg/kg to mice in the 2-year studies (NTP, 1990). Furfural exposure produced only some evidence of carcinogenic activity in male rats based on the presence of cholangiocarcinoma in two animals, clear evidence of carcinogenic activity in male mice and some evidence of carcinogenic activity in female mice based on increased incidences of hepatocellular neoplasms. There was no evidence of carcinogenic activity in female rats.

Thus, 5-(hydroxymethyl)-2-furfural was less toxic than furfural, and both compounds were much less toxic than furan.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of 5-(hydroxymethyl)-2-furfural in male or female F344/N rats administered 188, 375, or 750 mg/kg. There was *no evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in male B6C3F1 mice administered 188 or 375 mg/kg. There was *some evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in female B6C3F1 mice based on increased incidences of hepatocellular adenoma in the 188 and 375 mg/kg groups.

Administration of 5-(hydroxymethyl)-2-furfural was associated with increased incidences of lesions of the olfactory and respiratory epithelium of the nose in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF 5-(HYDROXYMETHYL)-2-FURFURAL

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	1
Moribund	20	9	9	10
Natural deaths	8	7	9	4
Survivors				
Terminal sacrifice	22	34	31	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma			2 (4%)	
Mesentery	(9)	(8)	(7)	(6)
Fibrosarcoma, metastatic, spleen			1 (14%)	
Liposarcoma, metastatic, uncertain primary site				1 (17%)
Neurofibrosarcoma			1 (14%)	1 (17%)
Pancreas	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, spleen			1 (2%)	
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Acinus, adenoma	1 (2%)			
Salivary glands	(50)	(50)	(49)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(1)	(0)	(0)
Squamous cell papilloma		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		2 (4%)		
Schwannoma malignant			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		2 (4%)		
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	5 (10%)	6 (12%)	6 (12%)	6 (12%)
Pheochromocytoma malignant		1 (2%)	1 (2%)	
Bilateral, pheochromocytoma benign				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Carcinoma	2 (4%)		1 (2%)	
Parathyroid gland	(49)	(48)	(48)	(48)
Adenoma			1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	15 (30%)	16 (32%)	15 (30%)	8 (16%)
Pars distalis, carcinoma				1 (2%)
Pars intermedia, carcinoma		1 (2%)		
Thyroid gland	(50)	(49)	(48)	(48)
C-cell, adenoma	1 (2%)	6 (12%)	12 (25%)	4 (8%)
C-cell, carcinoma		1 (2%)	2 (4%)	2 (4%)
Follicular cell, adenoma	1 (2%)			
Follicular cell, carcinoma	2 (4%)	1 (2%)		2 (4%)
General Body System				
Peritoneum	(1)			
Genital System				
Coagulating gland	(1)	(0)	(0)	(4)
Epididymis	(50)	(50)	(50)	(50)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	4 (8%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	
Bilateral, adenoma		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	1 (2%)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	40 (80%)	35 (70%)	37 (74%)	40 (80%)
Interstitial cell, adenoma	8 (16%)	10 (20%)	11 (22%)	7 (14%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(10)	(6)	(4)	(4)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung		1 (17%)		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		1 (17%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Spleen	(50)	(50)	(50)	(50)
Fibroma			1 (2%)	
Leiomyosarcoma	1 (2%)			
Thymus	(48)	(47)	(49)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Fibroadenoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Skin	(50)	(49)	(50)	(50)
Basal cell adenoma		1 (2%)	1 (2%)	
Basal cell adenoma, multiple	1 (2%)			
Keratoacanthoma	2 (4%)	5 (10%)	3 (6%)	
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Squamous cell carcinoma	1 (2%)	2 (4%)	1 (2%)	
Squamous cell papilloma				1 (2%)
Trichoepithelioma			1 (2%)	
Sebaceous gland, adenoma		1 (2%)	1 (2%)	
Subcutaneous tissue, fibroma	5 (10%)	4 (8%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibroma, multiple		1 (2%)		
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)		1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chondroma			1 (2%)	
Hemangiosarcoma			1 (2%)	
Osteosarcoma				1 (2%)
Skeletal muscle	(3)	(1)	(2)	(2)
Hemangiosarcoma			1 (50%)	
Liposarcoma, metastatic, uncertain primary site				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant		1 (2%)	1 (2%)	
Carcinoma, metastatic, pituitary gland		1 (2%)		
Oligodendroglioma benign			1 (2%)	
Spinal cord	(1)	(1)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma				2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)		
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Osteosarcoma, metastatic, bone				1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		2 (4%)		
Mediastinum, osteosarcoma, metastatic, bone				1 (2%)
Nose	(50)	(49)	(48)	(49)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(0)	(0)	(0)	(2)
Pinna, neural crest tumor				1 (50%)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(49)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Renal tubule, adenoma				1 (2%)
Renal tubule, carcinoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	28 (56%)	27 (54%)	31 (62%)	18 (36%)
Lymphoma malignant			1 (2%)	
Mesothelioma benign		1 (2%)		
Mesothelioma malignant	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	49	49
Total primary neoplasms	127	141	147	112
Total animals with benign neoplasms	50	49	49	48
Total benign neoplasms	87	99	101	79
Total animals with malignant neoplasms	35	35	36	27
Total malignant neoplasms	40	41	46	32
Total animals with metastatic neoplasms		4	1	2
Total metastatic neoplasms		9	2	13
Total animals with malignant neoplasms of uncertain primary site				1
Total animals with uncertain neoplasms-benign or malignant				1
Total uncertain neoplasms				1

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Adrenal Medulla: Pheochromocytoma Benign				
Overall rate[a]	5/50 (10%)	6/50 (12%)	6/50 (12%)	7/50 (14%)
Adjusted rate[b]	12.2%	13.2%	13.8%	15.6%
Terminal rate[c]	4/22 (18%)	4/34 (12%)	5/31 (16%)	6/35 (17%)
First incidence (days)	666	693	688	720
Poly-3 test[d]	P=0.376	P=0.569	P=0.539	P=0.443
Adrenal Medulla: Pheochromocytoma Benign or Malignant				
Overall rate	5/50 (10%)	7/50 (14%)	7/50 (14%)	7/50 (14%)
Adjusted rate	12.2%	15.4%	16.1%	15.6%
Terminal rate	4/22 (18%)	4/34 (12%)	6/31 (19%)	6/35 (17%)
First incidence (days)	666	693	688	720
Poly-3 test	P=0.416	P=0.451	P=0.417	P=0.443
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	9.6%	4.4%	6.9%	4.5%
Terminal rate	1/22 (5%)	2/34 (6%)	3/31 (10%)	2/35 (6%)
First incidence (days)	589	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.304N	P=0.299N	P=0.479N	P=0.302N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	15/50 (30%)	16/50 (32%)	15/50 (30%)	8/50 (16%)
Adjusted rate	34.6%	34.5%	34.1%	17.8%
Terminal rate	7/22 (32%)	10/34 (29%)	13/31 (42%)	8/35 (23%)
First incidence (days)	519	589	610	727 (T)
Poly-3 test	P=0.037N	P=0.583N	P=0.571N	P=0.057N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	15/50 (30%)	16/50 (32%)	15/50 (30%)	9/50 (18%)
Adjusted rate	34.6%	34.5%	34.1%	20.1%
Terminal rate	7/22 (32%)	10/34 (29%)	13/31 (42%)	9/35 (26%)
First incidence (days)	519	589	610	727 (T)
Poly-3 test	P=0.064N	P=0.583N	P=0.571N	P=0.094N
Preputial Gland: Adenoma				
Overall rate	5/50 (10%)	5/50 (10%)	1/50 (2%)	1/50 (2%)
Adjusted rate	11.9%	10.8%	2.3%	2.2%
Terminal rate	0/22 (0%)	3/34 (9%)	1/31 (3%)	0/35 (0%)
First incidence (days)	541	436	727 (T)	720
Poly-3 test	P=0.030N	P=0.564N	P=0.094N	P=0.086N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	14.2%	10.8%	4.6%	2.2%
Terminal rate	0/22 (0%)	3/34 (9%)	2/31 (7%)	0/35 (0%)
First incidence (days)	541	436	727 (T)	720
Poly-3 test	P=0.022N	P=0.433N	P=0.124N	P=0.046N
Skin: Keratoacanthoma				
Overall rate	2/50 (4%)	5/50 (10%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.9%	10.9%	6.9%	0.0%
Terminal rate	1/22 (5%)	3/34 (9%)	2/31 (7%)	0/35 (0%)
First incidence (days)	666	587	693	—[e]
Poly-3 test	P=0.089N	P=0.262	P=0.525	P=0.218N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Skin: Squamous Cell Papilloma or Keratocanthoma				
Overall rate	2/50 (4%)	5/50 (10%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.9%	10.9%	6.9%	2.2%
Terminal rate	1/22 (5%)	3/34 (9%)	2/31 (7%)	1/35 (3%)
First incidence (days)	666	587	693	727 (T)
Poly-3 test	P=0.209N	P=0.262	P=0.525	P=0.469N
Skin: Squamous Cell Papilloma, Keratocanthoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	1/50 (2%)
Adjusted rate	7.3%	10.9%	9.1%	2.2%
Terminal rate	2/22 (9%)	3/34 (9%)	2/31 (7%)	1/35 (3%)
First incidence (days)	666	587	610	727 (T)
Poly-3 test	P=0.148N	P=0.417	P=0.535	P=0.274N
Skin: Squamous Cell Papilloma, Keratocanthoma, Trichoepithelioma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	6/50 (12%)	6/50 (12%)	1/50 (2%)
Adjusted rate	9.7%	13.1%	13.5%	2.2%
Terminal rate	3/22 (14%)	3/34 (9%)	3/31 (10%)	1/35 (3%)
First incidence (days)	666	587	490	727 (T)
Poly-3 test	P=0.095N	P=0.439	P=0.420	P=0.152N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	5/50 (10%)	5/50 (10%)	1/50 (2%)	2/50 (4%)
Adjusted rate	12.0%	11.1%	2.3%	4.5%
Terminal rate	2/22 (9%)	5/34 (15%)	0/31 (0%)	2/35 (6%)
First incidence (days)	519	727 (T)	707	727 (T)
Poly-3 test	P=0.084N	P=0.581N	P=0.092N	P=0.187N
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	6/50 (12%)	6/50 (12%)	1/50 (2%)	2/50 (4%)
Adjusted rate	14.3%	13.3%	2.3%	4.5%
Terminal rate	2/22 (9%)	6/34 (18%)	0/31 (0%)	2/35 (6%)
First incidence (days)	519	727 (T)	707	727 (T)
Poly-3 test	P=0.039N	P=0.567N	P=0.050N	P=0.111N
Testes: Adenoma				
Overall rate	48/50 (96%)	45/50 (90%)	48/50 (96%)	47/50 (94%)
Adjusted rate	97.5%	94.3%	97.9%	97.6%
Terminal rate	22/22 (100%)	33/34 (97%)	30/31 (97%)	34/35 (97%)
First incidence (days)	489	548	483	533
Poly-3 test	P=0.475	P=0.362N	P=0.727	P=0.770
Thyroid Gland (C-cell): Adenoma				
Overall rate	1/50 (2%)	6/49 (12%)	12/48 (25%)	4/48 (8%)
Adjusted rate	2.5%	13.6%	27.9%	9.1%
Terminal rate	1/22 (5%)	6/34 (18%)	8/31 (26%)	4/35 (11%)
First incidence (days)	727 (T)	727 (T)	490	727 (T)
Poly-3 test	P=0.301	P=0.069	P<0.001	P=0.201
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	7/49 (14%)	14/48 (29%)	6/48 (13%)
Adjusted rate	2.5%	15.8%	32.5%	13.7%
Terminal rate	1/22 (5%)	7/34 (21%)	10/31 (32%)	5/35 (14%)
First incidence (days)	727 (T)	727 (T)	490	720
Poly-3 test	P=0.144	P=0.039	P<0.001	P=0.067

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/49 (2%)	0/48 (0%)	2/48 (4%)
Adjusted rate	7.3%	2.3%	0.0%	4.6%
Terminal rate	2/22 (9%)	1/34 (3%)	0/31 (0%)	1/35 (3%)
First incidence (days)	589	727 (T)	—	720
Poly-3 test	P=0.459N	P=0.281N	P=0.116N	P=0.472N
All Organs: Mononuclear Cell Leukemia				
Overall rate	28/50 (56%)	27/50 (54%)	31/50 (62%)	18/50 (36%)
Adjusted rate	59.5%	56.3%	66.8%	38.6%
Terminal rate	9/22 (41%)	16/34 (47%)	18/31 (58%)	12/35 (34%)
First incidence (days)	489	366	603	349
Poly-3 test	P=0.026N	P=0.457N	P=0.300	P=0.031N
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.8%	4.4%	4.6%	8.7%
Terminal rate	0/22 (0%)	2/34 (6%)	1/31 (3%)	2/35 (6%)
First incidence (days)	532	727 (T)	605	533
Poly-3 test	P=0.247	P=0.666N	P=0.679N	P=0.382
All Organs: Benign or Malignant Mesothelioma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.8%	6.6%	4.6%	8.7%
Terminal rate	0/22 (0%)	3/34 (9%)	1/31 (3%)	2/35 (6%)
First incidence (days)	532	727 (T)	605	533
Poly-3 test	P=0.317	P=0.535	P=0.679N	P=0.382
All Organs: Benign Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	49/50 (98%)	48/50 (96%)
Adjusted rate	100.0%	99.7%	100.0%	99.6%
Terminal rate	22/22 (100%)	34/34 (100%)	31/31 (100%)	35/35 (100%)
First incidence (days)	489	436	483	533
Poly-3 test	P=0.992N	P=1.000N	P=1.000N	P=1.000N
All Organs: Malignant Neoplasms				
Overall rate	35/50 (70%)	35/50 (70%)	36/50 (72%)	28/50 (56%)
Adjusted rate	72.5%	72.0%	74.7%	58.3%
Terminal rate	12/22 (55%)	22/34 (65%)	20/31 (65%)	18/35 (51%)
First incidence (days)	489	366	483	349
Poly-3 test	P=0.067N	P=0.568N	P=0.497	P=0.101N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	99.9%
Terminal rate	22/22 (100%)	34/34 (100%)	31/31 (100%)	35/35 (100%)
First incidence (days)	489	366	483	349
Poly-3 test	P=1.000N	—[f]	P=1.000N	P=1.000N

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	1
Moribund	20	9	9	10
Natural deaths	8	7	9	4
Survivors				
Terminal sacrifice	22	34	31	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Periesophageal tissue, hemorrhage			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)			
Parasite metazoan	3 (6%)	5 (10%)	3 (6%)	7 (14%)
Epithelium, ulcer	1 (2%)			
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	4 (8%)	7 (14%)	4 (8%)	7 (14%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		2 (4%)
Basophilic focus	25 (50%)	32 (64%)	27 (54%)	34 (68%)
Clear cell focus	4 (8%)	6 (12%)	11 (22%)	20 (40%)
Degeneration, cystic			1 (2%)	
Eosinophilic focus	1 (2%)		2 (4%)	2 (4%)
Fibrosis	1 (2%)			1 (2%)
Hematopoietic cell proliferation	5 (10%)	5 (10%)	3 (6%)	7 (14%)
Hemorrhage	1 (2%)			1 (2%)
Hepatodiaphragmatic nodule	4 (8%)	6 (12%)	6 (12%)	3 (6%)
Inflammation, chronic active	25 (50%)	34 (68%)	30 (60%)	38 (76%)
Mixed cell focus	16 (32%)	17 (34%)	16 (32%)	17 (34%)
Bile duct, hyperplasia	49 (98%)	47 (94%)	47 (94%)	48 (96%)
Centrilobular, hepatocyte, degeneration	2 (4%)	1 (2%)	5 (10%)	3 (6%)
Hepatocyte, degeneration, cystic	8 (16%)	17 (34%)	15 (30%)	10 (20%)
Hepatocyte, fatty change	14 (28%)	7 (14%)	7 (14%)	2 (4%)
Hepatocyte, hyperplasia		1 (2%)	1 (2%)	
Hepatocyte, necrosis		4 (8%)	2 (4%)	
Hepatocyte, vacuolization cytoplasmic	18 (36%)	24 (48%)	16 (32%)	24 (48%)
Mesentery	(9)	(8)	(7)	(6)
Fat, fibrosis	5 (56%)	6 (75%)	4 (57%)	3 (50%)
Fat, hemorrhage		1 (13%)		
Fat, inflammation, chronic active	5 (56%)	5 (63%)	2 (29%)	2 (33%)
Fat, mineralization	2 (22%)	1 (13%)	2 (29%)	1 (17%)
Fat, necrosis	6 (67%)	6 (75%)	4 (57%)	4 (67%)
Fat, pigmentation		2 (25%)		1 (17%)

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus		1 (2%)		
Cyst	1 (2%)			
Inflammation, chronic active	1 (2%)			
Pigmentation	1 (2%)			
Acinus, atrophy	23 (46%)	21 (42%)	25 (50%)	23 (46%)
Acinus, hyperplasia		1 (2%)	3 (6%)	1 (2%)
Duct, cyst			1 (2%)	
Salivary glands	(50)	(50)	(49)	(49)
Atrophy, focal				1 (2%)
Inflammation, chronic active			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic active	4 (8%)		1 (2%)	
Epithelium, hyperplasia	2 (4%)	1 (2%)		
Epithelium, ulcer	3 (6%)		1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)			1 (2%)
Epithelium, erosion	1 (2%)	2 (4%)	2 (4%)	
Epithelium, hyperplasia				1 (2%)
Tongue		(1)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	48 (96%)	49 (98%)	49 (98%)	48 (96%)
Mineralization			1 (2%)	1 (2%)
Pigmentation		1 (2%)		
Atrium, fibrosis				2 (4%)
Atrium, thrombosis	5 (10%)	4 (8%)	3 (6%)	2 (4%)
Valve, thrombosis				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule			1 (2%)	1 (2%)
Degeneration, fatty			1 (2%)	
Hematopoietic cell proliferation	9 (18%)	6 (12%)	2 (4%)	9 (18%)
Hyperplasia	20 (40%)	7 (14%)	11 (22%)	18 (36%)
Hypertrophy	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Necrosis		1 (2%)	1 (2%)	
Vacuolization cytoplasmic	36 (72%)	25 (50%)	25 (50%)	28 (56%)
Capsule, inflammation, chronic active			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Fibrosis	2 (4%)			
Hemorrhage		1 (2%)		
Hyperplasia	19 (38%)	26 (52%)	17 (34%)	13 (26%)
Pigmentation	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)		2 (4%)	
Parathyroid gland	(49)	(48)	(48)	(48)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, angiectasis	15 (30%)	16 (32%)	19 (38%)	14 (28%)
Pars distalis, cyst	8 (16%)	3 (6%)	3 (6%)	4 (8%)
Pars distalis, cyst, multiple	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Pars distalis, hyperplasia	20 (40%)	20 (40%)	18 (36%)	23 (46%)
Pars distalis, pigmentation	14 (28%)	18 (36%)	14 (28%)	11 (22%)
Pars intermedia, angiectasis		1 (2%)		
Pars intermedia, cyst			1 (2%)	
Pars intermedia, pigmentation	3 (6%)	2 (4%)		
Thyroid gland	(50)	(49)	(48)	(48)
Pigmentation	1 (2%)			
Ultimobranchial cyst	2 (4%)		1 (2%)	1 (2%)
Bilateral, C-cell, hyperplasia		1 (2%)		
C-cell, hyperplasia	11 (22%)	16 (33%)	8 (17%)	11 (23%)
Follicle, cyst		4 (8%)	1 (2%)	3 (6%)
Follicular cell, hyperplasia	1 (2%)		1 (2%)	1 (2%)
General Body System				
Peritoneum	(1)			
Genital System				
Coagulating gland	(1)	(0)	(0)	(4)
Inflammation				1 (25%)
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	4 (8%)			2 (4%)
Preputial gland	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	43 (86%)	46 (92%)	44 (88%)	46 (92%)
Mineralization				1 (2%)
Bilateral, hyperplasia		1 (2%)		
Duct, ectasia	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Cyst, multiple			1 (2%)	
Inflammation, chronic active	22 (44%)	27 (54%)	36 (72%)	30 (60%)
Epithelium, hyperplasia	10 (20%)	13 (26%)	14 (28%)	17 (34%)
Epithelium, hypertrophy	14 (28%)	14 (28%)	21 (42%)	17 (34%)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Mineralization	32 (64%)	34 (68%)	30 (60%)	24 (48%)
Germinal epithelium, degeneration	5 (10%)	7 (14%)	8 (16%)	5 (10%)
Interstitial cell, hyperplasia	10 (20%)	11 (22%)	10 (20%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hyperplasia	19 (38%)	20 (40%)	27 (54%)	16 (32%)
Lymph node	(10)	(6)	(4)	(4)
Deep cervical, pigmentation	1 (10%)			
Pancreatic, hemorrhage		1 (17%)		

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Necrosis, lymphoid		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	5 (10%)			2 (4%)
Necrosis	1 (2%)			
Capsule, fibrosis			1 (2%)	
Lymphoid follicle, atrophy			1 (2%)	
Lymphoid follicle, hyperplasia			1 (2%)	
Thymus	(48)	(47)	(49)	(46)
Ectopic parathyroid gland			1 (2%)	1 (2%)
Thymocyte, necrosis		1 (2%)		
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Cyst				2 (4%)
Galactocele			1 (2%)	
Duct, dilatation	7 (14%)	15 (31%)	8 (16%)	4 (8%)
Skin	(50)	(49)	(50)	(50)
Cyst epithelial inclusion				1 (2%)
Inflammation, chronic active			1 (2%)	
Epidermis, hyperplasia			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		1 (2%)		
Skeletal muscle	(3)	(1)	(2)	(2)
Lymphatic, angiectasis				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	1 (2%)	2 (4%)		
Hemorrhage	4 (8%)	2 (4%)	1 (2%)	
Hydrocephalus		1 (2%)		2 (4%)
Cerebellum, necrosis		1 (2%)		
Spinal cord	(1)	(1)	(1)	(0)
Hemorrhage		1 (100%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion				1 (2%)
Fibrosis	1 (2%)			
Hemorrhage				1 (2%)
Inflammation, suppurative				1 (2%)
Inflammation, chronic active	22 (44%)	19 (38%)	24 (48%)	30 (60%)
Metaplasia, osseous	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Metaplasia, squamous		1 (2%)		
Pigmentation				1 (2%)
Alveolar epithelium, hyperplasia	9 (18%)	15 (30%)	13 (26%)	9 (18%)
Alveolar epithelium, metaplasia, squamous	1 (2%)			

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Alveolus, infiltration cellular, histiocyte	28 (56%)	30 (60%)	34 (68%)	36 (72%)
Bronchus, foreign body				1 (2%)
Bronchus, hyperplasia				1 (2%)
Perivascular, infiltration cellular, lymphoid	29 (58%)	28 (56%)	28 (56%)	32 (64%)
Nose	(50)	(49)	(48)	(49)
Foreign body	10 (20%)	14 (29%)	7 (15%)	9 (18%)
Inflammation, suppurative	3 (6%)	7 (14%)	5 (10%)	9 (18%)
Inflammation, chronic active	6 (12%)	9 (18%)	2 (4%)	5 (10%)
Thrombosis	2 (4%)	4 (8%)	6 (13%)	1 (2%)
Glands, dilatation	1 (2%)	1 (2%)		
Nasolacrimal duct, cyst			1 (2%)	
Nasolacrimal duct, inflammation, suppurative	2 (4%)	1 (2%)		
Nasolacrimal duct, inflammation, chronic	3 (6%)	4 (8%)	3 (6%)	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	6 (12%)			
Olfactory epithelium, cyst		1 (2%)		
Olfactory epithelium, degeneration	18 (36%)	22 (45%)	26 (54%)	29 (59%)
Olfactory epithelium, metaplasia, respiratory	2 (4%)	5 (10%)	3 (6%)	11 (22%)
Olfactory epithelium, metaplasia, squamous				1 (2%)
Olfactory epithelium, necrosis	1 (2%)			
Respiratory epithelium, accumulation, hyaline droplet	7 (14%)			
Respiratory epithelium, hyperplasia	28 (56%)	24 (49%)	18 (38%)	23 (47%)
Respiratory epithelium, metaplasia, squamous		2 (4%)	1 (2%)	16 (33%)
Respiratory epithelium, necrosis	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic active				1 (2%)
Special Senses System				
Ear	(0)	(0)	(0)	(2)
Eye	(50)	(50)	(50)	(50)
Lens, cataract	3 (6%)	1 (2%)		1 (2%)
Retina, degeneration	3 (6%)	1 (2%)		1 (2%)
Harderian gland	(49)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation, chronic active	5 (10%)	6 (12%)	3 (6%)	7 (14%)
Zymbal's gland				(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis				1 (2%)
Infarct		1 (2%)	1 (2%)	
Inflammation, suppurative				1 (2%)
Mineralization	19 (38%)	30 (60%)	22 (44%)	30 (60%)
Nephropathy	50 (100%)	49 (98%)	45 (90%)	47 (94%)
Thrombosis			1 (2%)	1 (2%)
Bilateral, infarct		1 (2%)		
Cortex, cyst	1 (2%)	1 (2%)		
Renal tubule, accumulation, hyaline droplet		1 (2%)	1 (2%)	1 (2%)
Renal tubule, hyperplasia			2 (4%)	
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation, chronic active				1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF 5-(HYDROXYMETHYL)-2-FURFURAL

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths				2
Moribund	14	7	13	7
Natural deaths	5	11	10	11
Survivors				
Died last week of study				1
Terminal sacrifice	31	32	27	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(49)
Intestine large, colon	(50)	(50)	(50)	(49)
Yolk sac carcinoma, metastatic, ovary		1 (2%)		
Intestine large, rectum	(50)	(49)	(50)	(50)
Adenoma	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(49)
Yolk sac carcinoma, metastatic, ovary		1 (2%)		
Mesentery	(7)	(10)	(9)	(9)
Yolk sac carcinoma, metastatic, ovary		1 (10%)		
Pancreas	(50)	(50)	(50)	(49)
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(49)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(49)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Adenoma	1 (2%)		1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma benign		1 (2%)		1 (2%)
Pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma		1 (2%)		
Carcinoma				1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	21 (42%)	22 (44%)	18 (36%)	22 (44%)
Pars distalis, adenoma, multiple	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Pars distalis, carcinoma	1 (2%)		1 (2%)	
Pars nervosa, adenoma				1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	11 (22%)	3 (6%)	6 (12%)	4 (8%)
C-cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Follicular cell, adenoma	1 (2%)		1 (2%)	
Follicular cell, carcinoma	2 (4%)	1 (2%)		
General Body System				
Peritoneum	(0)	(1)	(0)	(0)
Yolk sac carcinoma, metastatic, ovary		1 (100%)		
Tissue NOS	(0)	(0)	(0)	(1)
Schwannoma malignant				1 (100%)
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	3 (6%)	6 (12%)	4 (8%)
Ovary	(49)	(50)	(50)	(49)
Granulosa cell tumor benign		1 (2%)		1 (2%)
Left, yolk sac carcinoma		1 (2%)		
Right, yolk sac carcinoma, metastatic, ovary		1 (2%)		
Uterus	(50)	(50)	(50)	(49)
Hemangioma				1 (2%)
Polyp stromal	8 (16%)	6 (12%)	10 (20%)	5 (10%)
Sarcoma stromal		2 (4%)		
Bilateral, polyp stromal				1 (2%)
Vagina	(0)	(0)	(3)	(0)
Polyp			2 (67%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(1)	(1)	(1)	(4)
Mediastinal, carcinoma, metastatic, thyroid gland	1 (100%)			
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Spleen	(50)	(50)	(50)	(49)
Thymus	(48)	(47)	(48)	(43)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	2 (4%)		
Adenoma, multiple	1 (2%)			
Fibroadenoma	17 (34%)	16 (32%)	9 (18%)	17 (35%)
Fibroadenoma, multiple	12 (24%)	12 (24%)	14 (28%)	6 (12%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma			1 (2%)	
Squamous cell papilloma			1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)		1 (2%)
Subcutaneous tissue, fibrosarcoma		3 (6%)	2 (4%)	1 (2%)
Subcutaneous tissue, schwannoma malignant	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)			
Skeletal muscle	(1)	(0)	(0)	(0)
Osteosarcoma, metastatic, bone	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)	
Carcinoma, metastatic, pituitary gland	1 (2%)		1 (2%)	
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Fibrosarcoma, metastatic, skin		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla				1 (2%)
Schwannoma malignant, metastatic, tissue NOS				1 (2%)
Nose	(50)	(49)	(49)	(49)
Special Senses System				
Ear	(1)	(1)	(0)	(1)
Pinna, neural crest tumor		1 (100%)		1 (100%)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(49)	(50)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	9 (18%)	9 (18%)	8 (16%)	9 (18%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	46	44	42
Total primary neoplasms	99	89	87	83
Total animals with benign neoplasms	46	42	41	37
Total benign neoplasms	82	71	73	67
Total animals with malignant neoplasms	16	16	12	15
Total malignant neoplasms	17	17	14	15
Total animals with metastatic neoplasms	3	2	2	2
Total metastatic neoplasms	3	6	2	2
Total animals with malignant neoplasms of uncertain primary site			1	
Total animals with uncertain neoplasms-benign or malignant		1		1
Total uncertain neoplasms		1		1

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Clitoral Gland: Adenoma				
Overall rate[a]	3/50 (6%)	3/50 (6%)	6/50 (12%)	4/50 (8%)
Adjusted rate[b]	7.0%	6.9%	15.7%	9.3%
Terminal rate[c]	2/31 (7%)	1/32 (3%)	4/27 (15%)	3/30 (10%)
First incidence (days)	663	490	517	716
Poly-3 test[d]	P=0.358	P=0.656N	P=0.185	P=0.502
Mammary Gland: Fibroadenoma				
Overall rate	29/50 (58%)	28/50 (56%)	23/50 (46%)	23/50 (46%)
Adjusted rate	64.4%	62.8%	60.2%	52.2%
Terminal rate	20/31 (65%)	22/32 (69%)	17/27 (63%)	16/30 (53%)
First incidence (days)	503	572	627	602
Poly-3 test	P=0.111N	P=0.524N	P=0.430N	P=0.162N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	30/50 (60%)	30/50 (60%)	23/50 (46%)	23/50 (46%)
Adjusted rate	66.7%	66.8%	60.2%	52.2%
Terminal rate	21/31 (68%)	23/32 (72%)	17/27 (63%)	16/30 (53%)
First incidence (days)	503	572	627	602
Poly-3 test	P=0.058N	P=0.586	P=0.345N	P=0.112N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	24/50 (48%)	24/50 (48%)	20/50 (40%)	23/50 (46%)
Adjusted rate	52.6%	52.9%	48.9%	50.4%
Terminal rate	13/31 (42%)	16/32 (50%)	11/27 (41%)	12/30 (40%)
First incidence (days)	554	449	418	550
Poly-3 test	P=0.429N	P=0.572	P=0.448N	P=0.499N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	25/50 (50%)	24/50 (48%)	21/50 (42%)	23/50 (46%)
Adjusted rate	54.8%	52.9%	51.3%	50.4%
Terminal rate	14/31 (45%)	16/32 (50%)	12/27 (44%)	12/30 (40%)
First incidence (days)	554	449	418	550
Poly-3 test	P=0.369N	P=0.513N	P=0.457N	P=0.416N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	0.0%	6.9%	5.4%	2.3%
Terminal rate	0/31 (0%)	0/32 (0%)	1/27 (4%)	0/30 (0%)
First incidence (days)	—[e]	625	706	713
Poly-3 test	P=0.560	P=0.121	P=0.208	P=0.502
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.3%	9.2%	5.4%	4.6%
Terminal rate	1/31 (3%)	1/32 (3%)	1/27 (4%)	1/30 (3%)
First incidence (days)	728 (T)	625	706	713
Poly-3 test	P=0.569	P=0.182	P=0.452	P=0.503
Thyroid Gland (C-cell): Adenoma				
Overall rate	11/50 (22%)	3/50 (6%)	6/50 (12%)	4/50 (8%)
Adjusted rate	24.9%	7.1%	15.9%	9.3%
Terminal rate	7/31 (23%)	3/32 (9%)	4/27 (15%)	2/30 (7%)
First incidence (days)	503	728 (T)	627	713
Poly-3 test	P=0.069N	P=0.023N	P=0.236N	P=0.047N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	12/50 (24%)	4/50 (8%)	7/50 (14%)	6/50 (12%)
Adjusted rate	27.1%	9.4%	18.6%	13.9%
Terminal rate	8/31 (26%)	4/32 (13%)	5/27 (19%)	4/30 (13%)
First incidence (days)	503	728 (T)	627	713
Poly-3 test	P=0.149N	P=0.030N	P=0.258N	P=0.101N
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	6/50 (12%)	10/50 (20%)	6/50 (12%)
Adjusted rate	18.2%	14.1%	24.7%	13.8%
Terminal rate	5/31 (16%)	6/32 (19%)	5/27 (19%)	5/30 (17%)
First incidence (days)	455	728 (T)	390	602
Poly-3 test	P=0.423N	P=0.414N	P=0.322	P=0.394N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	8/50 (16%)	8/50 (16%)	10/50 (20%)	6/50 (12%)
Adjusted rate	18.2%	18.4%	24.7%	13.8%
Terminal rate	5/31 (16%)	6/32 (19%)	5/27 (19%)	5/30 (17%)
First incidence (days)	455	554	390	602
Poly-3 test	P=0.359N	P=0.599	P=0.322	P=0.394N
All Organs: Mononuclear Cell Leukemia				
Overall rate	9/50 (18%)	9/50 (18%)	8/50 (16%)	9/50 (18%)
Adjusted rate	20.2%	20.7%	20.8%	20.4%
Terminal rate	4/31 (13%)	7/32 (22%)	4/27 (15%)	6/30 (20%)
First incidence (days)	474	489	531	550
Poly-3 test	P=0.546	P=0.579	P=0.578	P=0.593
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	42/50 (84%)	41/50 (82%)	37/50 (74%)
Adjusted rate	96.1%	87.9%	92.5%	81.0%
Terminal rate	30/31 (97%)	28/32 (88%)	26/27 (96%)	25/30 (83%)
First incidence (days)	455	449	390	550
Poly-3 test	P=0.014N	P=0.114N	P=0.361N	P=0.016N
All Organs: Malignant Neoplasms				
Overall rate	16/50 (32%)	16/50 (32%)	13/50 (26%)	15/50 (30%)
Adjusted rate	34.5%	34.9%	33.1%	33.9%
Terminal rate	8/31 (26%)	8/32 (25%)	7/27 (26%)	10/30 (33%)
First incidence (days)	198	489	422	550
Poly-3 test	P=0.508N	P=0.569	P=0.538N	P=0.567N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	46/50 (92%)	44/50 (88%)	42/50 (84%)
Adjusted rate	96.8%	92.0%	95.3%	90.9%
Terminal rate	30/31 (97%)	28/32 (88%)	26/27 (96%)	27/30 (90%)
First incidence (days)	198	449	390	550
Poly-3 test	P=0.207N	P=0.270N	P=0.564N	P=0.200N

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths				2
Moribund	14	7	13	7
Natural deaths	5	11	10	11
Survivors				
Died last week of study				1
Terminal sacrifice	31	32	27	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(49)
Intestine large, colon	(50)	(50)	(50)	(49)
Parasite metazoan	1 (2%)		1 (2%)	4 (8%)
Intestine large, rectum	(50)	(49)	(50)	(50)
Diverticulum		1 (2%)		
Parasite metazoan	3 (6%)	6 (12%)	3 (6%)	3 (6%)
Intestine small, ileum	(50)	(50)	(50)	(49)
Parasite metazoan			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(49)
Peyer's patch, hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(49)
Angiectasis		1 (2%)	1 (2%)	2 (4%)
Basophilic focus	44 (88%)	47 (94%)	45 (90%)	42 (86%)
Clear cell focus		2 (4%)	2 (4%)	1 (2%)
Eosinophilic focus			1 (2%)	1 (2%)
Hematopoietic cell proliferation	7 (14%)	10 (20%)	8 (16%)	4 (8%)
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	6 (12%)	8 (16%)	8 (16%)	7 (14%)
Inflammation, chronic active	43 (86%)	40 (80%)	41 (82%)	39 (80%)
Mineralization			1 (2%)	
Mixed cell focus	9 (18%)	13 (26%)	8 (16%)	8 (16%)
Bile duct, hyperplasia	23 (46%)	22 (44%)	29 (58%)	24 (49%)
Centrilobular, hepatocyte, degeneration	2 (4%)			2 (4%)
Hepatocyte, degeneration, cystic			1 (2%)	1 (2%)
Hepatocyte, fatty change	6 (12%)	5 (10%)		2 (4%)
Hepatocyte, hyperplasia		1 (2%)		
Hepatocyte, necrosis				2 (4%)
Hepatocyte, vacuolization cytoplasmic	9 (18%)	5 (10%)	2 (4%)	2 (4%)
Mesentery	(7)	(10)	(9)	(9)
Fat, fibrosis	5 (71%)	8 (80%)	7 (78%)	8 (89%)
Fat, inflammation, chronic active	5 (71%)	7 (70%)	7 (78%)	5 (56%)
Fat, mineralization	4 (57%)	6 (60%)	3 (33%)	5 (56%)
Fat, necrosis	7 (100%)	9 (90%)	7 (78%)	9 (100%)
Fat, pigmentation				3 (33%)
Lymphatic, angiectasis			1 (11%)	

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(49)
Infiltration cellular, lymphoid		1 (2%)		
Inflammation, chronic active		2 (4%)	1 (2%)	
Acinus, atrophy	10 (20%)	11 (22%)	5 (10%)	10 (20%)
Acinus, hyperplasia			1 (2%)	
Duct, cyst		1 (2%)		3 (6%)
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(49)
Inflammation, chronic active	1 (2%)	2 (4%)		3 (6%)
Epithelium, hyperplasia	2 (4%)			3 (6%)
Epithelium, ulcer	1 (2%)	2 (4%)		2 (4%)
Stomach, glandular	(50)	(50)	(50)	(49)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	47 (94%)	49 (98%)	46 (92%)	47 (94%)
Mineralization	1 (2%)			
Atrium, thrombosis	2 (4%)			1 (2%)
Valve, inflammation, suppurative			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Accessory adrenal cortical nodule			1 (2%)	2 (4%)
Hematopoietic cell proliferation	7 (14%)	14 (28%)	9 (18%)	7 (14%)
Hyperplasia	12 (24%)	14 (28%)	13 (26%)	4 (8%)
Hypertrophy	5 (10%)	5 (10%)	1 (2%)	3 (6%)
Karyomegaly				1 (2%)
Necrosis	1 (2%)	1 (2%)		
Pigmentation			1 (2%)	
Vacuolization cytoplasmic	22 (44%)	25 (50%)	16 (32%)	20 (41%)
Bilateral, hemorrhage		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia	4 (8%)	3 (6%)	2 (4%)	4 (8%)
Infiltration cellular, lymphoid				2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Pars distalis, pars intermedia, pigmentation			1 (2%)	1 (2%)
Pars distalis, angiectasis	34 (68%)	34 (68%)	29 (58%)	34 (68%)
Pars distalis, cyst	7 (14%)	9 (18%)	11 (22%)	7 (14%)
Pars distalis, cyst, multiple	10 (20%)	12 (24%)	14 (28%)	9 (18%)
Pars distalis, hyperplasia	20 (40%)	13 (26%)	20 (40%)	20 (40%)
Pars distalis, pigmentation	27 (54%)	27 (54%)	27 (54%)	30 (60%)
Pars distalis, vacuolization cytoplasmic	1 (2%)			
Pars intermedia, angiectasis				1 (2%)
Pars intermedia, cyst	1 (2%)			4 (8%)
Pars intermedia, cyst, multiple	1 (2%)			1 (2%)
Pars intermedia, pigmentation	2 (4%)	1 (2%)	2 (4%)	
Rathke's cleft, cyst			1 (2%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst		1 (2%)		1 (2%)
C-cell, hyperplasia	14 (28%)	13 (26%)	13 (26%)	13 (26%)
Follicle, cyst	1 (2%)			1 (2%)
Follicular cell, hyperplasia				1 (2%)
General Body System				
Peritoneum	(0)	(1)	(0)	(0)
Tissue NOS	(0)	(0)	(0)	(1)
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Hyperplasia	10 (20%)	13 (26%)	7 (14%)	8 (16%)
Inflammation, chronic active	12 (24%)	26 (52%)	18 (36%)	10 (20%)
Bilateral, hyperplasia		1 (2%)	3 (6%)	1 (2%)
Duct, cyst	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Ovary	(49)	(50)	(50)	(49)
Atrophy		1 (2%)		
Cyst	11 (22%)	9 (18%)	6 (12%)	5 (10%)
Bilateral, cyst		1 (2%)		
Uterus	(50)	(50)	(50)	(49)
Hemorrhage	1 (2%)		1 (2%)	
Endometrium, cyst		2 (4%)	2 (4%)	1 (2%)
Vagina	(0)	(0)	(3)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	9 (18%)	9 (18%)	9 (18%)	8 (16%)
Hyperplasia, histiocytic			1 (2%)	
Lymph node	(1)	(1)	(1)	(4)
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Hyperplasia, lymphoid	1 (2%)			
Spleen	(50)	(50)	(50)	(49)
Accessory spleen				1 (2%)
Hematopoietic cell proliferation	2 (4%)	3 (6%)	6 (12%)	2 (4%)
Lymphoid follicle, hyperplasia		1 (2%)		
Thymus	(48)	(47)	(48)	(43)
Ectopic parathyroid gland	2 (4%)	6 (13%)	2 (4%)	1 (2%)
Ectopic thyroid			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Cyst				1 (2%)
Galactocele	17 (34%)	17 (34%)	21 (42%)	17 (35%)
Hyperplasia, cystic		1 (2%)	1 (2%)	
Duct, dilatation	37 (74%)	40 (80%)	35 (70%)	38 (78%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)			1 (2%)
Subcutaneous tissue, inflammation, chronic active				1 (2%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis	1 (2%)			
Skeletal muscle	(1)	(0)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	2 (4%)	1 (2%)	2 (4%)	4 (8%)
Hemorrhage	1 (2%)			
Hydrocephalus	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Inflammation, chronic active	1 (2%)			
Necrosis	1 (2%)			
Spinal cord	(0)	(0)	(1)	(0)
Hemorrhage			1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Fibrosis				3 (6%)
Hemorrhage				1 (2%)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic active	31 (62%)	30 (60%)	28 (56%)	37 (74%)
Metaplasia, osseous	2 (4%)	1 (2%)	1 (2%)	
Pigmentation	3 (6%)	3 (6%)	5 (10%)	2 (4%)
Alveolar epithelium, hyperplasia	11 (22%)	10 (20%)	10 (20%)	8 (16%)
Alveolus, infiltration cellular, histiocyte	45 (90%)	46 (92%)	46 (92%)	37 (74%)
Bronchus, hyperplasia				3 (6%)
Bronchus, metaplasia, squamous				3 (6%)
Perivascular, infiltration cellular, lymphoid	40 (80%)	45 (90%)	43 (86%)	42 (84%)
Nose	(50)	(49)	(49)	(49)
Foreign body	3 (6%)	2 (4%)	1 (2%)	8 (16%)
Inflammation, suppurative				8 (16%)
Inflammation, chronic active	4 (8%)	3 (6%)	2 (4%)	7 (14%)
Thrombosis				1 (2%)
Glands, dilatation		1 (2%)	1 (2%)	
Nasolacrimal duct, inflammation, suppurative	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Nasolacrimal duct, inflammation, chronic	2 (4%)	2 (4%)	3 (6%)	12 (24%)
Olfactory epithelium, accumulation, hyaline droplet	34 (68%)	15 (31%)	22 (45%)	
Olfactory epithelium, degeneration	21 (42%)	35 (71%)	36 (73%)	28 (57%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	1 (2%)		11 (22%)
Olfactory epithelium, metaplasia, squamous				2 (4%)
Olfactory epithelium, necrosis			1 (2%)	
Respiratory epithelium, accumulation, hyaline droplet	9 (18%)	3 (6%)	4 (8%)	
Respiratory epithelium, hyperplasia	18 (36%)	13 (27%)	21 (43%)	20 (41%)
Respiratory epithelium, metaplasia, squamous	1 (2%)	1 (2%)		24 (49%)
Respiratory epithelium, necrosis		1 (2%)		2 (4%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Special Senses System				
Ear	(1)	(1)	(0)	(1)
Eye	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Lens, cataract	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Retina, degeneration	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Hyperplasia		1 (2%)		
Inflammation, chronic active	12 (24%)	12 (24%)	18 (37%)	15 (30%)
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Hydronephrosis	1 (2%)	1 (2%)		
Infarct	1 (2%)		1 (2%)	
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active		1 (2%)		1 (2%)
Mineralization	28 (56%)	17 (34%)	19 (38%)	25 (51%)
Nephropathy	43 (86%)	42 (84%)	39 (78%)	35 (71%)
Cortex, pelvis, cyst, multiple	1 (2%)			
Cortex, cyst	2 (4%)	1 (2%)		1 (2%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)			
Pelvis, inflammation, chronic active	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF 5-(HYDROXYMETHYL)-2-FURFURAL

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths				2
Moribund	6	9	4	1
Natural deaths	4	6	3	31
Survivors				
Died last week of study				1
Terminal sacrifice	40	35	43	14
Missing				1
Animals examined microscopically	50	50	50	49
Alimentary System				
Gallbladder	(49)	(50)	(49)	(49)
Intestine large, cecum	(50)	(50)	(50)	(49)
Carcinoma	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(49)
Leiomyosarcoma		1 (2%)		
Intestine small, duodenum	(50)	(50)	(50)	(49)
Carcinoma	2 (4%)			
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Intestine small, ileum	(49)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(49)
Hemangioma			1 (2%)	
Hemangiosarcoma	1 (2%)		1 (2%)	
Hemangiosarcoma, multiple			1 (2%)	
Hepatoblastoma	1 (2%)	4 (8%)	7 (14%)	
Hepatocellular adenoma	14 (28%)	10 (20%)	5 (10%)	6 (12%)
Hepatocellular adenoma, multiple	19 (38%)	20 (40%)	36 (72%)	4 (8%)
Hepatocellular carcinoma	5 (10%)	11 (22%)	11 (22%)	5 (10%)
Hepatocellular carcinoma, multiple	6 (12%)	3 (6%)	3 (6%)	1 (2%)
Hepatocholangiocarcinoma		1 (2%)	1 (2%)	1 (2%)
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Mesentery	(4)	(4)	(4)	(2)
Leiomyosarcoma, metastatic, intestine large, colon		1 (25%)		
Pancreas	(50)	(50)	(50)	(49)
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(49)
Squamous cell papilloma	2 (4%)		1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(49)
Tooth	(12)	(7)	(4)	(1)
Odontoma		3 (43%)	2 (50%)	
Cardiovascular System				
Blood vessel	(3)	(1)	(0)	(1)
Heart	(50)	(49)	(50)	(49)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Liposarcoma, metastatic, uncertain primary site		1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(49)
Adenoma			1 (2%)	
Subcapsular, adenoma	5 (10%)	3 (6%)	6 (12%)	
Subcapsular, adenoma, multiple			2 (4%)	
Adrenal medulla	(49)	(50)	(50)	(49)
Islets, pancreatic	(50)	(50)	(50)	(49)
Parathyroid gland	(41)	(48)	(48)	(37)
Pituitary gland	(48)	(50)	(50)	(49)
Thyroid gland	(50)	(50)	(50)	(49)
Follicular cell, adenoma	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Follicular cell, adenoma, multiple	1 (2%)			
Follicular cell, carcinoma			1 (2%)	
General Body System				
None				
Genital System				
Coagulating gland	(0)	(2)	(2)	(0)
Leiomyosarcoma, metastatic, intestine large, colon		1 (50%)		
Epididymis	(50)	(50)	(50)	(49)
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Preputial gland	(50)	(50)	(50)	(49)
Prostate	(50)	(50)	(50)	(49)
Seminal vesicle	(50)	(50)	(50)	(49)
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Testes	(50)	(50)	(50)	(49)
Interstitial cell, adenoma		1 (2%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Lymph node	(4)	(3)	(4)	
Hepatocholangiocarcinoma, metastatic, liver			1 (25%)	
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (25%)			
Mediastinal, hepatocholangiocarcinoma, metastatic, liver		1 (33%)	1 (25%)	
Mediastinal, liposarcoma, metastatic, uncertain primary site		1 (33%)		
Lymph node, mandibular	(49)	(50)	(49)	(48)
Lymph node, mesenteric	(50)	(49)	(49)	(49)
Carcinoma, metastatic, intestine large, cecum	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Spleen	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)			
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Thymus	(43)	(47)	(47)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Liposarcoma, metastatic, uncertain primary site		1 (2%)		

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Subcutaneous tissue, schwannoma malignant		1 (2%)		
Musculoskeletal System				
Skeletal muscle	(1)	(4)	(1)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (100%)			
Hemangiosarcoma				1 (100%)
Hepatocholangiocarcinoma, metastatic, liver		1 (25%)		
Leiomyosarcoma, metastatic, intestine large, colon		1 (25%)		
Liposarcoma, metastatic, uncertain primary site		1 (25%)		
Schwannoma malignant, metastatic, skin		1 (25%)		
Nervous System				
Brain	(50)	(50)	(50)	(49)
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Alveolar/bronchiolar adenoma	10 (20%)	3 (6%)	10 (20%)	2 (4%)
Alveolar/bronchiolar adenoma, multiple		2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma	7 (14%)	1 (2%)	1 (2%)	
Carcinoma, metastatic, Harderian gland	1 (2%)	1 (2%)		
Hepatoblastoma, metastatic, liver		1 (2%)		
Hepatocellular carcinoma, metastatic, liver	6 (12%)	4 (8%)	4 (8%)	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Liposarcoma, metastatic, uncertain primary site		1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Mediastinum, hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Mediastinum, leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Mediastinum, liposarcoma, metastatic, uncertain primary site		1 (2%)		
Serosa, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Nose	(50)	(50)	(50)	(47)
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Carcinoma, metastatic, Harderian gland		1 (2%)		
Harderian gland	(50)	(50)	(50)	(48)
Adenoma	11 (22%)	5 (10%)	7 (14%)	4 (8%)
Carcinoma	3 (6%)	2 (4%)	1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Leiomyosarcoma, metastatic, intestine large, colon		1 (2%)		
Renal tubule, adenoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(49)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	2 (4%)	1 (2%)	3 (6%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	43	47	20
Total primary neoplasms	93	74	106	29
Total animals with benign neoplasms	42	32	44	16
Total benign neoplasms	64	49	75	21
Total animals with malignant neoplasms	26	24	23	8
Total malignant neoplasms	29	25	31	8
Total animals with metastatic neoplasms	8	10	5	1
Total metastatic neoplasms	15	31	10	1
Total animals with malignant neoplasms of uncertain primary site		1		

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	5/49 (10%)	3/50 (6%)	9/50 (18%)	0/49 (0%)
Adjusted rate[b]	11.1%	6.9%	19.3%	0.0%
Terminal rate[c]	5/39 (13%)	3/35 (9%)	9/43 (21%)	0/14 (0%)
First incidence (days)	728 (T)	728 (T)	728 (T)	—[e]
Poly-3 test[d]	P=0.395N	P=0.374N	P=0.211	P=0.106N
Harderian Gland: Adenoma				
Overall rate	11/50 (22%)	5/50 (10%)	7/50 (14%)	4/49 (8%)
Adjusted rate	23.8%	11.4%	14.8%	14.8%
Terminal rate	10/40 (25%)	5/35 (14%)	5/43 (12%)	4/14 (29%)
First incidence (days)	699	728 (T)	399	728 (T)
Poly-3 test	P=0.188N	P=0.103N	P=0.198N	P=0.278N
Harderian Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	0/49 (0%)
Adjusted rate	6.5%	4.5%	2.2%	0.0%
Terminal rate	2/40 (5%)	1/35 (3%)	1/43 (2%)	0/14 (0%)
First incidence (days)	602	551	728 (T)	—
Poly-3 test	P=0.101N	P=0.523N	P=0.305N	P=0.245N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	13/50 (26%)	7/50 (14%)	8/50 (16%)	4/49 (8%)
Adjusted rate	27.9%	15.8%	16.9%	14.8%
Terminal rate	11/40 (28%)	6/35 (17%)	6/43 (14%)	4/14 (29%)
First incidence (days)	602	551	399	728 (T)
Poly-3 test	P=0.101N	P=0.127N	P=0.150N	P=0.172N
Liver: Hepatocellular Adenoma				
Overall rate	33/50 (66%)	30/50 (60%)	41/50 (82%)	10/49 (20%)
Adjusted rate	68.6%	65.3%	84.4%	34.5%
Terminal rate	28/40 (70%)	23/35 (66%)	37/43 (86%)	6/14 (43%)
First incidence (days)	327	446	394	449
Poly-3 test	P=0.055N	P=0.451N	P=0.049	P=0.003N
Liver: Hepatocellular Carcinoma				
Overall rate	11/50 (22%)	14/50 (28%)	14/50 (28%)	6/49 (12%)
Adjusted rate	22.6%	29.9%	29.8%	20.9%
Terminal rate	4/40 (10%)	6/35 (17%)	12/43 (28%)	3/14 (21%)
First incidence (days)	524	380	652	476
Poly-3 test	P=0.506	P=0.284	P=0.288	P=0.540N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	39/50 (78%)	36/50 (72%)	43/50 (86%)	14/49 (29%)
Adjusted rate	78.0%	74.9%	88.3%	45.7%
Terminal rate	29/40 (73%)	25/35 (71%)	38/43 (88%)	7/14 (50%)
First incidence (days)	327	380	394	449
Poly-3 test	P=0.021N	P=0.449N	P=0.133	P=0.002N
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	4/50 (8%)	7/50 (14%)	0/49 (0%)
Adjusted rate	2.2%	9.0%	15.0%	0.0%
Terminal rate	1/40 (3%)	3/35 (9%)	6/43 (14%)	0/14 (0%)
First incidence (days)	728 (T)	555	725	—
Poly-3 test	P=0.323	P=0.166	P=0.031	P=0.600N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	12/50 (24%)	18/50 (36%)	18/50 (36%)	6/49 (12%)
Adjusted rate	24.7%	38.0%	38.3%	20.9%
Terminal rate	5/40 (13%)	9/35 (26%)	15/43 (35%)	3/14 (21%)
First incidence (days)	524	380	652	476
Poly-3 test	P=0.494	P=0.117	P=0.112	P=0.461N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	39/50 (78%)	37/50 (74%)	43/50 (86%)	14/49 (29%)
Adjusted rate	78.0%	76.1%	88.3%	45.7%
Terminal rate	29/40 (73%)	25/35 (71%)	38/43 (88%)	7/14 (50%)
First incidence (days)	327	380	394	449
Poly-3 test	P=0.019N	P=0.505N	P=0.133	P=0.002N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	10/50 (20%)	5/50 (10%)	10/50 (20%)	3/49 (6%)
Adjusted rate	21.6%	11.4%	21.5%	10.8%
Terminal rate	9/40 (23%)	5/35 (14%)	9/43 (21%)	1/14 (7%)
First incidence (days)	699	728 (T)	725	589
Poly-3 test	P=0.286N	P=0.155N	P=0.590N	P=0.204N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/50 (14%)	1/50 (2%)	1/50 (2%)	0/49 (0%)
Adjusted rate	15.1%	2.3%	2.1%	0.0%
Terminal rate	6/40 (15%)	0/35 (0%)	0/43 (0%)	0/14 (0%)
First incidence (days)	651	629	394	—
Poly-3 test	P=0.004N	P=0.036N	P=0.028N	P=0.052N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	16/50 (32%)	6/50 (12%)	11/50 (22%)	3/49 (6%)
Adjusted rate	34.4%	13.6%	23.2%	10.8%
Terminal rate	14/40 (35%)	5/35 (14%)	9/43 (21%)	1/14 (7%)
First incidence (days)	651	629	394	589
Poly-3 test	P=0.032N	P=0.017N	P=0.164N	P=0.027N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	2/49 (4%)
Adjusted rate	4.3%	4.6%	6.4%	7.4%
Terminal rate	2/40 (5%)	2/35 (6%)	3/43 (7%)	2/14 (14%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.328	P=0.675	P=0.505	P=0.492
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	4/50 (8%)	2/49 (4%)
Adjusted rate	4.3%	4.6%	8.6%	7.4%
Terminal rate	2/40 (5%)	2/35 (6%)	4/43 (9%)	2/14 (14%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.266	P=0.675	P=0.342	P=0.492
Tooth: Odontoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/49 (0%)
Adjusted rate	0.0%	6.9%	4.3%	0.0%
Terminal rate	0/40 (0%)	3/35 (9%)	2/43 (5%)	0/14 (0%)
First incidence (days)	—	728 (T)	728 (T)	—
Poly-3 test	P=0.515	P=0.109	P=0.240	—[f]

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	1/49 (2%)
Adjusted rate	4.3%	0.0%	6.4%	3.7%
Terminal rate	2/40 (5%)	0/35 (0%)	3/43 (7%)	0/14 (0%)
First incidence (days)	728 (T)	—	728 (T)	726
Poly-3 test	P=0.431	P=0.249N	P=0.505	P=0.671N
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	0/49 (0%)
Adjusted rate	4.3%	2.3%	6.3%	0.0%
Terminal rate	1/40 (3%)	1/35 (3%)	1/43 (2%)	0/14 (0%)
First incidence (days)	713	728 (T)	485	—
Poly-3 test	P=0.435N	P=0.519N	P=0.512	P=0.370N
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	32/50 (64%)	44/50 (88%)	16/49 (33%)
Adjusted rate	87.3%	69.7%	90.6%	53.8%
Terminal rate	37/40 (93%)	25/35 (71%)	40/43 (93%)	10/14 (71%)
First incidence (days)	327	446	394	449
Poly-3 test	P=0.012N	P=0.024N	P=0.420	P<0.001N
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	24/50 (48%)	23/50 (46%)	8/49 (16%)
Adjusted rate	53.0%	49.2%	46.8%	27.9%
Terminal rate	17/40 (43%)	12/35 (34%)	17/43 (40%)	4/14 (29%)
First incidence (days)	524	380	394	476
Poly-3 test	P=0.040N	P=0.434N	P=0.342N	P=0.031N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	43/50 (86%)	47/50 (94%)	20/49 (41%)
Adjusted rate	98.0%	86.6%	94.0%	64.2%
Terminal rate	39/40 (98%)	29/35 (83%)	40/43 (93%)	11/14 (79%)
First incidence (days)	327	380	394	449
Poly-3 test	P<0.001N	P=0.035N	P=0.306N	P<0.001N

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

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths				2
Moribund	6	9	4	1
Natural deaths	4	6	3	31
Survivors				
Died last week of study				1
Terminal sacrifice	40	35	43	14
Missing				1
Animals examined microscopically	50	50	50	49
Alimentary System				
Gallbladder	(49)	(50)	(49)	(49)
Infiltration cellular, mononuclear cell	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Necrosis		1 (2%)		
Intestine large, cecum	(50)	(50)	(50)	(49)
Intestine large, colon	(50)	(50)	(50)	(49)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Intestine small, ileum	(49)	(50)	(50)	(49)
Inflammation, chronic active		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(49)
Inflammation, chronic active		2 (4%)	1 (2%)	
Peyer's patch, hyperplasia, lymphoid		2 (4%)		
Liver	(50)	(50)	(50)	(49)
Basophilic focus		4 (8%)	5 (10%)	1 (2%)
Clear cell focus	26 (52%)	21 (42%)	26 (52%)	1 (2%)
Cyst		1 (2%)		
Eosinophilic focus	7 (14%)	9 (18%)	16 (32%)	8 (16%)
Hematopoietic cell proliferation		2 (4%)		
Infarct	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, mononuclear cell	6 (12%)	9 (18%)	6 (12%)	1 (2%)
Inflammation, granulomatous		1 (2%)		
Inflammation, chronic active	37 (74%)	30 (60%)	38 (76%)	19 (39%)
Mineralization	1 (2%)		1 (2%)	2 (4%)
Mixed cell focus	5 (10%)	11 (22%)	15 (30%)	3 (6%)
Pigmentation		1 (2%)	1 (2%)	1 (2%)
Thrombosis			1 (2%)	
Bile duct, hyperplasia			1 (2%)	
Hepatocyte, necrosis	2 (4%)	4 (8%)	5 (10%)	1 (2%)
Hepatocyte, tension lipidosis	1 (2%)		2 (4%)	1 (2%)
Hepatocyte, vacuolization cytoplasmic	31 (62%)	28 (56%)	30 (60%)	10 (20%)
Mesentery	(4)	(4)	(4)	(2)
Pigmentation			1 (25%)	
Artery, inflammation, chronic active			1 (25%)	
Fat, fibrosis	2 (50%)	2 (50%)	2 (50%)	2 (100%)
Fat, inflammation, chronic active	2 (50%)	3 (75%)	3 (75%)	2 (100%)
Fat, mineralization	2 (50%)	2 (50%)	3 (75%)	2 (100%)
Fat, necrosis	2 (50%)	2 (50%)	2 (50%)	2 (100%)

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

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(49)
Acinus, atrophy	1 (2%)	2 (4%)		
Artery, inflammation, chronic active	2 (4%)	1 (2%)		
Salivary glands	(50)	(50)	(50)	(49)
Atrophy			1 (2%)	
Mineralization			1 (2%)	
Artery, mineralization	2 (4%)			
Stomach, forestomach	(50)	(50)	(50)	(49)
Inflammation, chronic active	1 (2%)	5 (10%)	3 (6%)	2 (4%)
Ulcer		1 (2%)	2 (4%)	
Epithelium, hyperkeratosis		2 (4%)	2 (4%)	4 (8%)
Epithelium, hyperplasia	1 (2%)	4 (8%)	4 (8%)	4 (8%)
Stomach, glandular	(50)	(50)	(50)	(49)
Dysplasia		1 (2%)		
Infiltration cellular, mast cell			1 (2%)	
Inflammation, chronic active		1 (2%)		
Mineralization		1 (2%)	2 (4%)	
Tooth	(12)	(7)	(4)	(1)
Inflammation, chronic active		1 (14%)		
Malformation	10 (83%)	3 (43%)	2 (50%)	1 (100%)
Gingiva, inflammation, chronic active	2 (17%)			
Cardiovascular System				
Blood vessel	(3)	(1)	(0)	(1)
Inflammation, chronic active	1 (33%)			
Heart	(50)	(49)	(50)	(49)
Cardiomyopathy			2 (4%)	2 (4%)
Inflammation, chronic active	1 (2%)			
Mineralization	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Thrombosis			1 (2%)	
Artery, inflammation, chronic active	1 (2%)	1 (2%)	3 (6%)	
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(49)
Accessory adrenal cortical nodule		2 (4%)	1 (2%)	
Degeneration, fatty	1 (2%)			
Hyperplasia				1 (2%)
Hypertrophy	13 (27%)	20 (40%)	13 (26%)	6 (12%)
Subcapsular, hyperplasia	45 (92%)	41 (82%)	46 (92%)	41 (84%)
Zona fasciculata, hyperplasia		4 (8%)	1 (2%)	
Zona glomerulosa, hyperplasia		1 (2%)		
Adrenal medulla	(49)	(50)	(50)	(49)
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia	1 (2%)			
Parathyroid gland	(41)	(48)	(48)	(37)
Cyst		2 (4%)		1 (3%)
Pituitary gland	(48)	(50)	(50)	(49)
Pars distalis, cyst	6 (13%)	2 (4%)	3 (6%)	2 (4%)
Pars distalis, hyperplasia			1 (2%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(49)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Follicle, cyst	5 (10%)	2 (4%)	1 (2%)	1 (2%)
Follicle, degeneration	9 (18%)	13 (26%)	9 (18%)	5 (10%)
Follicular cell, hyperplasia	17 (34%)	8 (16%)	13 (26%)	6 (12%)
General Body System				
None				
Genital System				
Coagulating gland	(0)	(2)	(2)	(0)
Atrophy			1 (50%)	
Inflammation, chronic active		1 (50%)		
Epididymis	(50)	(50)	(50)	(49)
Atrophy			1 (2%)	
Granuloma sperm	1 (2%)	1 (2%)		
Mineralization	2 (4%)			
Preputial gland	(50)	(50)	(50)	(49)
Infiltration cellular, mononuclear cell			1 (2%)	
Inflammation, chronic active	7 (14%)	3 (6%)	2 (4%)	
Bilateral, duct, ectasia			1 (2%)	
Duct, ectasia	10 (20%)	7 (14%)	7 (14%)	7 (14%)
Prostate	(50)	(50)	(50)	(49)
Inflammation, chronic active		1 (2%)		
Artery, inflammation, chronic active	1 (2%)	1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(49)
Atrophy			1 (2%)	
Inflammation, chronic active	1 (2%)			
Testes	(50)	(50)	(50)	(49)
Atrophy			1 (2%)	
Mineralization	2 (4%)	2 (4%)	4 (8%)	1 (2%)
Bilateral, germinal epithelium, degeneration			1 (2%)	
Germinal epithelium, degeneration			1 (2%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Myeloid cell, hyperplasia		1 (2%)		
Lymph node	(4)	(3)	(4)	(0)
Inguinal, hyperplasia, lymphoid	1 (25%)			
Mediastinal, hyperplasia, lymphoid	1 (25%)	1 (33%)		
Pancreatic, hematopoietic cell proliferation		1 (33%)		
Lymph node, mandibular	(49)	(50)	(49)	(48)
Hyperplasia, lymphoid	6 (12%)	4 (8%)	7 (14%)	
Lymph node, mesenteric	(50)	(49)	(49)	(49)
Hyperplasia, lymphoid	2 (4%)	3 (6%)	5 (10%)	
Infiltration cellular, plasma cell			1 (2%)	
Spleen	(50)	(50)	(50)	(49)
Hematopoietic cell proliferation	11 (22%)	16 (32%)	11 (22%)	2 (4%)
Lymphoid follicle, hyperplasia		3 (6%)	4 (8%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Hematopoietic System (continued)				
Thymus	(43)	(47)	(47)	(49)
Atrophy	18 (42%)	23 (49%)	16 (34%)	9 (18%)
Cyst	25 (58%)	19 (40%)	26 (55%)	23 (47%)
Ectopic parathyroid gland	2 (5%)	1 (2%)	1 (2%)	
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Inflammation, chronic active	2 (4%)	3 (6%)	2 (4%)	
Epidermis, hyperkeratosis		1 (2%)		
Epidermis, hyperplasia	2 (4%)	2 (4%)	1 (2%)	
Epidermis, ulcer	2 (4%)	3 (6%)	1 (2%)	
Subcutaneous tissue, inflammation, chronic active		1 (2%)		1 (2%)
Subcutaneous tissue, mineralization				1 (2%)
Subcutaneous tissue, necrosis				1 (2%)
Musculoskeletal System				
Skeletal muscle	(1)	(4)	(1)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(49)
Degeneration				3 (6%)
Artery, inflammation				1 (2%)
Cerebrum, hippocampus neuron, necrosis, focal			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation, chronic active	4 (8%)	2 (4%)	2 (4%)	
Mineralization		1 (2%)	1 (2%)	
Pigmentation			1 (2%)	
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)	2 (4%)	7 (14%)
Alveolus, infiltration cellular, histiocyte	3 (6%)	1 (2%)	2 (4%)	
Artery, mediastinum, inflammation, chronic active		1 (2%)		
Glands, inflammation, chronic active	1 (2%)	1 (2%)		
Nose	(50)	(50)	(50)	(47)
Edema	1 (2%)	2 (4%)		
Inflammation, chronic active		6 (12%)	18 (36%)	45 (96%)
Polyp, inflammatory		1 (2%)		
Glands, dilatation	16 (32%)	22 (44%)	47 (94%)	45 (96%)
Glands, hyperplasia	3 (6%)	7 (14%)	45 (90%)	45 (96%)
Glands, inflammation, chronic active	4 (8%)	12 (24%)	34 (68%)	43 (91%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	1 (2%)		
Olfactory epithelium, accumulation, hyaline droplet	13 (26%)	17 (34%)	29 (58%)	27 (57%)
Olfactory epithelium, degeneration	4 (8%)	2 (4%)	17 (34%)	39 (83%)
Olfactory epithelium, hyperplasia			2 (4%)	3 (6%)
Olfactory epithelium, metaplasia	1 (2%)	7 (14%)	38 (76%)	43 (91%)
Respiratory epithelium, accumulation, hyaline droplet	14 (28%)	17 (34%)	23 (46%)	31 (66%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Cornea, inflammation, chronic active	2 (4%)	1 (2%)		
Harderian gland	(50)	(50)	(50)	(48)
Hyperplasia	4 (8%)	5 (10%)	1 (2%)	1 (2%)
Inflammation, chronic active			1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Hydronephrosis				1 (2%)
Infarct	1 (2%)	1 (2%)	3 (6%)	
Metaplasia, osseous		1 (2%)	3 (6%)	
Mineralization	43 (86%)	43 (86%)	44 (88%)	29 (59%)
Nephropathy	49 (98%)	48 (96%)	46 (92%)	30 (61%)
Artery, inflammation, chronic active	3 (6%)	1 (2%)	3 (6%)	
Renal tubule, cyst	20 (40%)	12 (24%)	15 (30%)	
Renal tubule, dilatation	1 (2%)			
Renal tubule, hyperplasia			1 (2%)	
Renal tubule, pigmentation			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(49)
Mineralization			1 (2%)	1 (2%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF 5-(HYDROXYMETHYL)-2-FURFURAL

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	5	8	6
Natural deaths	7	2	10	22
Survivors				
Terminal sacrifice	39	42	32	22
Missing		1		
Animals examined microscopically	50	49	50	50
Alimentary System				
Gallbladder	(50)	(48)	(49)	(48)
Intestine large, cecum	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Intestine large, colon	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Intestine large, rectum	(50)	(49)	(50)	(50)
Intestine small, duodenum	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Intestine small, ileum	(50)	(49)	(50)	(49)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Intestine small, jejunum	(50)	(49)	(50)	(50)
Hemangioma		1 (2%)		
Liver	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hemangioma		1 (2%)		
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	10 (20%)	16 (33%)	19 (38%)	5 (10%)
Hepatocellular adenoma, multiple	4 (8%)	10 (20%)	7 (14%)	1 (2%)
Hepatocellular carcinoma	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Hepatocellular carcinoma, multiple			1 (2%)	1 (2%)
Mesentery	(12)	(7)	(6)	(1)
Carcinoma, metastatic, uterus		1 (14%)		
Fibrous histiocytoma, metastatic, skin		1 (14%)		
Hemangiosarcoma, metastatic, spleen			1 (17%)	
Hepatoblastoma, metastatic, liver	1 (8%)			
Pancreas	(49)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Salivary glands	(49)	(49)	(49)	(43)
Stomach, forestomach	(50)	(49)	(50)	(50)
Squamous cell papilloma	2 (4%)	1 (2%)		3 (6%)
Stomach, glandular	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Tongue	(1)			
Squamous cell carcinoma	1 (100%)			
Cardiovascular System				
Blood vessel	(1)	(1)	(3)	(2)
Heart	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hemangiosarcoma, metastatic, spleen			1 (2%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(49)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Adrenal medulla	(50)	(49)	(50)	(49)
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma		1 (2%)		
Parathyroid gland	(43)	(41)	(33)	(36)
Pituitary gland	(49)	(49)	(47)	(44)
Pars distalis, adenoma	2 (4%)	5 (10%)	4 (9%)	
Pars intermedia, adenoma	2 (4%)			
Thyroid gland	(48)	(49)	(49)	(47)
Bilateral, follicular cell, adenoma	1 (2%)		1 (2%)	
Follicular cell, adenoma		5 (10%)	1 (2%)	
Follicular cell, adenoma, multiple		1 (2%)		
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(49)	(47)
Ovary	(50)	(48)	(49)	(48)
Cystadenoma	3 (6%)	4 (8%)	2 (4%)	2 (4%)
Thecoma malignant		1 (2%)		
Oviduct	(2)	(1)	(2)	(0)
Uterus	(50)	(49)	(50)	(50)
Carcinoma		1 (2%)		
Hemangioma	2 (4%)			
Hemangiosarcoma				1 (2%)
Polyp stromal	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Sarcoma stromal			1 (2%)	
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Hemangiosarcoma		2 (4%)		
Hemangiosarcoma, metastatic, spleen	1 (2%)		1 (2%)	
Lymph node	(10)	(7)	(3)	(3)
Mediastinal, fibrous histiocytoma, metastatic, skin		1 (14%)		
Lymph node, mandibular	(49)	(49)	(46)	(43)
Lymph node, mesenteric	(49)	(48)	(49)	(49)
Carcinoma, metastatic, uterus		1 (2%)		
Fibrous histiocytoma		1 (2%)		
Hemangiosarcoma	1 (2%)			
Spleen	(49)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hemangiosarcoma	4 (8%)	2 (4%)	1 (2%)	
Thymus	(47)	(49)	(50)	(47)
Fibrous histiocytoma, metastatic, skin		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Integumentary System				
Mammary gland	(50)	(49)	(50)	(46)
Carcinoma	1 (2%)		1 (2%)	
Skin	(50)	(49)	(50)	(50)
Subcutaneous tissue, fibrosarcoma		2 (4%)		
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen	1 (2%)			
Subcutaneous tissue, sarcoma stromal, metastatic, uterus			1 (2%)	
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(49)	(49)	(50)	(50)
Vertebra, osteosarcoma			1 (2%)	
Skeletal muscle	(4)	(2)	(1)	(2)
Fibrous histiocytoma, metastatic, skin		1 (50%)		
Hemangiosarcoma, metastatic, spleen	1 (25%)			
Rhabdomyosarcoma				1 (50%)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Peripheral nerve	(1)	(0)	(1)	(0)
Spinal cord	(1)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)	1 (2%)	
Carcinoma, metastatic, mammary gland	1 (2%)			
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hemangioma				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)		1 (2%)	3 (6%)
Mediastinum, fibrous histiocytoma, metastatic, skin		1 (2%)		
Nose	(49)	(49)	(50)	(50)
Special Senses System				
Eye	(49)	(49)	(50)	(50)
Harderian gland	(50)	(48)	(50)	(48)
Adenoma	6 (12%)	3 (6%)	9 (18%)	3 (6%)
Bilateral, adenoma	1 (2%)			
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Hemangiosarcoma, metastatic, spleen			1 (2%)	
Urinary bladder	(50)	(49)	(49)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Sarcoma stromal, metastatic, uterus			1 (2%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(49)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	8 (16%)	6 (12%)	5 (10%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	34	39	42	24
Total primary neoplasms	55	72	62	27
Total animals with benign neoplasms	26	33	39	17
Total benign neoplasms	36	51	48	19
Total animals with malignant neoplasms	16	15	13	8
Total malignant neoplasms	19	21	14	8
Total animals with metastatic neoplasms	4	2	4	3
Total metastatic neoplasms	6	19	8	3

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

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	7/50 (14%)	3/49 (6%)	9/50 (18%)	3/50 (6%)
Adjusted rate[b]	15.4%	6.4%	19.4%	9.8%
Terminal rate[c]	7/39 (18%)	3/42 (7%)	5/32 (16%)	2/22 (9%)
First incidence (days)	727 (T)	727 (T)	531	675
Poly-3 test[d]	P=0.531N	P=0.146N	P=0.411	P=0.362N
Liver: Hepatocellular Adenoma				
Overall rate	14/50 (28%)	26/49 (53%)	26/50 (52%)	6/50 (12%)
Adjusted rate	30.8%	55.4%	56.5%	19.7%
Terminal rate	14/39 (36%)	24/42 (57%)	19/32 (59%)	6/22 (27%)
First incidence (days)	727 (T)	695	555	727 (T)
Poly-3 test	P=0.354N	P=0.013	P=0.009	P=0.216N
Liver: Hepatocellular Carcinoma				
Overall rate	2/50 (4%)	1/49 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.4%	2.1%	4.5%	12.6%
Terminal rate	2/39 (5%)	1/42 (2%)	1/32 (3%)	1/22 (5%)
First incidence (days)	727 (T)	727 (T)	693	507
Poly-3 test	P=0.097	P=0.490N	P=0.689	P=0.194
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	14/50 (28%)	26/49 (53%)	26/50 (52%)	10/50 (20%)
Adjusted rate	30.8%	55.4%	56.5%	31.5%
Terminal rate	14/39 (36%)	24/42 (57%)	19/32 (59%)	7/22 (32%)
First incidence (days)	727 (T)	695	555	507
Poly-3 test	P=0.408	P=0.013	P=0.009	P=0.572
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	3/50 (6%)	1/49 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	6.6%	2.1%	4.5%	12.6%
Terminal rate	3/39 (8%)	1/42 (2%)	1/32 (3%)	1/22 (5%)
First incidence (days)	727 (T)	727 (T)	693	507
Poly-3 test	P=0.196	P=0.295N	P=0.506N	P=0.315
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	15/50 (30%)	26/49 (53%)	26/50 (52%)	10/50 (20%)
Adjusted rate	33.0%	55.4%	56.5%	31.5%
Terminal rate	15/39 (39%)	24/42 (57%)	19/32 (59%)	7/22 (32%)
First incidence (days)	727 (T)	695	555	507
Poly-3 test	P=0.482	P=0.023	P=0.017	P=0.541N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	2/49 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.4%	4.3%	4.4%	9.9%
Terminal rate	2/39 (5%)	2/42 (5%)	0/32 (0%)	3/22 (14%)
First incidence (days)	727 (T)	727 (T)	649	727 (T)
Poly-3 test	P=0.246	P=0.683N	P=0.692	P=0.328
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	3/49 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.2%	6.4%	2.2%	0.0%
Terminal rate	1/39 (3%)	3/42 (7%)	1/32 (3%)	0/22 (0%)
First incidence (days)	727 (T)	727 (T)	727 (T)	—[e]
Poly-3 test	P=0.299N	P=0.316	P=0.757	P=0.577N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	5/49 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rate	6.6%	10.7%	6.7%	9.9%
Terminal rate	3/39 (8%)	5/42 (12%)	1/32 (3%)	3/22 (14%)
First incidence (days)	727 (T)	727 (T)	649	727 (T)
Poly-3 test	P=0.461	P=0.372	P=0.659	P=0.470
Ovary: Cystadenoma				
Overall rate	3/50 (6%)	4/48 (8%)	2/49 (4%)	2/48 (4%)
Adjusted rate	6.6%	8.7%	4.6%	6.8%
Terminal rate	3/39 (8%)	4/42 (10%)	1/31 (3%)	2/21 (10%)
First incidence (days)	727 (T)	727 (T)	654	727 (T)
Poly-3 test	P=0.474N	P=0.506	P=0.515N	P=0.660
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/49 (4%)	5/49 (10%)	4/47 (9%)	0/44 (0%)
Adjusted rate	4.5%	10.7%	9.5%	0.0%
Terminal rate	2/38 (5%)	4/42 (10%)	3/30 (10%)	0/16 (0%)
First incidence (days)	727 (T)	703	626	—
Poly-3 test	P=0.422N	P=0.238	P=0.313	P=0.385N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma				
Overall rate	0/50 (0%)	3/49 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	6.3%	0.0%	0.0%
Terminal rate	0/39 (0%)	0/42 (0%)	0/32 (0%)	0/22 (0%)
First incidence (days)	—	549	—[f]	—
Poly-3 test	P=0.379N	P=0.128	—	—
Spleen: Hemangiosarcoma				
Overall rate	4/49 (8%)	2/49 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	8.8%	4.3%	2.2%	0.0%
Terminal rate	3/39 (8%)	2/42 (5%)	0/32 (0%)	0/22 (0%)
First incidence (days)	521	727 (T)	722	—
Poly-3 test	P=0.046N	P=0.322N	P=0.182N	P=0.131N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	2/50 (4%)	1/49 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	4.4%	2.1%	0.0%	9.9%
Terminal rate	2/39 (5%)	1/42 (2%)	0/32 (0%)	3/22 (14%)
First incidence (days)	727 (T)	727 (T)	—	727 (T)
Poly-3 test	P=0.291	P=0.490N	P=0.241N	P=0.328
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/48 (2%)	6/49 (12%)	2/49 (4%)	0/47 (0%)
Adjusted rate	2.3%	12.8%	4.6%	0.0%
Terminal rate	1/38 (3%)	6/42 (14%)	2/32 (6%)	0/22 (0%)
First incidence (days)	727 (T)	727 (T)	727 (T)	—
Poly-3 test	P=0.285N	P=0.068	P=0.502	P=0.579N
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	1/49 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	2.1%	6.7%	3.3%
Terminal rate	1/39 (3%)	1/42 (2%)	3/32 (9%)	1/22 (5%)
First incidence (days)	727 (T)	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.330	P=0.754N	P=0.299	P=0.664

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	1/50 (2%)	1/49 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.2%	2.1%	9.0%	3.3%
Terminal rate	1/39 (3%)	1/42 (2%)	4/32 (13%)	1/22 (5%)
First incidence (days)	727 (T)	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.270	P=0.754N	P=0.174	P=0.664
All Organs: Hemangiosarcoma				
Overall rate	5/50 (10%)	2/49 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	10.9%	4.3%	2.2%	3.3%
Terminal rate	4/39 (10%)	2/42 (5%)	0/32 (0%)	1/22 (5%)
First incidence (days)	521	727 (T)	722	727 (T)
Poly-3 test	P=0.085N	P=0.210N	P=0.108N	P=0.230N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	4/49 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	15.1%	8.6%	2.2%	6.6%
Terminal rate	5/39 (13%)	4/42 (10%)	0/32 (0%)	2/22 (9%)
First incidence (days)	521	727 (T)	722	727 (T)
Poly-3 test	P=0.062N	P=0.257N	P=0.034N	P=0.230N
All Organs: Malignant Lymphoma				
Overall rate	8/50 (16%)	6/49 (12%)	5/50 (10%)	2/50 (4%)
Adjusted rate	16.9%	12.7%	11.0%	6.6%
Terminal rate	5/39 (13%)	4/42 (10%)	2/32 (6%)	1/22 (5%)
First incidence (days)	230	603	654	718
Poly-3 test	P=0.120N	P=0.386N	P=0.304N	P=0.173N
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	33/49 (67%)	39/50 (78%)	17/50 (34%)
Adjusted rate	56.7%	70.3%	81.6%	55.5%
Terminal rate	24/39 (62%)	31/42 (74%)	27/32 (84%)	16/22 (73%)
First incidence (days)	623	695	531	675
Poly-3 test	P=0.296	P=0.120	P=0.006	P=0.552N
All Organs: Malignant Neoplasms				
Overall rate	16/50 (32%)	15/49 (31%)	13/50 (26%)	8/50 (16%)
Adjusted rate	32.8%	31.1%	28.3%	25.2%
Terminal rate	10/39 (26%)	10/42 (24%)	6/32 (19%)	4/22 (18%)
First incidence (days)	230	549	648	507
Poly-3 test	P=0.255N	P=0.514N	P=0.401N	P=0.322N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	34/50 (68%)	39/49 (80%)	42/50 (84%)	24/50 (48%)
Adjusted rate	69.0%	80.6%	86.6%	75.1%
Terminal rate	26/39 (67%)	33/42 (79%)	27/32 (84%)	19/22 (86%)
First incidence (days)	230	549	531	507
Poly-3 test	P=0.163	P=0.137	P=0.028	P=0.370

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

TABLE D3
Historical Incidence of Hepatocellular Neoplasms in Untreated Female B6C3F1 Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Water Gavage Studies			
Formamide	6/50	4/50	9/50
5-(Hydroxymethyl)-2-furfural	14/50	2/50	14/50
Overall Historical Incidence: Water Gavage Studies			
Total (%)	20/100 (20.0%)	6/100 (6.0%)	23/100 (23.0%)
Mean ± standard deviation	20.0% ± 11.3%	6.0% ± 2.8%	23.0% ± 7.1%
Range	12%-28%	4%-8%	18%-28%
Overall Historical Incidence: All Routes			
Total (%)	345/1,245 (27.7%)	131/1,245 (10.5%)	419/1,245 (33.7%)
Mean ± standard deviation	27.8% ± 17.0%	10.5% ± 7.7%	33.7% ± 19.1%
Range	2%-62%	0%-28%	8%-64%

[a] Data as of October 4, 2007

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	5	8	6
Natural deaths	7	2	10	22
Survivors				
Terminal sacrifice	39	42	32	22
Missing		1		
Animals examined microscopically	50	49	50	50
Alimentary System				
Gallbladder	(50)	(48)	(49)	(48)
Infiltration cellular, mononuclear cell	3 (6%)	2 (4%)		
Intestine large, cecum	(50)	(49)	(50)	(50)
Lymphoid tissue, hyperplasia	1 (2%)			
Intestine large, colon	(50)	(49)	(50)	(50)
Artery, inflammation, chronic active	1 (2%)		1 (2%)	
Intestine large, rectum	(50)	(49)	(50)	(50)
Intestine small, duodenum	(50)	(49)	(50)	(50)
Intestine small, ileum	(50)	(49)	(50)	(49)
Intestine small, jejunum	(50)	(49)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid			1 (2%)	
Liver	(50)	(49)	(50)	(50)
Basophilic focus		6 (12%)	5 (10%)	
Clear cell focus	1 (2%)	1 (2%)		
Eosinophilic focus	6 (12%)	14 (29%)	6 (12%)	3 (6%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	1 (2%)	
Hemorrhage			1 (2%)	
Infiltration cellular, mononuclear cell	32 (64%)	45 (92%)	34 (68%)	27 (54%)
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic active	40 (80%)	45 (92%)	41 (82%)	36 (72%)
Mineralization		1 (2%)	1 (2%)	
Mixed cell focus	4 (8%)	5 (10%)	6 (12%)	
Pigmentation			1 (2%)	
Thrombosis			1 (2%)	
Bile duct, cyst			1 (2%)	
Hepatocyte, necrosis	2 (4%)		3 (6%)	1 (2%)
Hepatocyte, tension lipidosis	5 (10%)	4 (8%)	2 (4%)	
Hepatocyte, vacuolization cytoplasmic	34 (68%)	41 (84%)	34 (68%)	28 (56%)
Mesentery	(12)	(7)	(6)	(1)
Infiltration cellular, mononuclear cell	1 (8%)			
Artery, inflammation, chronic active				1 (100%)
Fat, fibrosis	9 (75%)	4 (57%)	3 (50%)	
Fat, inflammation, chronic active	6 (50%)	5 (71%)	3 (50%)	
Fat, metaplasia, osseous		1 (14%)		
Fat, mineralization	6 (50%)	3 (43%)	1 (17%)	
Fat, necrosis	9 (75%)	4 (57%)	3 (50%)	

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Alimentary System (continued)				
Pancreas	(49)	(49)	(50)	(50)
Cyst				1 (2%)
Inflammation, chronic active				1 (2%)
Acinus, atrophy		1 (2%)	2 (4%)	
Acinus, hypertrophy	1 (2%)			
Salivary glands	(49)	(49)	(49)	(43)
Artery, inflammation, chronic active		1 (2%)	1 (2%)	1 (2%)
Stomach, forestomach	(50)	(49)	(50)	(50)
Inflammation, chronic active		2 (4%)		
Ulcer		1 (2%)		
Epithelium, hyperkeratosis	2 (4%)	2 (4%)		1 (2%)
Epithelium, hyperplasia	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Stomach, glandular	(50)	(49)	(50)	(50)
Mineralization	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Tongue	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(1)	(1)	(3)	(2)
Aorta, inflammation, chronic active				1 (50%)
Heart	(50)	(49)	(50)	(50)
Cardiomyopathy	1 (2%)		1 (2%)	
Mineralization		1 (2%)		
Thrombosis			1 (2%)	
Artery, inflammation, chronic active		3 (6%)	2 (4%)	1 (2%)
Valve, inflammation, suppurative	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(49)
Accessory adrenal cortical nodule		2 (4%)	1 (2%)	
Hematopoietic cell proliferation			1 (2%)	
Mineralization			1 (2%)	
Subcapsular, hyperplasia	48 (96%)	49 (100%)	50 (100%)	49 (100%)
Zona fasciculata, hyperplasia		1 (2%)	2 (4%)	1 (2%)
Adrenal medulla	(50)	(49)	(50)	(49)
Hyperplasia			1 (2%)	
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	2 (4%)			
Parathyroid gland	(43)	(41)	(33)	(36)
Cyst	2 (5%)			
Pituitary gland	(49)	(49)	(47)	(44)
Pars distalis, angiectasis	1 (2%)			1 (2%)
Pars distalis, cyst		2 (4%)		
Pars distalis, hyperplasia	7 (14%)	11 (22%)	4 (9%)	
Thyroid gland	(48)	(49)	(49)	(47)
Ectopic thymus		1 (2%)	1 (2%)	
Inflammation, chronic active	4 (8%)	7 (14%)	5 (10%)	2 (4%)
Ultimobranchial cyst			1 (2%)	
Follicle, cyst	2 (4%)	2 (4%)		1 (2%)
Follicle, degeneration	10 (21%)	14 (29%)	15 (31%)	4 (9%)
Follicular cell, hyperplasia	10 (21%)	12 (24%)	11 (22%)	3 (6%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(49)	(47)
Inflammation, chronic active			1 (2%)	
Ovary	(50)	(48)	(49)	(48)
Atrophy	12 (24%)	5 (10%)	14 (29%)	20 (42%)
Cyst	9 (18%)	14 (29%)	19 (39%)	11 (23%)
Hyperplasia, adenomatous			1 (2%)	
Mineralization	2 (4%)	1 (2%)		1 (2%)
Pigmentation	5 (10%)	3 (6%)		2 (4%)
Bilateral, cyst	2 (4%)	1 (2%)		
Oviduct	(2)	(1)	(2)	(0)
Infiltration cellular, mononuclear cell	1 (50%)			
Uterus	(50)	(49)	(50)	(50)
Angiectasis				1 (2%)
Hemorrhage			1 (2%)	
Inflammation, chronic active			1 (2%)	
Thrombosis				1 (2%)
Endometrium, hyperplasia, cystic	40 (80%)	43 (88%)	42 (84%)	32 (64%)
Lymphatic, angiectasis		1 (2%)		
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Lymph node	(10)	(7)	(3)	(3)
Mediastinal, hyperplasia, lymphoid	6 (60%)	1 (14%)		1 (33%)
Renal, hematopoietic cell proliferation	1 (10%)			
Lymph node, mandibular	(49)	(49)	(46)	(43)
Hyperplasia, lymphoid	3 (6%)	9 (18%)	2 (4%)	2 (5%)
Infiltration cellular, mast cell			1 (2%)	
Pigmentation	1 (2%)			
Lymph node, mesenteric	(49)	(48)	(49)	(49)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	6 (12%)	1 (2%)		
Inflammation, chronic active	1 (2%)			
Artery, inflammation, chronic active		1 (2%)		
Spleen	(49)	(49)	(50)	(50)
Hematopoietic cell proliferation	9 (18%)	6 (12%)	5 (10%)	3 (6%)
Lymphoid follicle, hyperplasia	8 (16%)		1 (2%)	2 (4%)
Thymus	(47)	(49)	(50)	(47)
Atrophy	8 (17%)	3 (6%)	5 (10%)	2 (4%)
Cyst	32 (68%)	29 (59%)	25 (50%)	14 (30%)
Ectopic parathyroid gland	9 (19%)	10 (20%)	8 (16%)	2 (4%)
Hyperplasia, lymphoid		1 (2%)	3 (6%)	
Integumentary System				
Mammary gland	(50)	(49)	(50)	(46)
Hyperplasia, cystic			1 (2%)	
Skin	(50)	(49)	(50)	(50)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Musculoskeletal System				
Bone	(49)	(49)	(50)	(50)
Fibrous osteodystrophy	3 (6%)	3 (6%)	1 (2%)	4 (8%)
Skeletal muscle	(4)	(2)	(1)	(2)
Infiltration cellular, lymphoid	1 (25%)			
Infiltration cellular, mononuclear cell	1 (25%)			
Inflammation, chronic active	1 (25%)			1 (50%)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Compression		1 (2%)	2 (4%)	
Degeneration			1 (2%)	
Hemorrhage			1 (2%)	
Hydrocephalus	1 (2%)			
Artery, inflammation, chronic active			1 (2%)	1 (2%)
Cerebrum, neuron, necrosis			1 (2%)	
Peripheral nerve	(1)	(0)	(1)	(0)
Sciatic, demyelination			1 (100%)	
Spinal cord	(1)	(0)	(1)	(0)
Demyelination			1 (100%)	
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Infiltration cellular, mononuclear cell		1 (2%)		
Inflammation, chronic active	3 (6%)			
Mineralization	2 (4%)			
Alveolar epithelium, hyperplasia		5 (10%)		2 (4%)
Alveolus, infiltration cellular, histiocyte		3 (6%)		
Mediastinum, infiltration cellular, mononuclear cell	1 (2%)			
Nose	(49)	(49)	(50)	(50)
Inflammation, chronic active		1 (2%)	14 (28%)	41 (82%)
Glands, dilatation	12 (24%)	36 (73%)	48 (96%)	47 (94%)
Glands, hyperplasia		7 (14%)	42 (84%)	43 (86%)
Glands, inflammation, chronic active	1 (2%)	6 (12%)	21 (42%)	38 (76%)
Nasolacrimal duct, inflammation, suppurative			2 (4%)	3 (6%)
Olfactory epithelium, accumulation, hyaline droplet	1 (2%)	1 (2%)	27 (54%)	25 (50%)
Olfactory epithelium, degeneration	2 (4%)	1 (2%)	34 (68%)	24 (48%)
Olfactory epithelium, hyperplasia			8 (16%)	24 (48%)
Olfactory epithelium, metaplasia	1 (2%)	5 (10%)	30 (60%)	40 (80%)
Respiratory epithelium, accumulation, hyaline droplet	4 (8%)	4 (8%)	36 (72%)	27 (54%)
Special Senses System				
Eye	(49)	(49)	(50)	(50)
Atrophy			2 (4%)	
Anterior chamber, inflammation, suppurative	1 (2%)			
Cornea, hyperplasia	1 (2%)			
Cornea, inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Lens, cataract			1 (2%)	
Harderian gland	(50)	(48)	(50)	(48)
Hyperplasia		2 (4%)	4 (8%)	2 (4%)
Inflammation, chronic active		1 (2%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Atypia cellular		1 (2%)		
Infarct		4 (8%)	1 (2%)	
Infiltration cellular, mononuclear cell				1 (2%)
Metaplasia, osseous	2 (4%)	2 (4%)	1 (2%)	
Mineralization	13 (26%)	3 (6%)	5 (10%)	2 (4%)
Nephropathy	32 (64%)	42 (86%)	33 (66%)	26 (52%)
Artery, inflammation, chronic active		1 (2%)	1 (2%)	
Renal tubule, cyst				1 (2%)
Urinary bladder	(50)	(49)	(49)	(50)
Infiltration cellular, mononuclear cell				1 (2%)
Artery, inflammation, chronic active			1 (2%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Two independent assays for bacterial mutagenicity were conducted with 5-(hydroxymethyl)-2-furfural. The first study was performed as reported by Mortelmans *et al.* (1986). 5-(Hydroxymethyl)-2-furfural was sent to the laboratory as a coded aliquot. It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA102, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. The second assay, conducted with the same lot of 5-(hydroxymethyl)-2-furfural tested in the 2-year studies, used a slightly modified protocol (activation only with rat liver S9) and also employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. At both laboratories, 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 five doses of 5-(hydroxymethyl)-2-furfural. In the absence of toxicity, 10,000 µg per plate was the high dose. All trials were repeated with the same or a higher S9 fraction except TA1535.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) in each animal per treatment group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

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

Results

In the study conducted at BioReliance Corporation, 5-(hydroxymethyl)-2-furfural was weakly mutagenic in *S. typhimurium* strain TA100 in the absence of exogenous metabolic activation (S9) over a concentration range of 100 to 10,000 µg/plate; no mutagenic activity was detected in TA100 with S9 or in strains TA97, TA98, TA102, or TA1535, with or without S9 (Table E1). In the study conducted at SITEK Research Laboratories, no mutagenicity was detected over a concentration range of 1,500 to 10,000 µg/plate, with or without S9, in TA98 or TA100 or *E. coli* WP2 *uvrA*/pKM101. No increases in the frequencies of micronucleated NCEs were observed in peripheral blood of male or female mice treated with 5-(hydroxymethyl)-2-furfural by gavage for 3 months (Table E2); in addition, no significant dose-related changes in the percentage of immature PCEs were observed, suggesting that the chemical did not cause bone marrow toxicity.

TABLE E1
Mutagenicity of 5-(Hydroxymethyl)-2-furfural in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2
Study performed at BioReliance Corporation[c]			
TA102	0	372 ± 26.0	218 ± 2.0
	100	386 ± 17.0	215 ± 7.0
	333	391 ± 10.0	223 ± 7.0
	1,000	395 ± 19.0	207 ± 6.0
	3,333	365 ± 2.0	218 ± 17.0
	10,000	415 ± 3.0	191 ± 8.0
Trial summary		Negative	Negative
Positive control[d]		1,030 ± 11.0	822 ± 49.0

Strain	Dose (µg/plate)	+hamster S9 10%	+hamster S9 30%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA102	0	307 ± 18.0	394 ± 28.0	302 ± 26.0	398 ± 25.0	392 ± 11.0
(continued)	100	294 ± 12.0	412 ± 53.0		290 ± 19.0	400 ± 23.0
	333	279 ± 18.0	455 ± 10.0	316 ± 6.0	365 ± 15.0	394 ± 14.0
	1,000	301 ± 14.0	471 ± 7.0	300 ± 21.0	351 ± 24.0	362 ± 35.0
	3,333	237 ± 24.0	483 ± 19.0	299 ± 9.0	361 ± 2.0	373 ± 31.0
	5,000			261 ± 26.0		
	10,000	172 ± 16.0	391 ± 14.0	237 ± 20.0	361 ± 10.0	340 ± 8.0
Trial summary		Negative	Equivocal	Negative	Negative	Negative
Positive control		2,358 ± 21.0	1,747 ± 28.0	1,491 ± 60.0	2,416 ± 79.0	1,266 ± 50.0

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+hamster S9 10%	+hamster S9 30%
TA100	0	167 ± 22.0	193 ± 1.0	114 ± 2.0	87 ± 6.0	152 ± 11.0
	100	179 ± 5.0	206 ± 15.0	112 ± 8.0	92 ± 7.0	169 ± 9.0
	333	172 ± 11.0	225 ± 5.0	117 ± 9.0	91 ± 1.0	157 ± 12.0
	1,000	175 ± 6.0	215 ± 5.0	108 ± 5.0	92 ± 6.0	159 ± 7.0
	3,333	237 ± 3.0	236 ± 14.0	130 ± 2.0	95 ± 5.0	169 ± 9.0
	10,000	318 ± 13.0	265 ± 11.0	172 ± 1.0	95 ± 7.0	148 ± 5.0
Trial summary		Weakly Positive	Weakly Positive	Weakly Positive	Negative	Negative
Positive control		509 ± 10.0	644 ± 39.0	409 ± 9.0	222 ± 22.0	703 ± 19.0

TABLE E1
Mutagenicity of 5-(Hydroxymethyl)-2-furfural in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate +rat S9 10%	Revertants/Plate +rat S9 30%
Study performed at BioReliance Corporation (continued)			
TA100 (continued)	0	97 ± 5.0	158 ± 20.0
	100	102 ± 6.0	158 ± 4.0
	333	101 ± 2.0	151 ± 4.0
	1,000	99 ± 4.0	162 ± 6.0
	3,333	95 ± 5.0	164 ± 15.0
	10,000	114 ± 3.0	160 ± 9.0
Trial summary		Negative	Negative
Positive control		309 ± 28.0	482 ± 21.0

Strain	Dose (µg/plate)	–S9 Trial 1
TA1535	0	11 ± 1.0
	100	9 ± 1.0
	333	11 ± 2.0
	1,000	11 ± 2.0
	3,333	10 ± 2.0
	10,000	5 ± 1.0
Trial summary		Negative
Positive control		142 ± 23.0

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA97	0	112 ± 9.0	180 ± 6.0	176 ± 12.0	204 ± 17.0	201 ± 8.0	199 ± 10.0
	100	118 ± 10.0	221 ± 7.0	215 ± 12.0	170 ± 14.0	182 ± 13.0	207 ± 16.0
	333	103 ± 7.0	192 ± 9.0	170 ± 5.0	159 ± 10.0	209 ± 13.0	222 ± 2.0
	1,000	101 ± 3.0	207 ± 4.0	195 ± 10.0	159 ± 10.0	169 ± 5.0	195 ± 10.0
	3,333	99 ± 8.0	128 ± 8.0	128 ± 9.0	147 ± 8.0	179 ± 15.0	178 ± 9.0
	10,000	97 ± 6.0	123 ± 1.0	138 ± 14.0	98 ± 9.0	109 ± 5.0	136 ± 7.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		345 ± 10.0	498 ± 37.0	435 ± 9.0	546 ± 46.0	677 ± 11.0	415 ± 18.0
TA98	0	15 ± 2.0	19 ± 1.0	15 ± 3.0	25 ± 1.0	12 ± 1.0	25 ± 4.0
	100	16 ± 3.0	19 ± 3.0	20 ± 4.0	24 ± 4.0	17 ± 2.0	23 ± 2.0
	333	19 ± 3.0	27 ± 2.0	17 ± 1.0	20 ± 3.0	16 ± 3.0	27 ± 1.0
	1,000	19 ± 2.0	15 ± 2.0	18 ± 3.0	23 ± 1.0	15 ± 3.0	18 ± 1.0
	3,333	14 ± 3.0	18 ± 3.0	17 ± 3.0	21 ± 1.0	15 ± 2.0	19 ± 2.0
	10,000	7 ± 2.0	15 ± 3.0	15 ± 0.0	20 ± 1.0	17 ± 1.0	17 ± 1.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		353 ± 10.0	251 ± 7.0	403 ± 38.0	457 ± 15.0	222 ± 18.0	188 ± 38.0

TABLE E1
Mutagenicity of 5-(Hydroxymethyl)-2-furfural in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SITEK Research Laboratories					
TA100	0	72 ± 5.0	70 ± 3.0	90 ± 7.0	63 ± 3.0
	1,500	47 ± 4.0	59 ± 6.0	84 ± 5.0	57 ± 2.0
	3,500	50 ± 6.0	45 ± 5.0	83 ± 6.0	56 ± 1.0
	5,000	46 ± 3.0	41 ± 1.0	82 ± 3.0	62 ± 2.0
	7,500	40 ± 3.0	37 ± 4.0	89 ± 2.0	64 ± 3.0
	10,000	23 ± 1.0[e]	28 ± 2.0	76 ± 7.0	47 ± 7.0
Trial summary		Negative	Negative	Negative	Negative
Positive control		380 ± 37.0	554 ± 3.0	868 ± 76.0	1,050 ± 28.0
TA98	0	20 ± 2.0	20 ± 2.0	28 ± 3.0	24 ± 2.0
	1,500	18 ± 3.0	28 ± 3.0	24 ± 3.0	33 ± 5.0
	3,500	21 ± 1.0	19 ± 2.0	28 ± 4.0	27 ± 3.0
	5,000	24 ± 2.0	18 ± 1.0	27 ± 3.0	26 ± 1.0
	7,500	18 ± 4.0	16 ± 2.0	22 ± 2.0	21 ± 2.0
	10,000	12 ± 1.0	12 ± 1.0	24 ± 2.0	21 ± 3.0
Trial summary		Negative	Negative	Negative	Negative
Positive control		512 ± 24.0	648 ± 26.0	1,270 ± 95.0	1,071 ± 42.0
***Escherichia coli* WP2 *uvrA*/pKM101 (Analogous to TA102)**					
	0	143 ± 5.0	200 ± 7.0	195 ± 12.0	218 ± 15.0
	1,500	180 ± 5.0	239 ± 2.0	182 ± 7.0	253 ± 11.0
	3,500	193 ± 4.0	221 ± 19.0	180 ± 15.0	276 ± 10.0
	5,000	173 ± 5.0	206 ± 24.0	179 ± 8.0	250 ± 13.0
	7,500	180 ± 5.0	210 ± 10.0	189 ± 4.0	278 ± 10.0
	10,000	164 ± 2.0	195 ± 12.0	207 ± 13.0	231 ± 11.0
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,654 ± 53.0	1,814 ± 111.0	996 ± 15.0	869 ± 45.0

[a] 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The detailed protocol is presented by Mortelmans *et al*. (1986).
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and 1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), mitomycin-C (TA102), and methyl methanesulfonate (pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene or sterigmatocystin was used for TA102.
[e] Slight toxicity

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with 5-(Hydroxymethyl)-2-furfural by Gavage for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)
Male					
Deionized water[d]	0	10	3.90 ± 0.48		3.9 ± 0.2
5-(Hydroxymethyl)-2-furfural	47	10	3.50 ± 0.43	0.6793	4.0 ± 0.3
	94	10	2.60 ± 0.37	0.9469	4.0 ± 0.2
	188	10	3.70 ± 0.58	0.5909	4.2 ± 0.1
	375	10	3.00 ± 0.65	0.8611	4.3 ± 0.1
	750	9	3.00 ± 0.41	0.8538	4.5 ± 0.1
			P=0.789[e]		
Female					
Deionized water	0	10	1.70 ± 0.50		4.3 ± 0.2
5-(Hydroxymethyl)-2-furfural	47	10	2.50 ± 0.50	0.1083	4.5 ± 0.3
	94	10	2.90 ± 0.43	0.0383	4.4 ± 0.3
	188	10	1.80 ± 0.47	0.4328	4.7 ± 0.3
	375	9	2.33 ± 0.65	0.1646	4.1 ± 0.3
	750	10	2.40 ± 0.37	0.1369	4.3 ± 0.3
			P=0.371		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al*. (1990).
[b] NCE=normochromatic erythrocyte
Mean ± standard error
[c] Pairwise comparison with the vehicle control; dosed group values are significant at $P \leq 0.005$ (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at $P \leq 0.025$ (ILS, 1990)

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male						
Hematology						
n						
Day 4	9	9	9	10	10	8
Day 23	10	10	10	10	10	10
Week 14	10	9	8	8	7	8
Hematocrit (%)						
Day 4	39.1 ± 0.5	39.6 ± 0.5	39.0 ± 0.4	39.3 ± 0.6	43.6 ± 1.3	38.4 ± 0.5
Day 23	41.3 ± 0.6	41.3 ± 0.3	41.2 ± 0.3	41.6 ± 0.3	41.8 ± 0.6	42.7 ± 0.5
Week 14	47.7 ± 0.5	46.6 ± 0.6	48.6 ± 0.2	47.5 ± 0.4	47.4 ± 0.6	47.9 ± 0.4
Hemoglobin (g/dL)						
Day 4	12.9 ± 0.1	13.1 ± 0.2	12.9 ± 0.1	13.0 ± 0.2	14.3 ± 0.4	12.6 ± 0.2
Day 23	14.1 ± 0.2	14.0 ± 0.1	14.1 ± 0.1	14.1 ± 0.1	14.2 ± 0.2	14.5 ± 0.1
Week 14	15.7 ± 0.1	15.4 ± 0.2	16.0 ± 0.1	15.8 ± 0.1	15.7 ± 0.2	15.8 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	6.71 ± 0.10	6.83 ± 0.12	6.72 ± 0.07	6.71 ± 0.10	7.51 ± 0.26	6.58 ± 0.08
Day 23	7.20 ± 0.08	7.18 ± 0.08	7.13 ± 0.07	7.19 ± 0.06	7.20 ± 0.11	7.39 ± 0.11
Week 14	9.00 ± 0.09	8.88 ± 0.13	9.15 ± 0.05	9.09 ± 0.07	9.00 ± 0.12	9.10 ± 0.07
Reticulocytes (10^6/µL)						
Day 4	0.30 ± 0.04	0.29 ± 0.03	0.27 ± 0.02	0.30 ± 0.02	0.28 ± 0.02	0.26 ± 0.02
Day 23	0.11 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01
Week 14	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Nucleated erythrocytes (10^3/µL)						
Day 4	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.03	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
Day 23	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.04 ± 0.02
Week 14	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Mean cell volume (fL)						
Day 4	58.2 ± 0.4	57.9 ± 0.3	58.0 ± 0.3	58.6 ± 0.2	58.2 ± 0.4	58.4 ± 0.2
Day 23	57.4 ± 0.2	57.6 ± 0.2	57.7 ± 0.2	57.9 ± 0.2	58.0 ± 0.2	57.9 ± 0.3
Week 14	52.9 ± 0.2	52.5 ± 0.3	53.2 ± 0.2	52.3 ± 0.3	52.7 ± 0.2	52.6 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.3 ± 0.2	19.2 ± 0.2	19.2 ± 0.2	19.4 ± 0.2	19.1 ± 0.2	19.2 ± 0.1
Day 23	19.6 ± 0.1	19.5 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.8 ± 0.1	19.6 ± 0.1
Week 14	17.5 ± 0.1	17.4 ± 0.1	17.5 ± 0.1	17.3 ± 0.1	17.4 ± 0.1	17.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.1 ± 0.2	33.1 ± 0.2	33.1 ± 0.2	33.1 ± 0.2	32.9 ± 0.2	32.9 ± 0.2
Day 23	34.1 ± 0.1	34.0 ± 0.2	34.2 ± 0.1	33.9 ± 0.2	34.0 ± 0.1	33.9 ± 0.2
Week 14	33.0 ± 0.2	33.1 ± 0.1	32.8 ± 0.2	33.1 ± 0.2	33.0 ± 0.2	33.1 ± 0.2
Platelets (10^3/µL)						
Day 4	912.3 ± 20.9	881.0 ± 22.4	894.1 ± 25.1	868.7 ± 21.0	916.2 ± 27.2	886.3 ± 25.3
Day 23	871.2 ± 14.2	889.2 ± 27.2	852.3 ± 15.1	815.8 ± 17.7*	814.6 ± 16.8*	789.1 ± 10.6**
Week 14	717.9 ± 14.4	692.8 ± 11.7	709.1 ± 10.8	726.0 ± 12.2	685.9 ± 17.7	688.5 ± 8.1
Leukocytes (10^3/µL)						
Day 4	6.79 ± 0.31	7.67 ± 0.31	7.26 ± 0.31	7.81 ± 0.44	8.50 ± 0.32**	7.44 ± 0.19
Day 23	8.88 ± 0.41	8.47 ± 0.37	8.27 ± 0.31	8.67 ± 0.46	9.05 ± 0.34	8.88 ± 0.32
Week 14	8.66 ± 0.52	8.49 ± 0.62	9.75 ± 0.31	8.64 ± 1.02	9.47 ± 0.41	8.85 ± 0.49
Segmented neutrophils (10^3/µL)						
Day 4	0.83 ± 0.03	0.90 ± 0.05	0.89 ± 0.06	1.00 ± 0.06	1.08 ± 0.05*	0.80 ± 0.08
Day 23	1.00 ± 0.07	0.92 ± 0.08	0.81 ± 0.05	0.76 ± 0.05*	0.91 ± 0.03	0.76 ± 0.02*
Week 14	1.31 ± 0.11	1.45 ± 0.08	1.21 ± 0.05	1.39 ± 0.12	1.60 ± 0.19	1.33 ± 0.11

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	9	9	9	10	10	8
Day 23	10	10	10	10	10	10
Week 14	10	9	8	8	7	8
Bands (10^3/µL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)						
Day 4	5.81 ± 0.29	6.65 ± 0.27	6.29 ± 0.31	6.64 ± 0.42	7.30 ± 0.36*	6.50 ± 0.22
Day 23	7.64 ± 0.39	7.32 ± 0.37	7.23 ± 0.27	7.72 ± 0.43	7.94 ± 0.33	7.91 ± 0.29
Week 14	7.20 ± 0.43	6.87 ± 0.58	8.42 ± 0.31	7.08 ± 0.91	7.66 ± 0.33	7.21 ± 0.47
Monocytes (10^3/µL)						
Day 4	0.15 ± 0.02	0.11 ± 0.01	0.08 ± 0.00	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.05
Day 23	0.21 ± 0.03	0.19 ± 0.06	0.17 ± 0.03	0.16 ± 0.04	0.17 ± 0.04	0.18 ± 0.05
Week 14	0.12 ± 0.02	0.13 ± 0.03	0.11 ± 0.04	0.17 ± 0.03	0.20 ± 0.03	0.26 ± 0.05*
Basophils (10^3/µL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01**	0.02 ± 0.01	0.03 ± 0.01
Day 23	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.02
Week 14	0.03 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.05 ± 0.03
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	9
Urea nitrogen (mg/dL)						
Day 4	8.3 ± 0.6	8.1 ± 0.5	7.1 ± 0.5	5.8 ± 0.4	10.1 ± 1.9	6.2 ± 0.4*
Day 23	11.8 ± 0.5	12.3 ± 0.3	11.4 ± 0.4	10.1 ± 0.5	10.1 ± 0.3	11.1 ± 0.5
Week 14	13.5 ± 0.5	14.0 ± 0.4	13.4 ± 0.5	12.7 ± 0.3	13.0 ± 0.8	11.7 ± 0.4*
Creatinine (mg/dL)						
Day 4	0.40 ± 0.00	0.40 ± 0.00	0.41 ± 0.01	0.40 ± 0.00	0.43 ± 0.02*	0.40 ± 0.00
Day 23	0.47 ± 0.03	0.51 ± 0.03	0.47 ± 0.02	0.50 ± 0.02	0.48 ± 0.01	0.48 ± 0.01
Week 14	0.55 ± 0.02	0.55 ± 0.02	0.53 ± 0.02	0.55 ± 0.02	0.58 ± 0.01	0.53 ± 0.02
Total protein (g/dL)						
Day 4	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	5.8 ± 0.2	5.0 ± 0.1
Day 23	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.0 ± 0.1
Week 14	6.8 ± 0.0	6.7 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.5 ± 0.1**	6.6 ± 0.1
Albumin (g/dL)						
Day 4	3.9 ± 0.0	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.1	4.4 ± 0.1	3.8 ± 0.0
Day 23	4.6 ± 0.1	4.5 ± 0.0	4.6 ± 0.0	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.0
Week 14	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.0	4.8 ± 0.1	4.9 ± 0.0

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	9
Alanine aminotransferase (IU/L)						
Day 4	76 ± 3	78 ± 2	78 ± 3	78 ± 2	63 ± 5	73 ± 2
Day 23	68 ± 1	55 ± 1**	61 ± 2*	58 ± 2**	63 ± 2	57 ± 2**
Week 14	129 ± 18	164 ± 26	103 ± 8	91 ± 5	102 ± 9	79 ± 5*
Alkaline phosphatase (IU/L)						
Day 4	853 ± 27	882 ± 18	856 ± 18	877 ± 20	827 ± 27	791 ± 21
Day 23	579 ± 14	612 ± 15	591 ± 11	592 ± 15	591 ± 21	606 ± 10
Week 14	253 ± 9	246 ± 7	249 ± 7	242 ± 8	258 ± 11	219 ± 4*
Creatine kinase (IU/L)						
Day 4	296 ± 40	381 ± 71	361 ± 40	303 ± 39	337 ± 39	287 ± 29
Day 23	260 ± 23	287 ± 51	256 ± 21[b]	331 ± 61	244 ± 22	331 ± 30
Week 14	137 ± 10	117 ± 20	157 ± 28	141 ± 30	158 ± 23	190 ± 37
Sorbitol dehydrogenase (IU/L)						
Day 4	14 ± 1	14 ± 1	13 ± 1	13 ± 1	16 ± 1	13 ± 1
Day 23	21 ± 1	17 ± 1	17 ± 1	21 ± 1	20 ± 1	18 ± 1
Week 14	47 ± 11	52 ± 9	29 ± 3	23 ± 1	34 ± 5	28 ± 4
Bile acids (µmol/L)						
Day 4	34.3 ± 4.5	44.1 ± 5.6	43.1 ± 4.5	40.5 ± 5.7	25.5 ± 2.3	28.6 ± 3.9
Day 23	22.2 ± 2.5	21.0 ± 2.5	24.5 ± 2.6	19.8 ± 1.7	18.8 ± 1.8	23.2 ± 2.3
Week 14	18.7 ± 1.4	21.1 ± 1.4	17.2 ± 1.7	16.0 ± 0.9	18.4 ± 0.5	18.6 ± 0.8
Female						
Hematology						
n						
Day 4	10	10	10	10	9	8
Day 23	10	10	10	10	10	8
Week 14	10	9	9	7	9	6
Hematocrit (%)						
Day 4	44.7 ± 1.2	42.4 ± 0.8	40.2 ± 0.4**	41.3 ± 0.5**	40.0 ± 0.4**	40.9 ± 0.6**
Day 23	43.3 ± 0.6	42.7 ± 0.6	42.8 ± 0.5	43.3 ± 0.4	42.8 ± 0.4	44.1 ± 0.7
Week 14	44.8 ± 0.6	44.9 ± 0.4	45.1 ± 0.4	45.8 ± 0.8	45.6 ± 0.4	46.3 ± 0.7
Hemoglobin (g/dL)						
Day 4	14.9 ± 0.4	14.0 ± 0.2	13.4 ± 0.2**	13.7 ± 0.1**	13.4 ± 0.2**	13.6 ± 0.2**
Day 23	14.7 ± 0.2	14.5 ± 0.2	14.6 ± 0.2	14.9 ± 0.1	14.7 ± 0.2	15.1 ± 0.2
Week 14	14.9 ± 0.2	15.0 ± 0.1	15.2 ± 0.1	15.2 ± 0.2	15.3 ± 0.1	15.5 ± 0.2*
Erythrocytes (10^6/µL)						
Day 4	7.84 ± 0.23	7.34 ± 0.15	6.93 ± 0.08**	7.11 ± 0.12**	6.89 ± 0.09**	7.12 ± 0.10**
Day 23	7.49 ± 0.12	7.38 ± 0.10	7.44 ± 0.09	7.49 ± 0.09	7.47 ± 0.09	7.73 ± 0.12
Week 14	8.00 ± 0.12	8.09 ± 0.08	8.11 ± 0.07	8.19 ± 0.15	8.16 ± 0.07	8.27 ± 0.14

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	9	8
Day 23	10	10	10	10	10	8
Week 14	10	9	9	7	9	6
Reticulocytes (10^6/μL)						
Day 4	0.18 ± 0.02	0.21 ± 0.03	0.26 ± 0.02	0.24 ± 0.03	0.22 ± 0.03	0.19 ± 0.01
Day 23	0.13 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.01
Week 14	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 4	0.02 ± 0.01	0.07 ± 0.03	0.02 ± 0.01	0.06 ± 0.03	0.02 ± 0.02	0.06 ± 0.03
Day 23	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Week 14	0.02 ± 0.02	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 4	57.1 ± 0.4	57.8 ± 0.2	58.0 ± 0.2	58.1 ± 0.3	58.1 ± 0.2	57.4 ± 0.2
Day 23	57.8 ± 0.2	57.8 ± 0.1	57.6 ± 0.2	57.8 ± 0.4	57.3 ± 0.3	57.1 ± 0.1*
Week 14	56.0 ± 0.3	55.6 ± 0.3	55.6 ± 0.2	56.0 ± 0.1	55.9 ± 0.1	56.0 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.0 ± 0.1	19.0 ± 0.2	19.4 ± 0.1	19.2 ± 0.2	19.4 ± 0.1	19.1 ± 0.1
Day 23	19.6 ± 0.2	19.6 ± 0.1	19.7 ± 0.1	19.9 ± 0.2	19.7 ± 0.1	19.5 ± 0.1
Week 14	18.7 ± 0.1	18.6 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	18.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.3 ± 0.2	33.0 ± 0.3	33.5 ± 0.2	33.2 ± 0.2	33.4 ± 0.2	33.2 ± 0.1
Day 23	33.9 ± 0.2	33.9 ± 0.2	34.2 ± 0.2	34.4 ± 0.2	34.5 ± 0.1	34.2 ± 0.1
Week 14	33.3 ± 0.2	33.4 ± 0.2	33.6 ± 0.2	33.1 ± 0.2	33.6 ± 0.2	33.5 ± 0.2
Platelets (10^3/μL)						
Day 4	918.1 ± 39.4	812.4 ± 21.9	873.2 ± 22.3	801.5 ± 23.2	847.3 ± 29.4	846.6 ± 28.5
Day 23	807.9 ± 20.4	785.0 ± 15.6	813.6 ± 11.4	799.2 ± 15.2	822.1 ± 16.7	752.3 ± 12.6
Week 14	737.7 ± 17.6	676.4 ± 20.0	714.8 ± 12.6	688.6 ± 26.2	713.7 ± 14.2	716.2 ± 18.4
Leukocytes (10^3/μL)						
Day 4	9.27 ± 0.50	9.05 ± 0.31	8.40 ± 0.50	8.84 ± 0.28	8.56 ± 0.38	8.69 ± 0.51
Day 23	7.89 ± 0.36	8.52 ± 0.33	8.35 ± 0.42	9.49 ± 0.49	9.14 ± 0.40	8.85 ± 0.47
Week 14	7.16 ± 0.52	6.91 ± 0.49	7.02 ± 0.67	6.76 ± 0.41	8.17 ± 0.61	6.48 ± 0.34
Segmented neutrophils (10^3/μL)						
Day 4	0.85 ± 0.04	0.82 ± 0.06	0.67 ± 0.05	0.95 ± 0.07	0.74 ± 0.06	0.87 ± 0.06
Day 23	0.74 ± 0.05	0.99 ± 0.07	0.82 ± 0.07	1.14 ± 0.13*	0.81 ± 0.05	0.76 ± 0.05
Week 14	1.44 ± 0.19	1.35 ± 0.13	1.47 ± 0.09	1.02 ± 0.16	1.34 ± 0.10	1.07 ± 0.06
Bands (10^3/μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 4	8.13 ± 0.47	7.98 ± 0.25	7.50 ± 0.46	7.64 ± 0.25	7.63 ± 0.35	7.62 ± 0.50
Day 23	7.04 ± 0.35	7.37 ± 0.29	7.40 ± 0.37	8.22 ± 0.48	8.20 ± 0.39	7.95 ± 0.44
Week 14	5.56 ± 0.42	5.39 ± 0.35	5.29 ± 0.59	5.57 ± 0.34	6.54 ± 0.55	5.26 ± 0.35
Monocytes (10^3/μL)						
Day 4	0.27 ± 0.07	0.25 ± 0.06	0.20 ± 0.03	0.21 ± 0.05	0.17 ± 0.03	0.15 ± 0.06
Day 23	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.03	0.07 ± 0.03
Week 14	0.14 ± 0.03	0.12 ± 0.03	0.21 ± 0.04	0.14 ± 0.02	0.22 ± 0.04	0.14 ± 0.02

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	9	8
Day 23	10	10	10	10	10	8
Week 14	10	9	9	7	9	6
Basophils (10^3/μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.02
Day 23	0.04 ± 0.02	0.10 ± 0.03	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0.02	0.08 ± 0.01
Week 14	0.03 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.03 ± 0.02	0.07 ± 0.02	0.02 ± 0.02
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	8
Day 23	10	10	10	10	10	8
Week 14	10	10	10	10	10	7
Urea nitrogen (mg/dL)						
Day 4	11.4 ± 1.1	10.0 ± 0.7	8.1 ± 0.7	7.5 ± 0.5*	7.9 ± 0.3	16.1 ± 3.8
Day 23	11.6 ± 0.4	13.0 ± 0.5	13.9 ± 0.5	12.0 ± 0.4	10.2 ± 0.5	10.8 ± 0.3
Week 14	16.4 ± 0.5	15.4 ± 0.5	15.0 ± 0.5	15.1 ± 0.5	14.7 ± 0.6*	12.6 ± 0.5**
Creatinine (mg/dL)						
Day 4	0.49 ± 0.02	0.49 ± 0.01	0.48 ± 0.01	0.46 ± 0.02	0.47 ± 0.02	0.48 ± 0.02
Day 23	0.54 ± 0.03	0.51 ± 0.01	0.58 ± 0.06	0.52 ± 0.01	0.50 ± 0.00	0.51 ± 0.01
Week 14	0.61 ± 0.01	0.59 ± 0.01	0.63 ± 0.02	0.66 ± 0.02	0.63 ± 0.02	0.63 ± 0.02
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1
Day 23	6.1 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Week 14	7.0 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.6 ± 0.1*	7.2 ± 0.1	6.7 ± 0.1
Albumin (g/dL)						
Day 4	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.0	4.4 ± 0.1
Day 23	4.8 ± 0.1	4.6 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.1
Week 14	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.2 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	52 ± 5	65 ± 3	64 ± 3	61 ± 3	62 ± 1	67 ± 4
Day 23	43 ± 1	46 ± 2	43 ± 2	44 ± 1	45 ± 2	44 ± 1
Week 14	66 ± 5	83 ± 9	64 ± 3	74 ± 7	65 ± 4	55 ± 1
Alkaline phosphatase (IU/L)						
Day 4	621 ± 24	666 ± 22	639 ± 24	637 ± 16	624 ± 15	621 ± 28
Day 23	411 ± 15	414 ± 10	390 ± 11	399 ± 10	394 ± 6	391 ± 7
Week 14	198 ± 11	227 ± 7	204 ± 9	210 ± 5	204 ± 5	203 ± 5
Creatine kinase (IU/L)						
Day 4	476 ± 131	273 ± 19	254 ± 19	290 ± 32	303 ± 14	306 ± 26
Day 23	302 ± 25	337 ± 29[b]	351 ± 43	323 ± 31	399 ± 38	305 ± 26
Week 14	278 ± 72	201 ± 44	161 ± 59	281 ± 67	230 ± 55	209 ± 89

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	8
Day 23	10	10	10	10	10	8
Week 14	10	10	10	10	10	7
Sorbitol dehydrogenase (IU/L)						
Day 4	18 ± 1	15 ± 1	13 ± 0**	15 ± 1	14 ± 1	14 ± 1
Day 23	22 ± 1	19 ± 1*	19 ± 1	21 ± 0	20 ± 1	21 ± 1
Week 14	21 ± 1	22 ± 1	19 ± 1	24 ± 3	24 ± 4	19 ± 1
Bile acids (µmol/L)						
Day 4	18.5 ± 1.5	19.9 ± 2.4	24.1 ± 2.5	22.2 ± 2.3	19.9 ± 1.5	25.9 ± 3.6
Day 23	20.8 ± 3.2	20.2 ± 1.8	19.0 ± 2.1	18.6 ± 2.9	14.5 ± 1.6	22.0 ± 2.3
Week 14	19.7 ± 2.3	18.9 ± 2.0	18.6 ± 2.1	26.3 ± 4.3	20.9 ± 2.8	19.6 ± 3.9

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	47 mg/kg	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg
Male						
Hematology						
n	10	10	10	10	10	9
Hematocrit (%)	48.2 ± 0.5	48.9 ± 1.0	49.7 ± 1.0	48.5 ± 0.5	48.9 ± 0.9	49.0 ± 0.7
Hemoglobin (g/dL)	16.2 ± 0.2	16.5 ± 0.3	16.7 ± 0.3	16.3 ± 0.2	16.6 ± 0.3	16.4 ± 0.2
Erythrocytes (10^6/µL)	10.31 ± 0.10	10.55 ± 0.24	10.77 ± 0.24	10.49 ± 0.11	10.56 ± 0.19	10.64 ± 0.16
Reticulocytes (10^6/µL)	0.04 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	46.7 ± 0.2	46.3 ± 0.1	46.3 ± 0.1	46.2 ± 0.1	46.3 ± 0.1	46.1 ± 0.1*
Mean cell hemoglobin (pg)	15.8 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.7 ± 0.1	15.4 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.1	33.7 ± 0.2	33.5 ± 0.1	33.6 ± 0.1	33.9 ± 0.2	33.5 ± 0.1
Platelets (10^3/µL)	766.3 ± 20.8	773.2 ± 40.8	736.6 ± 26.4	804.3 ± 36.9	761.4 ± 35.7	787.9 ± 45.0
Leukocytes (10^3/µL)	5.68 ± 0.26	4.92 ± 0.54	4.41 ± 0.33*	4.67 ± 0.41	5.85 ± 0.46	4.01 ± 0.34*
Segmented neutrophils (10^3/µL)	0.62 ± 0.04	0.81 ± 0.15	0.64 ± 0.05	0.67 ± 0.09	0.90 ± 0.11	0.54 ± 0.06
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	4.95 ± 0.25	4.02 ± 0.40	3.66 ± 0.29*	3.89 ± 0.36	4.81 ± 0.40	3.39 ± 0.31*
Monocytes (10^3/µL)	0.05 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.09 ± 0.03	0.04 ± 0.01
Female						
Hematology						
n	10	10	10	10	9	10
Hematocrit (%)	47.4 ± 0.6	48.9 ± 0.4	45.9 ± 0.4	47.5 ± 0.5	49.3 ± 0.8	46.2 ± 1.3
Hemoglobin (g/dL)	16.1 ± 0.2	16.7 ± 0.1	15.7 ± 0.2	16.1 ± 0.2	16.7 ± 0.3	15.6 ± 0.4
Erythrocytes (10^6/µL)	10.07 ± 0.14	10.52 ± 0.09	9.77 ± 0.11	10.14 ± 0.13	10.41 ± 0.17	9.85 ± 0.27
Reticulocytes (10^6/µL)	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.15 ± 0.02
Nucleated erythrocytes (10^3/µL)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.2 ± 0.2	46.6 ± 0.2	47.1 ± 0.2	46.8 ± 0.1	47.3 ± 0.1	47.0 ± 0.1
Mean cell hemoglobin (pg)	16.0 ± 0.1	15.9 ± 0.1	16.1 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.1	34.1 ± 0.1	34.1 ± 0.1	33.9 ± 0.1	33.8 ± 0.1	33.8 ± 0.1
Platelets (10^3/µL)	784.0 ± 39.1	811.8 ± 30.1	799.0 ± 37.2	742.5 ± 34.8	615.7 ± 43.9	754.0 ± 45.8
Leukocytes (10^3/µL)	5.68 ± 0.46	4.37 ± 0.34	4.35 ± 0.42	5.38 ± 0.39	4.54 ± 0.39	5.28 ± 0.90
Segmented neutrophils (10^3/µL)	0.88 ± 0.11	0.70 ± 0.12	0.56 ± 0.05	0.77 ± 0.10	0.68 ± 0.11	1.21 ± 0.55
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	4.70 ± 0.38	3.59 ± 0.28	3.70 ± 0.38	4.54 ± 0.34	3.78 ± 0.38	4.00 ± 0.45
Monocytes (10^3/µL)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.01	0.01 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.09 ± 0.03	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.03 ± 0.01	0.06 ± 0.01

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test
[a] Data are given as mean ± standard error. Ratios were calculated and statistical tests were performed on unrounded data.

APPENDIX G
URINALYSIS AND METABOLITE DATA

TABLE G1
Urinalysis and Metabolite Data for Rats in the 3-Week Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male						
n	5	5	5	5	5	4
Creatinine (mg/dL)	88.06 ± 7.34	89.20 ± 11.72	87.16 ± 11.89	95.12 ± 11.77	65.94 ± 6.52	54.15 ± 5.61*
Volume (mL/24 hours)	5.7 ± 0.6	6.7 ± 0.8	6.7 ± 0.7	5.7 ± 0.6	8.0 ± 0.4*	7.3 ± 0.6
5-(Hydroxymethyl)-2-furoic acid (µg/mL)	3 ± 2	1,930 ± 312	3,534 ± 1,090	8,670 ± 1,858	12,352 ± 1,583	25,176 ± 939
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)	4.1 ± 0.3	70.7 ± 9.8	96.0 ± 31.9	232.3 ± 28.4	267.0 ± 53.0	482.9 ± 40.1
Female						
n	4	5	5	5	5	5
Creatinine (mg/dL)	60.20 ± 4.70	78.30 ± 4.85	70.70 ± 13.86	71.18 ± 6.30	64.58 ± 4.11	66.74 ± 9.58
Volume (mL/24 hours)	7.0 ± 1.1	6.0 ± 0.6	4.4 ± 0.6	5.9 ± 0.5	5.8 ± 0.3	6.8 ± 1.4
5-(Hydroxymethyl)-2-furoic acid (µg/mL)	2 ± 2	1,407 ± 513	3,359 ± 402	6,092 ± 654	12,924 ± 1,511	21,348 ± 4,321
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)	5.7 ± 0.5	132.1 ± 32.9	278.6 ± 116.4	330.9 ± 85.7	530.5 ± 103.4	730.1 ± 314.2

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

TABLE G2
Urinalysis and Metabolite Data for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male						
n						
Day 17	9	10	10	10	10	9
Day 45	10	9	10	10	10	9
Week 14	9	10	9	10	10	9
Creatinine (mg/dL)						
Day 17	100.16 ± 4.84	104.99 ± 3.91	109.81 ± 5.00	98.83 ± 5.76	81.92 ± 3.55*	65.90 ± 6.09**
Day 45	145.88 ± 8.14	119.81 ± 8.17	163.69 ± 10.10	123.59 ± 7.89	109.96 ± 5.80**	79.98 ± 3.08**
Week 14	136.68 ± 9.59	130.07 ± 8.17	132.30 ± 10.21	104.23 ± 4.70**	97.42 ± 2.85**	64.14 ± 1.63**
Volume (mL/24 hours)						
Day 17	5.5 ± 0.3	5.9 ± 0.4	5.1 ± 0.6	5.8 ± 0.3	6.1 ± 0.4	8.2 ± 0.8**
Day 45	5.8 ± 0.5	7.1 ± 0.7	4.7 ± 0.6	6.8 ± 0.6	6.4 ± 0.5	9.4 ± 0.8**
Week 14	8.0 ± 0.7	8.4 ± 0.8	7.9 ± 0.9	9.9 ± 0.7	9.8 ± 0.4*	15.4 ± 0.7**
5-(Hydroxymethyl)-2-furoic acid (µg/mL)						
Day 17	1 ± 1	1,604 ± 248.7	3,356 ± 590	7,321 ± 1,604	12,878 ± 3,270	14,645 ± 4,853
Day 45	25 ± 32	1,982 ± 555[b]	5,660 ± 1,696	9,471 ± 1,783	22,995 ± 5,961	38,204 ± 9,850[d]
Week 14	(6) ± (2)	3,675 ± 1,169	8,630 ± 2,202	12,232 ± 2,218	22,340 ± 8,016[c]	32,001 ± 1,349
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)						
Day 17	5.1	93.2 ± 25.0	175.3 ± 63.6	315.3 ± 90.1	392.5 ± 71.2	340.4 ± 106.4
Day 45	—[e]	94.0 ± 32.7[b]	349.3 ± 113.2	380.7 ± 108.2	665.8 ± 227.2	752.8 ± 300.1
Week 14	(3.1) ± (1.3)	284.0 ± 106.6	390.7 ± 133.9	437.4 ± 122.9	472.4 ± 203.9[c]	548.2 ± 68.0[d]
Female						
n						
Day 17	10	10	10	10	10	8
Day 45	9	10	10	9	10	8
Week 14	10	9	10	10	10	7
Creatinine (mg/dL)						
Day 17	83.60 ± 4.06	87.95 ± 3.41	80.48 ± 4.86	93.35 ± 7.24	85.64 ± 6.26	69.15 ± 3.64
Day 45	109.82 ± 9.18	117.97 ± 3.45	112.08 ± 11.42	115.98 ± 8.75	100.54 ± 5.82	74.34 ± 6.13**
Week 14	124.43 ± 12.58	117.49 ± 5.19	106.07 ± 10.25	104.08 ± 6.82	76.27 ± 4.67**	53.11 ± 2.52**
Volume (mL/24 hours)						
Day 17	6.2 ± 0.6	5.5 ± 0.4	5.4 ± 0.5	5.0 ± 0.8	5.2 ± 0.5	6.0 ± 0.3
Day 45	5.0 ± 0.5	4.6 ± 0.2	5.5 ± 0.7	4.4 ± 0.4[b]	5.0 ± 0.4	7.0 ± 0.5
Week 14	5.8 ± 0.7	5.5 ± 0.2	6.3 ± 0.6	6.2 ± 0.8	7.6 ± 0.9	11.2 ± 0.9**
5-(Hydroxymethyl)-2-furoic acid (µg/mL)						
Day 17	12 ± 22	385 ± 94	737 ± 285	2,905 ± 998	5,397 ± 1,407	9,839 ± 5,056[c]
Day 45	12 ± 22[b]	1,050 ± 319	2,341 ± 1,081	5,707 ± 1,196	10,949 ± 3,668	19,774 ± 5,137
Week 14	(8) ± (4)	3,127 ± 546	6,302 ± 2,385	11,666 ± 2,270	19,667 ± 4,910	25,879 ± 3,004
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)						
Day 17	5.2	62.4 ± 17.7	117.3 ± 51.6	369.0 ± 142.1	598.3 ± 536.0	493.8 ± 122.6[c]
Day 45	5.1 ± 1.3[b]	109.9 ± 29.5	270.1 ± 114.2	465.6 ± 99.2	620.7 ± 167.5	807.5 ± 129.5
Week 14	11.0 ± 4.6	372.2 ± 69.4	441.7 ± 150.0	509.8 ± 156.7	686.6 ± 314.9	746 ± 179

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test (creatinine and volume)
** $P \leq 0.01$
[a] Data are given as mean ± standard error (creatinine and volume) or mean ± standard deviation (metabolites). Statistical tests were performed on unrounded data. Values in parentheses are below the experimental limit of quantitation although greater than the level of detection (LOD). 5-(Hydroxymethyl)-2-furoic acid LOD = 0.3 µg/mL; 5-(hydroxymethyl)-2-furoylglycine LOD = 0.4 µg/mL.
[b] n=10
[c] n=9
[d] n=7
[e] Below LOD

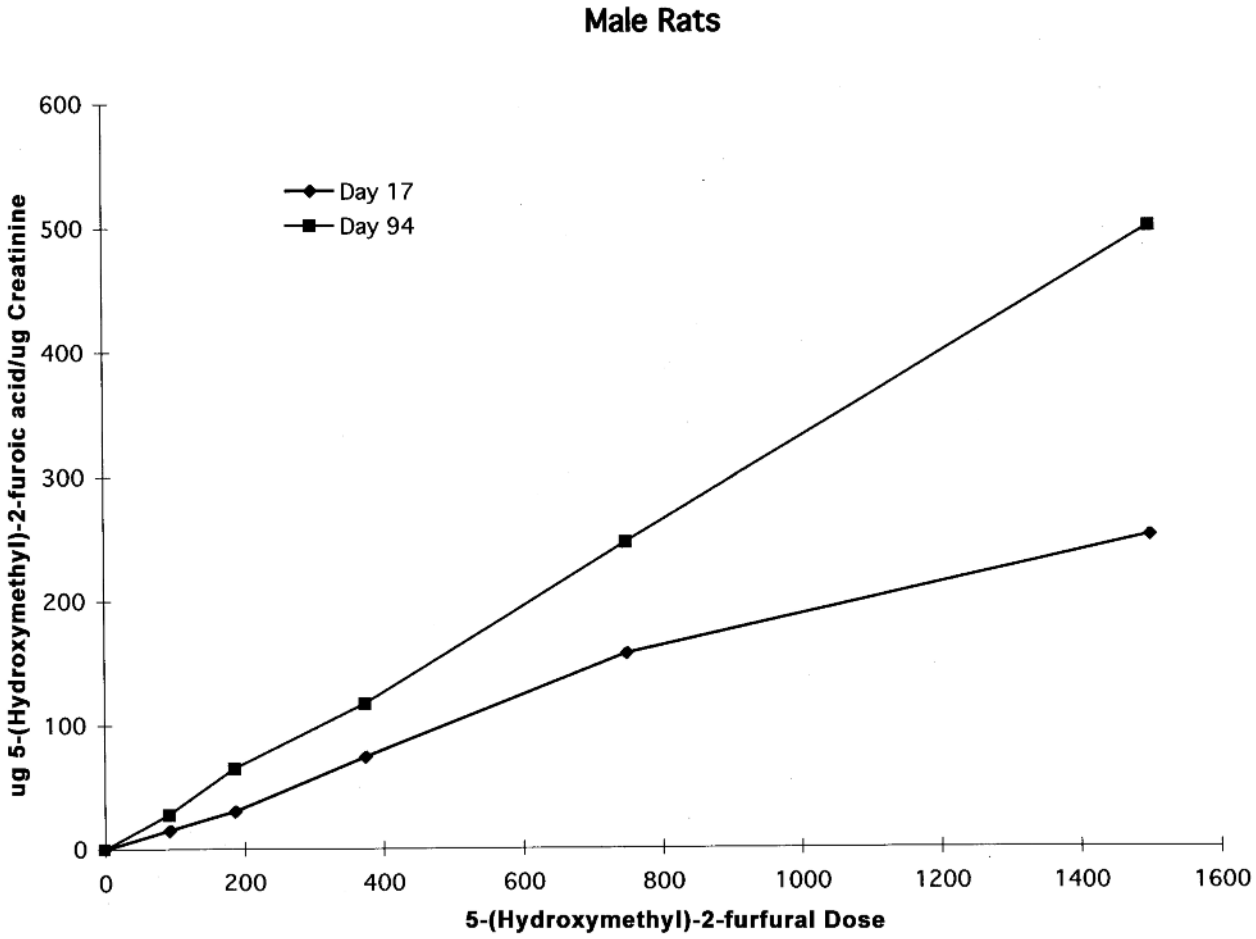

FIGURE G1
Ratio of 5-(Hydroxymethyl)-2-furoic Acid to Creatinine in the Urine of Male Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

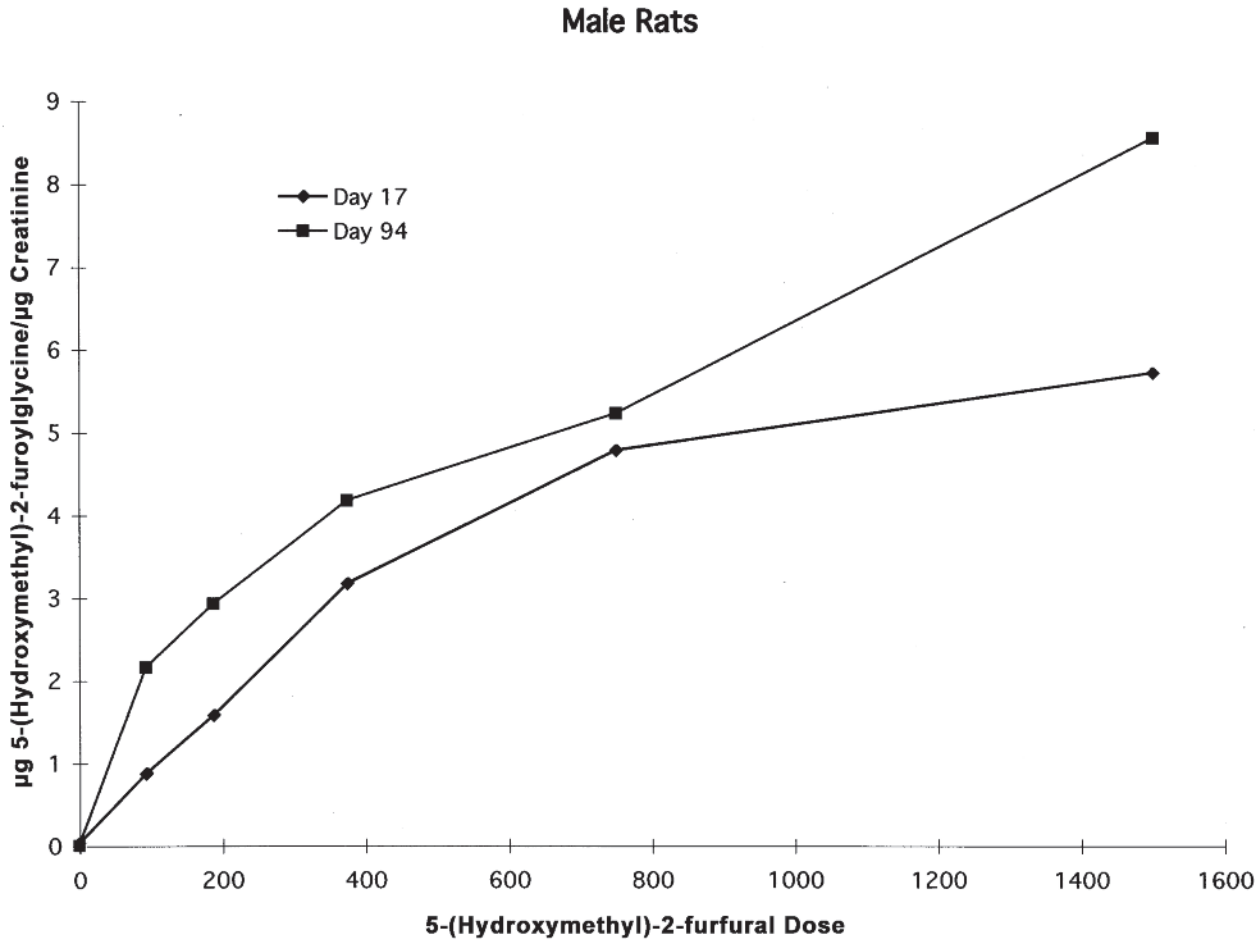

FIGURE G2
Ratio of 5-(Hydroxymethyl)-2-furoylglycine to Creatinine in the Urine of Male Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

TABLE G3
Urinalysis and Metabolite Data for Mice in the 3-Week Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
n	5	5	5	5	5	2
Male						
Creatinine (mg/dL)	56.70 ± 3.86	58.68 ± 3.56	57.12 ± 6.33	49.90 ± 2.54	51.38 ± 5.03	47.60 ± 5.10
Volume (mL/24 hours)	0.7 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
5-(Hydroxymethyl)-2-furoic acid (µg/mL)	27 ± 32	1,312 ± 485	3,337 ± 578	6,249 ± 614	11,961 ± 1,569	20,987 ± 3,916
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)	4.2 ± 2.3	241.4 ± 77.3	496.5 ± 94.5	596.2 ± 81.7	1403 ± 219	2937 ± 586
Female						
Creatinine (mg/dL)	49.60 ± 2.61	51.46 ± 1.01	55.84 ± 1.73	46.24 ± 5.50	50.42 ± 5.03	46.40 ± 16.00
Volume (mL/24 hours)	0.8 ± 0.1	1.0 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.4
5-(Hydroxymethyl)-2-furoic acid (µg/mL)	51 ± 25	1,755 ± 257	3,449 ± 1,001	7,359 ± 2,565	15,278 ± 3,017	15,043 ± 2,541
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)	4.5 ± 3.1	71.9 ± 8.4	117.2 ± 33.2	193.4 ± 75.0	458.5 ± 145.3	511.8 ± 209.7

[a] Data are given as mean ± standard error (creatinine and volume) or mean ± standard deviation (metabolites). Statistical tests were performed on unrounded data. Data (creatinine and volume) are not significantly different from the vehicle control group by Dunn's test.

TABLE G4
Urinalysis and Metabolite Data for Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	47 mg/kg	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg
Male						
n						
Day 17	7	9	8	8	10	8
Day 45	7	10	9	10	9	10
Week 14	10	10	10	10	9	10
Creatinine (mg/dL)						
Day 17	62.66 ± 5.62	45.74 ± 2.23	57.69 ± 3.09	57.35 ± 4.57	45.68 ± 4.32	54.39 ± 5.77
Day 45	49.96 ± 7.25	47.17 ± 4.09	39.00 ± 4.51	43.18 ± 4.72	38.47 ± 2.71	45.53 ± 4.77
Week 14	33.59 ± 2.90	40.52 ± 2.91	35.76 ± 4.10	35.29 ± 3.40	34.17 ± 3.46	39.32 ± 2.73
Volume (mL/24 hours)						
Day 17	0.2 ± 0.0	0.8 ± 0.1**	0.6 ± 0.2	0.4 ± 0.1	1.1 ± 0.2**	0.7 ± 0.2[b]
Day 45	0.7 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	1.4 ± 0.2*	0.7 ± 0.1
Week 14	0.8 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
5-(Hydroxymethyl)-2-furoic acid (µg/mL)						
Day 17	8 ± 11	154 ± 176[c]	162 ± 279	266 ± 242	776 ± 746	5,282 ± 7,173
Day 45	11 ± 6[d]	861 ± 480	907 ± 591	2,162 ± 1,245	4,194 ± 2,631[d]	7,574 ± 4,556[e]
Week 14	(18) ± (15)[d]	824 ± 403[b]	2,025 ± 1,221[b]	4,169 ± 1,927	6,277 ± 2,834	12,389 ± 4,608
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)						
Day 17	16.0 ± 11.2	37.1 ± 38.6[c]	46.0 ± 59.7	80.8 ± 61.3	192.5 ± 173.9	1,185 ± 1,610
Day 45	—[d,f]	216.0 ± 131.1	292.9 ± 174.2	596.9 ± 343.4	827.7 ± 393.1[d]	1,875 ± 985.5[e]
Week 14	(12.2) ± (15.7)[d]	374.6 ± 251.4[b]	750.0 ± 501.5[b]	1,111.6 ± 568.0	1,361.0 ± 457.4	2,171 ± 796

TABLE G4
Urinalysis and Metabolite Data for Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	47 mg/kg	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg
Female						
n						
Day 17	10	10	10	10	10	9
Day 45	10	9	10	10	10	9
Week 14	8	8	8	9	8	10
Creatinine (mg/dL)						
Day 17	53.18 ± 2.30	49.43 ± 3.26	56.86 ± 1.93	50.92 ± 4.27	49.67 ± 1.80	46.01 ± 2.14
Day 45	59.55 ± 5.20	60.10 ± 4.98	54.42 ± 2.01	53.89 ± 2.80	56.26 ± 5.36	54.78 ± 3.05
Week 14	44.75 ± 2.56	55.06 ± 3.93	51.60 ± 4.68	57.13 ± 3.21	47.55 ± 3.35	45.49 ± 4.84
Volume (mL/24 hours)						
Day 17	0.9 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
Day 45	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Week 14	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	1.3 ± 0.1
5-(Hydroxymethyl)-2-furoic acid (µg/mL)						
Day 17	13 ± 8	162 ± 149	498 ± 615	1,136 ± 694	3,649 ± 3,872	7,181 ± 2,029[c]
Day 45	38 ± 0	1,103 ± 706[c]	1,508 ± 509	2,906 ± 1,096	6,309 ± 1,939	10,258 ± 1,606
Week 14	(6) ± (9)	627 ± 357[e]	1,341 ± 552	2,649 ± 1,369[d]	5,686 ± 3,207	11,869 ± 4,950
5-(Hydroxymethyl)-2-furoylglycine (µg/mL)						
Day 17	10.3 ± 2.9	15.5 ± 9.0	40.4 ± 37.8	65.2 ± 40.0	106.9 ± 31.9	299.8 ± 78.9[c]
Day 45	(14.2)[g]	56.0 ± 29.4[c]	93.1 ± 26.0	151.7 ± 69.6	246.9 ± 108.9	449.8 ± 69.3
Week 14	(7.2) ± (3.2)	41.3 ± 46.7[e]	87.5 ± 38.9	130.8 ± 66.1[d]	289.2 ± 170.4	571.2 ± 260.1

* Significantly different (P#0.05) from the vehicle control group by Dunn's test (volume)
** P#0.01
[a] Data are given as mean ± standard error (creatinine and volume) or mean ± standard deviation (metabolites). Statistical tests were performed on unrounded data. Values in parentheses are below the experimental limit of quantitation although greater than the level of detection (LOD).
[b] 5-(Hydroxymethyl)-2-furoic acid LOD = 0.3 µg/mL; 5-(hydroxymethyl)-2-furoylglycine LOD = 0.4 µg/mL.
[c] n=9
[d] n=10
[e] n=8
[f] n=7
[g] Below LOD
n=1

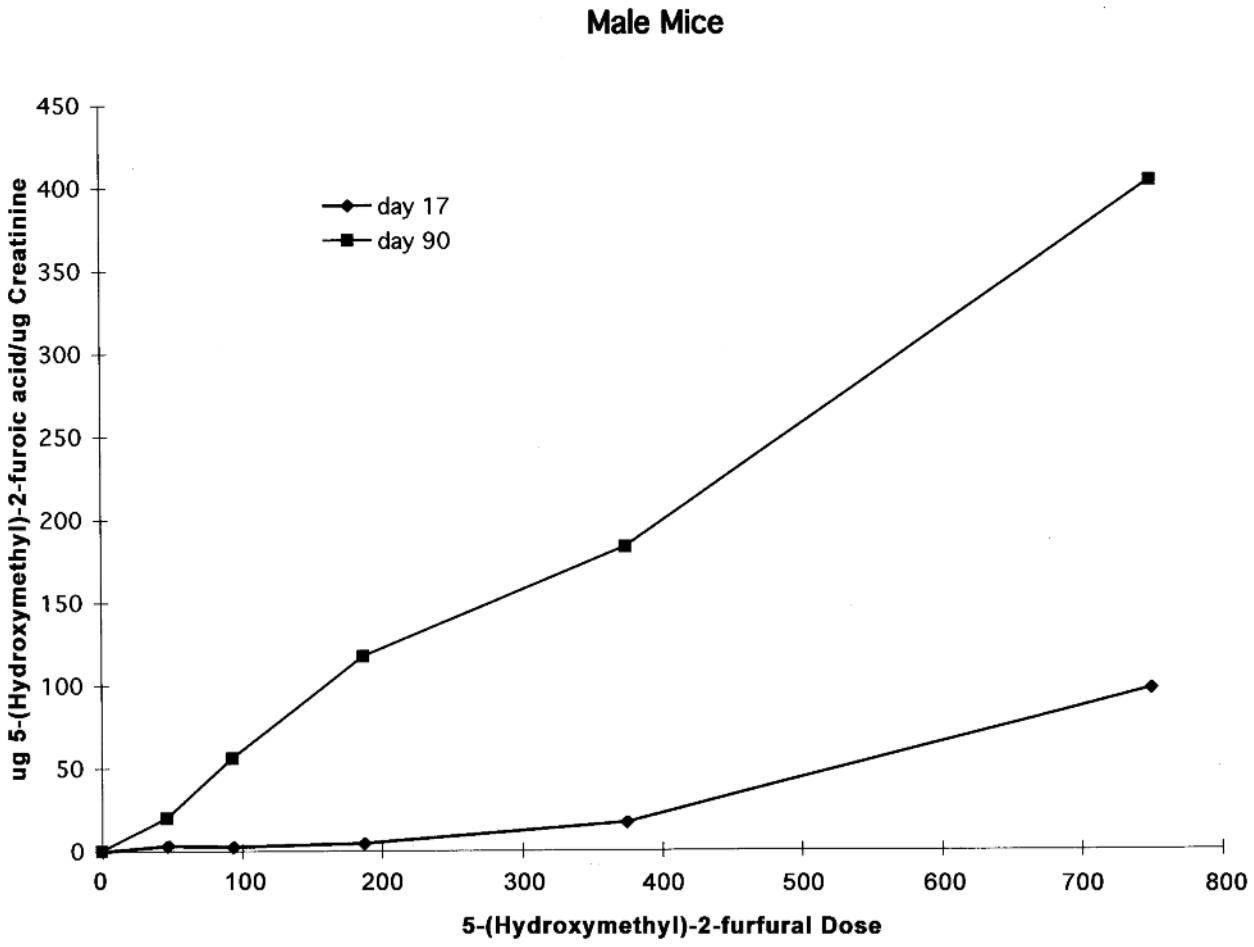

FIGURE G3
Ratio of 5-(Hydroxymethyl)-2-furoic Acid to Creatinine in the Urine of Male Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

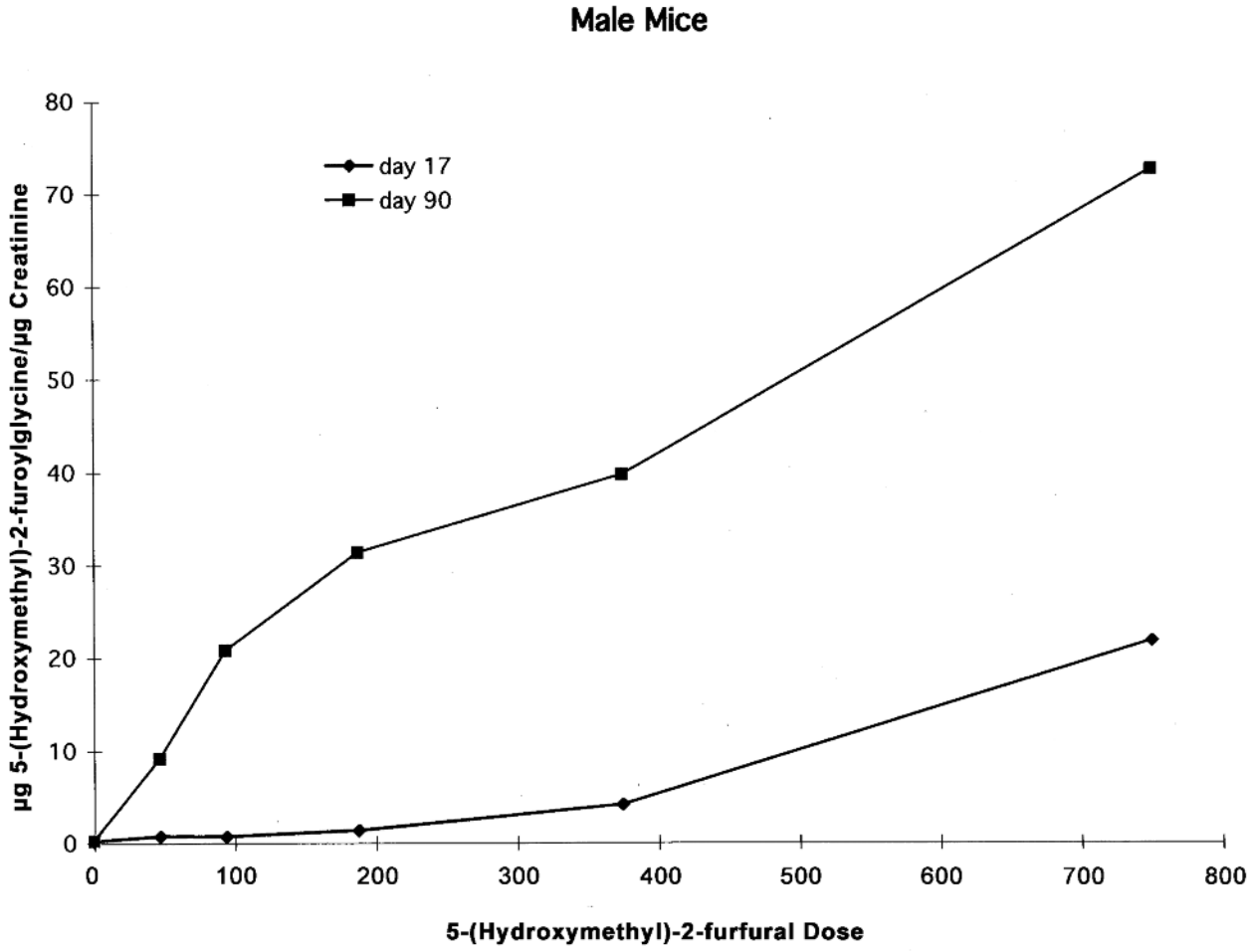

FIGURE G4
Ratio of 5-(Hydroxymethyl)-2-furoylglycine to Creatinine in the Urine of Male Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural

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

TABLE H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Week Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male						
n	5	5	5	5	5	4
Necropsy body wt	199 ± 3	207 ± 3	198 ± 4	200 ± 6	207 ± 3	181 ± 8*
Heart						
Absolute	0.765 ± 0.025	0.786 ± 0.023	0.736 ± 0.016	0.778 ± 0.017	0.764 ± 0.017	0.677 ± 0.048
Relative	3.840 ± 0.136	3.806 ± 0.070	3.724 ± 0.089	3.901 ± 0.162	3.700 ± 0.076	3.742 ± 0.112
R. Kidney						
Absolute	0.821 ± 0.022	0.869 ± 0.010	0.847 ± 0.019	0.876 ± 0.027	0.861 ± 0.014	0.797 ± 0.031
Relative	4.122 ± 0.122	4.210 ± 0.046	4.285 ± 0.071	4.375 ± 0.083	4.171 ± 0.087	4.423 ± 0.110
Liver						
Absolute	10.528 ± 0.382	9.813 ± 0.177	9.636 ± 0.373	9.811 ± 0.491	10.627 ± 0.471	8.911 ± 0.462*
Relative	52.855 ± 2.082	47.530 ± 0.777	48.761 ± 1.864	48.975 ± 1.854	51.516 ± 2.529	49.466 ± 2.228
Lung						
Absolute	1.335 ± 0.041	1.460 ± 0.150	1.220 ± 0.037	1.306 ± 0.080	1.719 ± 0.200	1.482 ± 0.160
Relative	6.693 ± 0.131	7.041 ± 0.642	6.177 ± 0.223	6.587 ± 0.612	8.284 ± 0.877	8.246 ± 0.940
R. Testis						
Absolute	1.290 ± 0.026	1.282 ± 0.013	1.236 ± 0.013	1.257 ± 0.030	1.240 ± 0.016	1.215 ± 0.035
Relative	6.471 ± 0.085	6.216 ± 0.123	6.257 ± 0.085	6.287 ± 0.145	6.006 ± 0.056*	6.744 ± 0.117
Thymus						
Absolute	0.413 ± 0.009	0.445 ± 0.015	0.384 ± 0.012	0.360 ± 0.009	0.444 ± 0.018	0.368 ± 0.039
Relative	2.070 ± 0.038	2.162 ± 0.101	1.946 ± 0.069	1.804 ± 0.053	2.152 ± 0.096	2.031 ± 0.165
Female						
n	5	5	5	5	5	5
Necropsy body wt	139 ± 2	140 ± 2	140 ± 2	137 ± 4	139 ± 3	134 ± 3
Heart						
Absolute	0.570 ± 0.015	0.557 ± 0.014	0.569 ± 0.018	0.559 ± 0.016	0.565 ± 0.010	0.530 ± 0.017
Relative	4.106 ± 0.130	3.986 ± 0.104	4.074 ± 0.093	4.080 ± 0.151	4.078 ± 0.087	3.947 ± 0.145
R. Kidney						
Absolute	0.615 ± 0.024	0.624 ± 0.015	0.613 ± 0.028	0.622 ± 0.016	0.639 ± 0.014	0.623 ± 0.016
Relative	4.419 ± 0.132	4.464 ± 0.083	4.389 ± 0.169	4.531 ± 0.078	4.612 ± 0.116	4.633 ± 0.035
Liver						
Absolute	6.775 ± 0.225	6.760 ± 0.253	6.137 ± 0.199	6.503 ± 0.320	6.259 ± 0.194	6.310 ± 0.239
Relative	48.745 ± 1.539	48.313 ± 1.527	44.037 ± 1.626	47.240 ± 1.465	45.163 ± 1.416	46.922 ± 1.169
Lung						
Absolute	1.012 ± 0.055[b]	1.021 ± 0.075	1.226 ± 0.186	1.115 ± 0.068	1.230 ± 0.065	1.007 ± 0.061
Relative	7.336 ± 0.409[b]	7.289 ± 0.489	8.740 ± 1.220	8.144 ± 0.593	8.902 ± 0.567	7.480 ± 0.335
Thymus						
Absolute	0.349 ± 0.009	0.347 ± 0.019	0.333 ± 0.017	0.342 ± 0.017	0.354 ± 0.015	0.308 ± 0.018
Relative	2.514 ± 0.068	2.489 ± 0.158	2.388 ± 0.105	2.480 ± 0.064	2.552 ± 0.093	2.287 ± 0.121

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

TABLE H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
Male						
n	10	10	10	10	10	9
Necropsy body wt	335 ± 7	337 ± 7	329 ± 8	339 ± 4	318 ± 6	311 ± 7*
Heart						
Absolute	1.030 ± 0.029	1.020 ± 0.021	1.019 ± 0.026	1.021 ± 0.015	0.972 ± 0.024	0.968 ± 0.031
Relative	3.078 ± 0.060	3.031 ± 0.075	3.099 ± 0.050	3.010 ± 0.027	3.061 ± 0.051	3.114 ± 0.053
R. Kidney						
Absolute	1.044 ± 0.025	1.059 ± 0.034	1.059 ± 0.029	1.064 ± 0.017	1.051 ± 0.026	1.135 ± 0.021
Relative	3.117 ± 0.028	3.133 ± 0.062	3.218 ± 0.042	3.136 ± 0.024	3.309 ± 0.058**	3.661 ± 0.053**
Liver						
Absolute	11.81 ± 0.43	12.67 ± 0.40	11.95 ± 0.47	12.47 ± 0.31	11.77 ± 0.38	12.10 ± 0.48
Relative	35.291 ± 1.075	37.499 ± 0.720	36.233 ± 0.713	36.775 ± 0.817	37.002 ± 0.809	38.922 ± 1.039*
Lung						
Absolute	1.869 ± 0.077	1.821 ± 0.115	1.750 ± 0.090	1.739 ± 0.062	1.719 ± 0.077	1.763 ± 0.070
Relative	5.592 ± 0.231	5.381 ± 0.283	5.333 ± 0.270	5.123 ± 0.147	5.415 ± 0.231	5.676 ± 0.174
R. Testis						
Absolute	1.476 ± 0.023	1.496 ± 0.021	1.497 ± 0.026	1.519 ± 0.025	1.464 ± 0.032	1.516 ± 0.022
Relative	4.416 ± 0.059	4.439 ± 0.035	4.561 ± 0.072	4.482 ± 0.063	4.608 ± 0.041*	4.895 ± 0.099**
Thymus						
Absolute	0.350 ± 0.012	0.334 ± 0.012	0.334 ± 0.015	0.334 ± 0.018	0.305 ± 0.010	0.316 ± 0.007
Relative	1.045 ± 0.031	0.990 ± 0.034	1.012 ± 0.026	0.986 ± 0.052	0.963 ± 0.035	1.020 ± 0.023
Female						
n	10	10	10	10	10	7
Necropsy body wt	196 ± 4	193 ± 3	193 ± 4	197 ± 3	195 ± 2	190 ± 2
Heart						
Absolute	0.678 ± 0.016	0.660 ± 0.008	0.657 ± 0.012	0.672 ± 0.011	0.676 ± 0.018	0.654 ± 0.007
Relative	3.461 ± 0.098	3.430 ± 0.044	3.402 ± 0.039	3.419 ± 0.060	3.471 ± 0.084	3.446 ± 0.042
R. Kidney						
Absolute	0.702 ± 0.019	0.685 ± 0.019	0.696 ± 0.016	0.714 ± 0.018	0.710 ± 0.019	0.743 ± 0.012
Relative	3.571 ± 0.043	3.552 ± 0.078	3.601 ± 0.060	3.626 ± 0.051	3.641 ± 0.076	3.916 ± 0.040**
Liver						
Absolute	6.772 ± 0.186	6.574 ± 0.205	6.831 ± 0.114	6.990 ± 0.139	6.877 ± 0.156	6.670 ± 0.157
Relative	34.472 ± 0.617	34.084 ± 0.718	35.415 ± 0.635	35.527 ± 0.364	35.314 ± 0.811	35.141 ± 0.614
Lung						
Absolute	1.185 ± 0.027	1.108 ± 0.037	1.163 ± 0.025	1.354 ± 0.097	1.212 ± 0.062	1.190 ± 0.061
Relative	6.049 ± 0.167	5.759 ± 0.206	6.024 ± 0.117	6.891 ± 0.506	6.201 ± 0.268	6.278 ± 0.343
Thymus						
Absolute	0.267 ± 0.011	0.269 ± 0.010	0.247 ± 0.011	0.265 ± 0.005	0.284 ± 0.009	0.244 ± 0.008
Relative	1.367 ± 0.067	1.397 ± 0.047	1.274 ± 0.039	1.351 ± 0.033	1.459 ± 0.050	1.289 ± 0.043

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

TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Week Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg	1,500 mg/kg
n	5	5	5	5	5	2
Male						
Necropsy body wt	23.3 ± 0.6	23.3 ± 0.3	22.3 ± 0.5	22.7 ± 0.3	22.2 ± 0.3	21.2 ± 0.8*
Heart						
Absolute	0.131 ± 0.005	0.153 ± 0.007*	0.121 ± 0.004	0.129 ± 0.003	0.144 ± 0.008	0.129 ± 0.008
Relative	5.619 ± 0.138	6.557 ± 0.255	5.397 ± 0.116	5.663 ± 0.123	6.520 ± 0.447	6.094 ± 0.162
R. Kidney						
Absolute	0.233 ± 0.008	0.222 ± 0.005	0.216 ± 0.006	0.225 ± 0.004	0.216 ± 0.006	0.199 ± 0.003*
Relative	10.013 ± 0.271	9.540 ± 0.144	9.665 ± 0.347	9.898 ± 0.211	9.698 ± 0.191	9.416 ± 0.192
Liver						
Absolute	1.215 ± 0.052	1.260 ± 0.029	1.151 ± 0.055	1.259 ± 0.039	1.183 ± 0.033	1.111 ± 0.054
Relative	52.100 ± 1.039	54.158 ± 1.135	51.448 ± 1.938	55.498 ± 2.070	53.237 ± 1.039	52.482 ± 0.668
Lung						
Absolute	0.188 ± 0.005	0.208 ± 0.017	0.177 ± 0.007	0.205 ± 0.018	0.217 ± 0.007	0.190 ± 0.026
Relative	8.102 ± 0.397	8.924 ± 0.643	7.946 ± 0.314	9.018 ± 0.764	9.771 ± 0.444	8.928 ± 0.889
R. Testis						
Absolute	0.099 ± 0.003	0.093 ± 0.004	0.091 ± 0.003	0.087 ± 0.006	0.089 ± 0.003	0.097 ± 0.004
Relative	4.270 ± 0.096	3.991 ± 0.178	4.069 ± 0.129	3.832 ± 0.292	4.006 ± 0.146	4.563 ± 0.004
Thymus						
Absolute	0.033 ± 0.002	0.038 ± 0.004	0.039 ± 0.003	0.040 ± 0.001	0.038 ± 0.001	0.038 ± 0.003
Relative	1.441 ± 0.114	1.627 ± 0.155	1.731 ± 0.118	1.778 ± 0.017	1.693 ± 0.063	1.804 ± 0.206
Female						
Necropsy body wt	19.4 ± 0.5	19.9 ± 0.3	19.1 ± 0.3	19.7 ± 0.6	19.6 ± 0.2	19.4 ± 0.8
Heart						
Absolute	0.114 ± 0.006	0.135 ± 0.009	0.136 ± 0.012	0.120 ± 0.006	0.129 ± 0.010	0.167 ± 0.009*
Relative	5.844 ± 0.208	6.804 ± 0.467	7.076 ± 0.590	6.111 ± 0.410	6.585 ± 0.523	8.601 ± 0.106*
R. Kidney						
Absolute	0.153 ± 0.004	0.161 ± 0.005	0.153 ± 0.005	0.164 ± 0.008	0.158 ± 0.004	0.157 ± 0.007
Relative	7.874 ± 0.193	8.074 ± 0.196	7.979 ± 0.189	8.319 ± 0.292	8.082 ± 0.232	8.140 ± 0.677
Liver						
Absolute	1.076 ± 0.047	1.077 ± 0.039	1.048 ± 0.055	1.031 ± 0.043	1.053 ± 0.023	1.071 ± 0.017
Relative	55.361 ± 1.559	54.141 ± 1.313	54.645 ± 2.198	52.270 ± 1.290	53.783 ± 1.123	55.439 ± 3.002
Lung						
Absolute	0.196 ± 0.021	0.199 ± 0.015	0.202 ± 0.014	0.196 ± 0.004	0.211 ± 0.023	0.243 ± 0.027
Relative	10.072 ± 0.968	10.041 ± 0.779	10.557 ± 0.807	9.982 ± 0.230	10.816 ± 1.237	12.523 ± 0.910
Thymus						
Absolute	0.058 ± 0.003	0.051 ± 0.003	0.050 ± 0.003	0.061 ± 0.004	0.050 ± 0.003	0.048 ± 0.006
Relative	2.979 ± 0.157	2.547 ± 0.158	2.633 ± 0.128	3.148 ± 0.290	2.533 ± 0.149	2.470 ± 0.380

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

TABLE H4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	47 mg/kg	94 mg/kg	188 mg/kg	375 mg/kg	750 mg/kg
Male						
n	10	10	10	10	10	9
Necropsy body wt	36.0 ± 0.9	36.1 ± 1.0	35.4 ± 1.0	33.5 ± 0.7	34.9 ± 0.5	31.0 ± 0.4**
Heart						
Absolute	0.173 ± 0.005	0.181 ± 0.008	0.174 ± 0.010	0.160 ± 0.004	0.160 ± 0.004	0.149 ± 0.003*
Relative	4.815 ± 0.117	5.057 ± 0.271	4.913 ± 0.235	4.789 ± 0.120	4.590 ± 0.127	4.827 ± 0.114
R. Kidney						
Absolute	0.305 ± 0.009	0.293 ± 0.009	0.290 ± 0.008	0.272 ± 0.006**	0.268 ± 0.008**	0.250 ± 0.005**
Relative	8.487 ± 0.171	8.131 ± 0.170	8.208 ± 0.156	8.152 ± 0.190	7.673 ± 0.151**	8.093 ± 0.186
Liver						
Absolute	1.551 ± 0.055	1.620 ± 0.043	1.517 ± 0.054	1.411 ± 0.036	1.489 ± 0.037	1.393 ± 0.033*
Relative	43.004 ± 0.774	44.992 ± 1.227	42.915 ± 0.879	42.262 ± 1.057	42.645 ± 0.784	44.981 ± 1.077
Lung						
Absolute	0.269 ± 0.010	0.280 ± 0.009	0.297 ± 0.019	0.228 ± 0.016	0.246 ± 0.011	0.273 ± 0.014
Relative	7.481 ± 0.202	7.837 ± 0.378	8.418 ± 0.536	6.844 ± 0.499	7.070 ± 0.338	8.813 ± 0.433
R. Testis						
Absolute	0.122 ± 0.004	0.122 ± 0.003	0.120 ± 0.003	0.117 ± 0.002	0.119 ± 0.004	0.120 ± 0.003
Relative	3.400 ± 0.133	3.388 ± 0.079	3.409 ± 0.102	3.490 ± 0.063	3.406 ± 0.109	3.875 ± 0.114**
Thymus						
Absolute	0.046 ± 0.002	0.044 ± 0.002	0.049 ± 0.004	0.037 ± 0.002	0.045 ± 0.003	0.040 ± 0.002
Relative	1.278 ± 0.042	1.211 ± 0.057	1.362 ± 0.095	1.119 ± 0.055	1.291 ± 0.072	1.301 ± 0.063
Female						
n	10	10	10	10	9	10
Necropsy body wt	28.3 ± 0.7	26.5 ± 0.5	28.3 ± 0.6	28.9 ± 0.6	27.8 ± 0.4	26.9 ± 0.7
Heart						
Absolute	0.140 ± 0.004	0.148 ± 0.007	0.134 ± 0.003	0.142 ± 0.004	0.146 ± 0.006	0.134 ± 0.006
Relative	4.961 ± 0.215	5.585 ± 0.243	4.739 ± 0.090	4.948 ± 0.215	5.261 ± 0.193	5.032 ± 0.294
R. Kidney						
Absolute	0.187 ± 0.003	0.186 ± 0.004	0.185 ± 0.004	0.184 ± 0.005	0.183 ± 0.004	0.180 ± 0.005
Relative	6.630 ± 0.120	7.043 ± 0.142	6.557 ± 0.114	6.362 ± 0.193	6.583 ± 0.099	6.725 ± 0.108
Liver						
Absolute	1.234 ± 0.034	1.225 ± 0.057	1.260 ± 0.031	1.305 ± 0.031	1.273 ± 0.030	1.180 ± 0.040
Relative	43.602 ± 0.727	46.222 ± 1.877	44.563 ± 0.796	45.231 ± 1.115	45.863 ± 1.092	43.987 ± 1.151
Lung						
Absolute	0.264 ± 0.014	0.267 ± 0.014	0.244 ± 0.011	0.264 ± 0.015	0.315 ± 0.012	0.268 ± 0.018
Relative	9.341 ± 0.494	10.073 ± 0.464	8.678 ± 0.463	9.206 ± 0.667	11.364 ± 0.510*	9.979 ± 0.580
Thymus						
Absolute	0.049 ± 0.001	0.051 ± 0.004	0.054 ± 0.002	0.053 ± 0.003	0.050 ± 0.002	0.048 ± 0.003
Relative	1.736 ± 0.057	1.896 ± 0.121	1.908 ± 0.053	1.854 ± 0.123	1.797 ± 0.058	1.793 ± 0.099

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

APPENDIX I
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE I1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	375 mg/kg	750 mg/kg	1,500 mg/kg
n	10	10	10	9
Weights (g)				
Necropsy body wt	335 ± 7	339 ± 4	318 ± 6	311 ± 7*
L. Cauda epididymis	0.1433 ± 0.0051	0.1441 ± 0.0046	0.1429 ± 0.0029	0.1410 ± 0.0047
L. Epididymis	0.4237 ± 0.0084	0.4262 ± 0.0087	0.4094 ± 0.0066	0.4200 ± 0.0059
L. Testis	1.5380 ± 0.0320	1.5683 ± 0.0173	1.4938 ± 0.0250	1.5066 ± 0.0208
Spermatid measurement				
Spermatid heads (10^6/g testis)	135.5 ± 10.6	144.4 ± 10.9	139.1 ± 7.6	137.5 ± 7.2
Spermatid heads (10^6/testis)	186.4 ± 15.4	208.3 ± 17.4	189.1 ± 11.6	188.6 ± 11.1
Epididymal spermatozoal measurements				
Sperm motility (%)	73.96 ± 0.66	74.81 ± 0.75	75.08 ± 0.71	74.66 ± 0.73
Sperm (10^6/g cauda epididymis)	541.1 ± 49.8	498.8 ± 25.0	614.8 ± 59.5	548.7 ± 27.2
Sperm (10^6/cauda epididymis)	77.52 ± 7.53	71.39 ± 3.21	87.65 ± 8.04	77.68 ± 5.27

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (tissue weights and spermatid and epididymal spermatozoal measurements).

TABLE I2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	375 mg/kg	750 mg/kg	1,500 mg/kg
Number weighed at necropsy	10	10	10	8
Necropsy body wt (g)	196 ± 4	197 ± 3	195 ± 2	187 ± 3
Proportion of regular cycling females[b]	8/10	8/10	6/10	2/8*
Estrous cycle length (days)	5.00 ± 0.16[c]	5.00 ± 0.00[c]	6.06 ± 0.67[d]	6.00 ± 1.00[e]
Estrous stages[f] (% of cycle)				
Diestrus	34.2	55.8	56.7	75.5
Proestrus	9.2	11.7	5.8	4.3
Estrus	23.3	20.8	25.0	13.8
Metestrus	11.7	9.2	10.8	6.4
Uncertain diagnoses	21.7	2.5	1.7	0.0

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (body weights and estrous cycle lengths).
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[e] Estrous cycle was longer than 12 days or unclear in 4 of 8 animals.
[f] Evidence shows that females given 1,500 mg/kg differ significantly (Wilks's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages. 1,500 mg/kg females spent significantly more time in diestrus and less time in proestrus, estrus, and metestrus than vehicle control females.

TABLE I3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
n	10	10	10	9
Weights (g)				
Necropsy body wt	36.0 ± 0.9	33.5 ± 0.7	34.9 ± 0.5	31.0 ± 0.4**
L. Cauda epididymis	0.0137 ± 0.0008	0.0135 ± 0.0004	0.0138 ± 0.0003	0.0139 ± 0.0005
L. Epididymis	0.0428 ± 0.0012	0.0416 ± 0.0008	0.0429 ± 0.0005	0.0440 ± 0.0011
L. Testis	0.1130 ± 0.0048	0.1108 ± 0.0043	0.1052 ± 0.0052	0.1119 ± 0.0038
Spermatid measurement				
Spermatid heads (10^6/g testis)	231.0 ± 12.8	232.7 ± 12.3	231.0 ± 6.5	226.0 ± 8.4
Spermatid heads (10^6/testis)	23.93 ± 1.49	23.20 ± 1.36	22.22 ± 0.88	22.73 ± 1.18
Epididymal spermatozoal measurements				
Sperm motility (%)	74.16 ± 0.69	78.87 ± 0.99**	75.67 ± 0.76	72.92 ± 0.63
Sperm (10^6/g cauda epididymis)	996.2 ± 97.3	1,086.7 ± 151.4	868.4 ± 80.7	963.5 ± 51.5
Sperm (10^6/cauda epididymis)	13.87 ± 1.60	14.64 ± 2.05	11.99 ± 1.12	13.40 ± 0.79

** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test (body weights) or Dunn's test (spermatid and epididymal spermatozoal measurements)
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (tissue weights).

TABLE I4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of 5-(Hydroxymethyl)-2-furfural[a]

	Vehicle Control	188 mg/kg	375 mg/kg	750 mg/kg
Number weighed at necropsy	10	10	9	10
Necropsy body wt (g)	28.3 ± 0.7	29.0 ± 0.6	27.8 ± 0.4	26.9 ± 0.7
Proportion of regular cycling females[b]	10/10	9/10	8/9	7/10
Estrous cycle length (days)	4.0 ± 0.1	3.9 ± 0.0[c]	4.1 ± 0.1	4.6 ± 0.6[d]
Estrous stages (% of cycle)				
Diestrus	31.7	33.3	34.3	33.3
Proestrus	5.0	0.0	0.9	1.7
Estrus	35.0	35.8	30.6	29.2
Metestrus	20.8	18.3	21.3	14.2
Uncertain diagnoses	7.5	12.5	13.0	21.7

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (body weights and estrous cycle lengths) or the Fisher exact test (proportion of regular cycling females). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.

APPENDIX J
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF 5-(HYDROXYMETHYL)-2-FURFURAL

5-(Hydroxymethyl)-2-furfural was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), in five lots (11129CQ-MRI796, 14901MQ, 34266-63, 34266-72, and 34266-76). Lot 11129CQ-MRI796 was used in the 3-week studies; lot 14901MQ was used in the 3-month studies; and lots 34266-63, 34266-72, and 34266-76 were used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the 5-(hydroxymethyl)-2-furfural studies are on file at the National Institute of Environmental Health Sciences.

Lots 11129CQ-MRI796, 14901MQ, and 34266-72 of the chemical, a yellow powder or light orange crystalline solid, were identified as 5-(hydroxymethyl)-2-furfural by the analytical chemistry laboratory using infrared spectroscopy and proton nuclear magnetic resonance spectroscopy; in addition, lot 34266-72 was identified as 5-(hydroxymethyl)-2-furfural by ultraviolet/visible spectroscopy, liquid chromatography/mass spectrometry, and melting point determination. The study laboratory identified all lots of the chemical as 5-(hydroxymethyl)-2-furfural by infrared spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1993a,b, 1997; *Sadtler*, 1996) of 5-(hydroxymethyl)-2-furfural. Representative infrared and proton nuclear magnetic resonance spectra are presented in Figures J1 and J2. The molecular ion determined by liquid chromatography/mass spectrometry was consistent with the molecular weight of the test chemical. The melting point was consistent with a literature value (MSDS, 2000).

The moisture content of lots 34266-63, 34266-72, and 34266-76 was determined prior to the 2-year studies by the analytical chemistry laboratory and periodically during the 2-year studies by Galbraith Laboratories, Inc. (Knoxville, TN), and Prevalere Life Sciences, Inc. (Whitesboro, NY). The purities of all lots were determined by the analytical chemistry and study laboratories using high-performance liquid chromatography (HPLC). In addition, the purity of lot 34266-72 was determined by the analytical chemistry laboratory using thin-layer chromatography (TLC). TLC was performed on 20 cm × 20 cm K6F silica gel 60 precoated (250 µm) plates (EM Science, Gibbstown, NJ). The plates were spotted with solutions of the test article and the reference standard (vanillin) and developed in a tank containing chloroform:ethanol (90:10) as the solvent system. The dried plates were examined using UV light (at 254 and 366 nm), visible light, iodine vapor, and a 2,4-dinitrophenyl hydrazine reagent spray.

Prior to the start of the 2-year studies, Karl Fischer titration of the bulk chemical indicated a water content of approximately 0.2%; periodic reanalyses indicated water content ranging from 0.1% to 2.9% during the studies. Analysis by TLC detected a single major spot and no impurities. For lot 11129CQ-MRI796, HPLC by the analytical chemistry laboratory using system A (Table J1) indicated one major peak and one impurity with an area of 0.22% relative to the total peak area. For lot 14901MQ, HPLC by the analytical chemistry laboratory using system B indicated one major peak and one impurity with an area of 0.07% relative to the total peak area. HPLC by the analytical chemistry laboratory using system C indicated one major peak and three impurities with an average combined area of 0.24% for lot 34266-63, 0.37% for lot 34266-72, and 0.32% for lot 34266-76 relative to the total peak area. The overall purity of all lots was determined to be 99% or greater.

Stability studies of lot 34266-76 of the bulk chemical were performed by the analytical chemistry laboratory. HPLC was performed using system D. These studies indicated that 5-(hydroxymethyl)-2-furfural was stable as a bulk chemical for 2 weeks when stored protected from light under a nitrogen headspace at temperatures up to 25° C. To ensure stability, the bulk chemical for the 3-week and 3-month studies was stored at refrigerated temperatures (approximately 5° C), protected from light in the original shipping containers. For the 2-year studies,

the bulk chemical was stored at less than or equal to –20° C in amber glass bottles under an argon headspace. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-week, 3-month, and 2-year studies using system D or a similar system, and no degradation of the bulk chemical was detected. No excessive moisture levels were detected in the bulk chemical during the 2-year studies.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing 5-(hydroxymethyl)-2-furfural with deionized water to give the required concentrations (Table J2). During preparation of the first formulation of the 3-month study, a small amount of insoluble material was noticed. Despite extensive attempts to characterize the material, its identity could not be conclusively determined. A filtration step added to the dose preparation procedure eliminated the problem. The dose formulations were stored at approximately 5° C in amber glass bottles with Teflon®-lined lids for up to 35 days.

Because all dose formulations in the studies were determined to be solutions, no homogeneity studies were required. Stability studies of 9.4 and 300 mg/mL dose formulations were performed by the analytical chemistry laboratory using HPLC by a system similar to system D (Table J1) on a different lot (13217 MG) of 5-(hydroxymethyl)-2-furfural obtained from Aldrich Chemical Company. Stability was confirmed for at least 35 days for dose formulations stored in glass vials at refrigerated (5° C) and ambient (25° C) temperatures and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of 5-(hydroxymethyl)-2-furfural were conducted by the study laboratory using HPLC by system D or a system similar to system D. During the 3-week studies, the dose formulations were analyzed once; all six dose formulations for rats and all five for mice were within 10% of the target concentrations (Table J3). Animal room samples of these dose formulations were also analyzed; all 11 animal room samples were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples were also analyzed (Table J4). Of the dose formulations analyzed and used in the studies, 13 of 15 for rats and all 15 for mice were within 10% of the target concentrations; all were within 12%; nine of 10 animal room samples for rats and all 10 animal room samples for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months (Table J5). Of the dose formulations analyzed and used in the studies, all 27 for rats and all 30 for mice were within 10% of the target concentrations; all nine animal room samples for rats and all 12 animal room samples for mice were within 10% of the target concentrations.

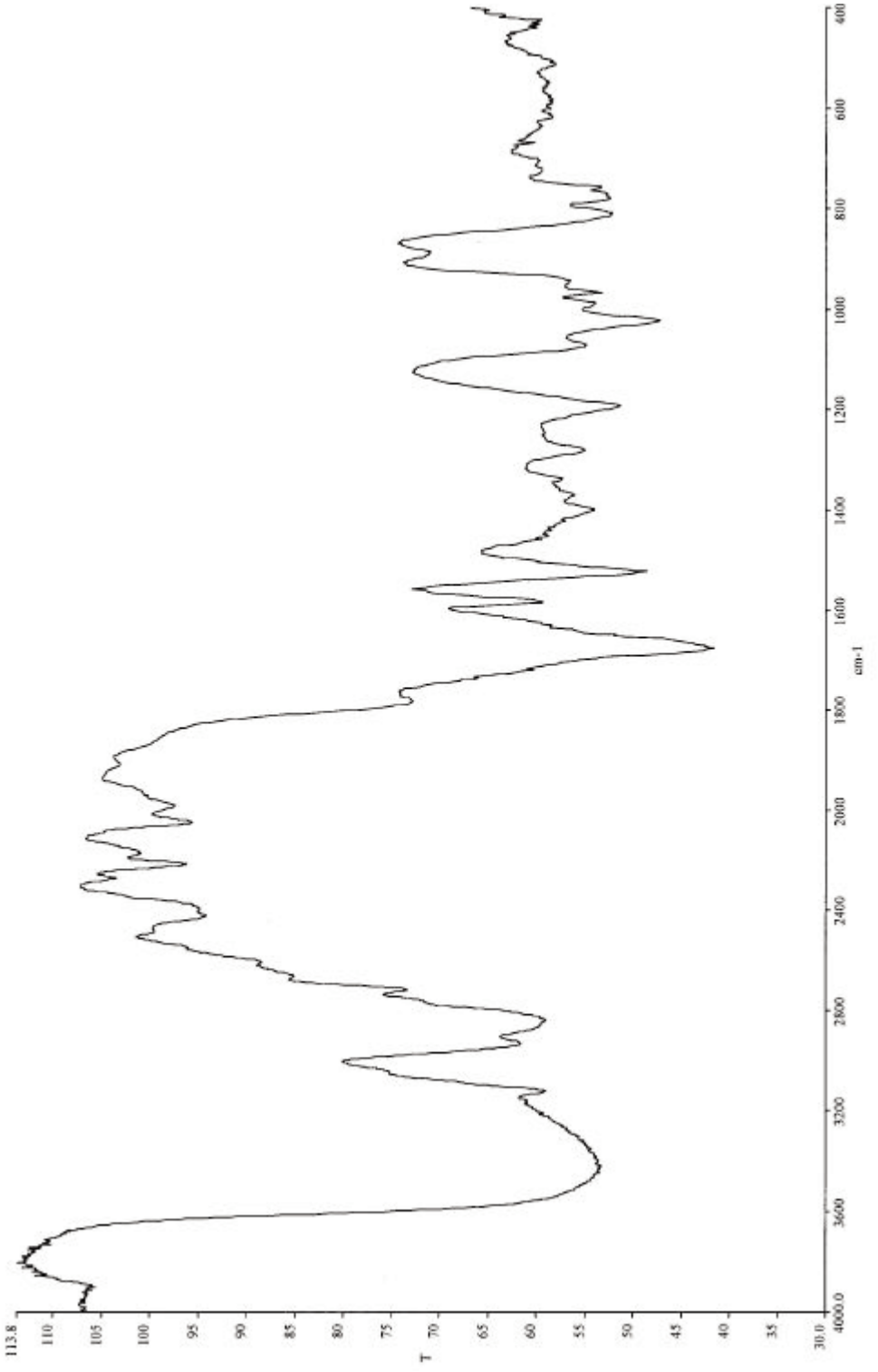

FIGURE J1
Infrared Absorption Spectrum of 5-(Hydroxymethyl)-2-furfural

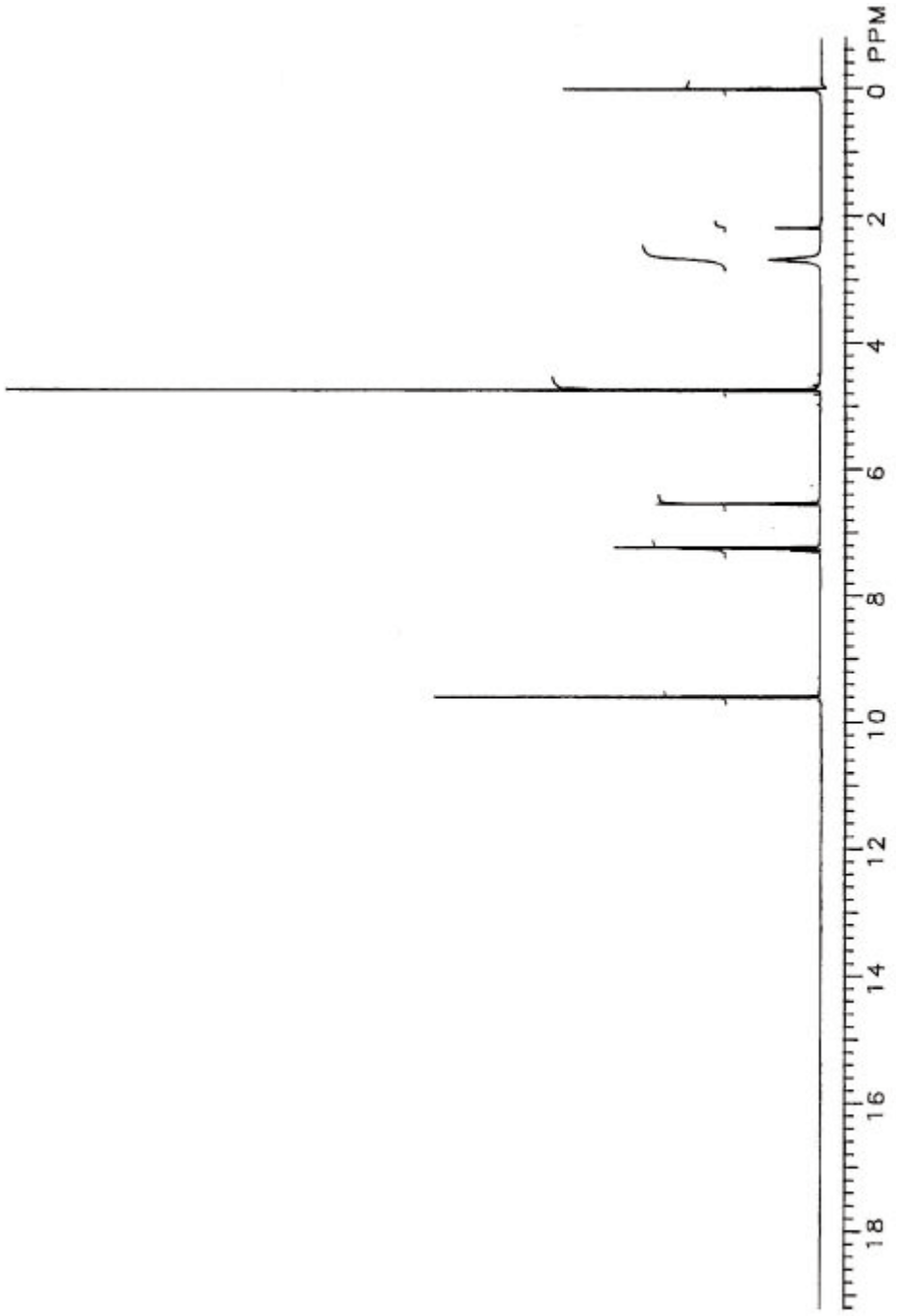

FIGURE J2
Proton Nuclear Magnetic Resonance Spectrum of 5-(Hydroxymethyl)-2-furfural

TABLE J1
High-Performance Liquid Chromatography Systems Used in the Gavage Studies of 5-(Hydroxymethyl)-2-furfural[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (285 nm) light	Phenomenex Hypersil® 5 C_{18}, 250 mm × 3.2 mm, 5 µm (Phenomenex, Torrance, CA)	A) Water and B) acetonitrile; 90% A:10% B for 7 minutes, then linear gradient to 50% A:50% B in 1 minute, held for 6 minutes, then linear return to initial conditions in 1 minute, held 5 minutes; flow rate 0.5 mL/minute
System B		
Ultraviolet (285 nm) light	Alltech Hypersil® ODS C_{18}, 250 mm × 3.2 mm, 5 µm (Alltech Associates, Inc., Deerfield, IL)	A) Water:acetonitrile (90:10) and B) acetonitrile; 100% A for 10 minutes, linear gradient to 100% B in 10 minutes, held for 5 minutes, then linear gradient to 100% A in 5 minutes; 15-minute equilibration; flow rate 0.5 mL/minute
System C		
Ultraviolet (285 nm) light	Alltech Alltima™ C_{18}, 250 mm × 3.2 mm, 5 µm (Alltech Associates, Inc.)	A) Water and B) acetonitrile; 90% A:10% B for 8 minutes, then linear gradient to 100% B in 10 minutes, held for 7 minutes, then linear return to initial conditions in 5 minutes; 10-minute equilibration; flow rate 0.58 mL/minute
System D		
Ultraviolet (285 nm) light	Alltech Alltima™ C_{18}, 250 mm × 4.6 mm, 5 µm (Alltech Associates, Inc.)	Water:acetonitrile (90:10), isocratic; flow rate 0.5 mL/minute; thymidine as internal standard

[a] The high-performance liquid chromatographs were manufactured by Agilent Technologies (Palo Alto, CA) (System B) or Waters Corporation (Milford, MA) (Systems A, C, and D).

TABLE J2
Preparation and Storage of Dose Formulations in the Gavage Studies of 5-(Hydroxymethyl)-2-furfural

3-Week Studies	3-Month Studies	2-Year Studies
Preparation		
The test article was melted at approximately 40° C and weighed into a volumetric flask. Deionized water was added to achieve approximately 90% of the final volume, and the flask was sealed, shaken, and/or sonicated to dissolve the chemical. The contents of the flask were then diluted to volume with deionized water. The dose formulations were prepared once.	The test article was weighed into a beaker or volumetric flask and diluted to the desired volume with deionized water. The contents of the vessel were then shaken and/or sonicated to dissolve the chemical. The dose formulations were prepared approximately monthly. During preparation of the first formulation of the 3-month study, a small amount of insoluble material was noticed. Despite extensive attempts to characterize the material, the identity of the material could not be conclusively determined. A filtration step added to the dose preparation procedure eliminated the problem.	A volume of deionized water equal to approximately ¼ of the final volume was placed in a calibrated mixing container. The target amount of the test article was weighed into a beaker, and deionized water was added to dissolve the chemical. The contents of the beaker were poured into the mixing container, and the beaker was rinsed three times with deionized water into the mixing container. The contents of the mixing container were diluted to volume with deionized water, capped, and shaken vigorously. A magnetic stir bar was added to the mixing container, which was again capped, and the contents were stirred vigorously on a stir plate for approximately 15 minutes. The resulting solution was filtered using a number 2 fluted filter (Whatman Laboratory Division, Kent, England) into a second calibrated mixing container. Due to discovery of evaporation during the prolonged filtration step, for dose formulations prepared on or after February 13, 2003, the calibrated mixing container used to collect the filtrate was diluted to the total volume with deionized water and then capped and shaken vigorously. The dose formulations were prepared approximately monthly.
Chemical Lot Numbers		
11129CQ-MRI796	14901MQ	34266-63 34266-72 34266-76
Maximum Storage Time		
35 days	35 days	35 days
Storage Conditions		
Stored in amber glass vials, sealed with Teflon®-lined lids at approximately 5° C.	Stored in amber glass vials, sealed with Teflon®-lined lids at approximately 5° C.	Stored in amber glass vials, sealed with Teflon®-lined lids at approximately 5° C.
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Week Gavage Studies of 5-(Hydroxymethyl)-2-furfural

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
December 9, 1996	December 11, 1996	18.8	18.95	+1
		37.5	37.35	0
		75	74.59	–1
		150	142.4	–5
		300	300.4	0
	January 9-10, 1997[b]	18.8	19.06	+1
		37.5	39.00	+4
		75	76.40	+2
		150	160.7	+7
		300	310.1	+3
January 6, 1997	January 9-10, 1997	300	301.2	0
	January 9-10, 1997[b]	300	303.3[c]	+1
Mice				
December 9, 1996	December 11, 1996	9.4	9.123	–3
		18.8	18.95	+1
		37.5	37.35	0
		75	74.59	–1
		150	142.4	–5
	January 9-10, 1997[b]	9.4	9.258	–2
		18.8	19.25	+2
		37.5	34.95	–7
		75	74.51	–1
		150	153.3	+2

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 18.8 mg/mL=94 mg/kg, 37.5 mg/mL=188 mg/kg, 75 mg/mL=375 mg/kg, 150 mg/mL=750 mg/kg, 300 mg/mL=1,500 mg/kg. For mice, dosing volume=10 mL/kg; 9.4 mg/mL=94 mg/kg, 18.8 mg/mL=188 mg/kg, 37.5 mg/mL=375 mg/kg, 75 mg/mL=750 mg/kg, 150 mg/mL=1,500 mg/kg
[b] Animal room samples
[c] Results of quintuplicate analyses

TABLE J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of 5-(Hydroxymethyl)-2-furfural

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
April 1, 1997	April 2-3, 1997	18.8	18.35	–2
		37.5	35.98	–4
		75	69.77	–7
		150	133.1[b]	–11
		300	334.1[b]	+11
	May 7-8, 1997[c]	18.8	18.66	–1
		37.5	37.96	+1
		75	74.60	–1
		150	137.9	–8
		300	359.3	+20
May 27, 1997	May 29-30, 1997	18.8	19.25	+2
		37.5	38.56	+3
		75	75.27	0
		150	153.0	+2
		300	303.9	+1
June 16, 1997	June 17, 1997	18.8	20.17	+7
		37.5	40.35	+8
		75	81.06	+8
		150	157.3	+5
		300	316.8	+6
	August 6-7, 1997[c]	18.8	18.53	–1
		37.5	37.03	–1
		75	73.58	–2
		150	149.8	0
		300	312.0	+4
Mice				
April 1, 1997	April 2-3, 1997	4.7	5.599[d]	+19
		9.4	9.232	–2
		18.8	18.35	–2
		37.5	35.98	–4
		75	69.77	–7
	May 7-8, 1997[c]	9.4	9.745	+4
		18.8	18.17	–3
		37.5	38.2	+2
		75	73.7	–2
April 2, 1997	April 3, 1997	4.7	4.810[e]	+2
	May 7-8, 1997[c]	4.7	4.969	+6

TABLE J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of 5-(Hydroxymethyl)-2-furfural

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
May 27, 1997	May 29-30, 1997	4.7	4.955	+5
		9.4	9.779	+4
		18.8	19.25	+2
		37.5	38.56	+3
		75	75.27	0
June 16, 1997	June 17, 1997	4.7	4.853	+3
		9.4	9.850	+5
		18.8	20.17	+7
		37.5	40.35	+8
		75	81.06	+8
	August 6-7, 1997[c]	4.7	4.756	+1
		9.4	9.547	+2
		18.8	18.98	+1
		37.5	36.60	–2
		75	76.35	+2

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 18.8 mg/mL=94 mg/kg, 37.5 mg/mL=188 mg/kg, 75 mg/mL=375 mg/kg, 150 mg/mL=750 mg/kg, 300 mg/mL=1,500 mg/kg. For mice, dosing volume=10 mL/kg; 4.7 mg/mL=47 mg/kg, 9.4 mg/mL=94 mg/kg, 18.8 mg/mL=188 mg/kg, 37.5 mg/mL=375 mg/kg, 75 mg/mL=750 mg/kg
[b] Formulation was outside the acceptable range of ± 10% of target concentration but used at NTP's direction.
[c] Animal room samples
[d] Remixed; not used in study
[e] Results of remix

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of 5-(Hydroxymethyl)-2-furfural

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a]	Difference from Target (%)
Rats				
February 20, 2002	February 20, 2002	37.5	40.01	+7
		75	80.63	+8
		150	162.1	+8
	March 27, 2002[b]	37.5	37.79	+1
		75	75.42	+1
		150	153.0	+2
May 14, 2002	May 15, 2002	37.5	38.02	+1
		75	76.79	+2
		150	153.5	+2
August 7, 2002	August 8, 2002	37.5	35.53	–5
		75	70.31	–6
		150	140.1	–7
October 29, 2002	October 29, 2002	37.5	39.58	+6
		75	81.87	+9
		150	164.4	+10
	December 4, 2002[b]	37.5	39.35	+5
		75	77.83	+4
		150	154.2	+3
January 15, 2003	January 16, 2003	37.5	39.71	+6
		75	80.42	+7
		150	165.9[c]	+11
January 21, 2003	January 21, 2003	150	154.2[d]	+3
April 8, 2003	April 9, 2003	37.5	37.27	–1
		75	75.49	+1
		150	152.0	+1
July 1, 2003	July 2-3, 2003	37.5	38.96	+4
		75	78.97	+5
		150	158.9	+6
	August 6, 2003[b]	37.5	37.91	+1
		75	75.68	+1
		150	151.8	+1
September 24, 2003	September 29, 2003	37.5	39.31	+5
		75	80.41	+7
		150	156.1	+4
December 16, 2003	December 16, 2003	37.5	39.09	+4
		75	79.77	+6
		150	162.6	+8

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of 5-(Hydroxymethyl)-2-furfural

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
July 18, 2001	July 23, 2001	18.8	18.93	+1
		37.5	38.19	+2
		75	74.70	0
	August 22-23, 2001[b]	18.8	19.52	+4
		37.5	39.48	+5
		75	79.23	+6
October 5, 2001	October 10, 2001	18.8	19.57	+4
		37.5	39.62	+6
		75	80.34	+7
December 28, 2001	January 3, 2002	18.8	19.09	+2
		37.5	39.54	+5
		75	78.20	+4
February 20, 2002	February 20, 2002	18.8	19.84	+6
		37.5	40.01	+7
		75	80.63	+8
	March 27, 2002[b]	18.8	19.11	+2
		37.5	38.98	+4
		75	77.51	+3
May 14, 2002	May 15, 2002	18.8	18.99	+1
		37.5	38.02	+1
		75	76.79	+2
August 7, 2002	August 8, 2002	18.8	18.07	–4
		37.5	35.53	–5
		75	70.31	–6
October 29, 2002	October 29, 2002	18.8	18.88	0
		37.5	39.58	+6
		75	81.87	+9
	December 4, 2002[b]	18.8	19.16	+2
		37.5	39.64	+6
		75	77.27	+3
January 15, 2003	January 16, 2003	18.8	19.40	+3
		37.5	39.71	+6
		75	80.42	+7

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of 5-(Hydroxymethyl)-2-furfural

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
April 8, 2003	April 9, 2003	18.8	18.95	+1
		37.5	37.27	–1
		75	75.49	+1
July 1, 2003	July 2-3, 2003	18.8	18.94 ± 0.59[e]	+1
		37.5	38.96	+4
		75	78.97	+5
	August 6, 2003[b]	18.8	19.51	+4
		37.5	38.79	+3
		75	76.27	+2

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 37.5 mg/mL=188 mg/kg, 75 mg/mL=375 mg/kg, 150 mg/mL=750 mg/kg. For mice, dosing volume=10 mL/kg; 18.8 mg/mL=188 mg/kg, 37.5 mg/mL=375 mg/kg, 75 mg/mL=750 mg/kg
[b] Animal room samples
[c] Remixed; not used in study
[d] Results of remix
[e] Results of triplicate analyses

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

TABLE K1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

TABLE K2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a] Per kg of finished product

TABLE K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.8 ± 0.56	13.7 – 15.7	31
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.6	31
Crude fiber (% by weight)	9.0 ± 0.45	8.0 – 9.9	31
Ash (% by weight)	5.2 ± 0.28	4.7 – 5.8	31
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	4,926 ± 109	3,060 – 8,900	31
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	8.3 ± 3.38	5.9 – 25.2	31
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	1.001 ± 0.059	0.873 – 1.140	31
Phosphorus (%)	0.605 ± 0.035	0.549 – 0.701	31
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.35 ± 0.155	0.14 – 0.50	31
Cadmium (ppm)	0.06 ± 0.021	0.04 – 0.10	31
Lead (ppm)	0.07 ± 0.026	0.05 – 0.17	31
Mercury (ppm)	<0.02		31
Selenium (ppm)	0.22 ± 0.050	0.14 – 0.36	31
Aflatoxins (ppb)	<5.00		31
Nitrate nitrogen (ppm)[c]	14.1 ± 3.78	6.85 – 23.2	31
Nitrite nitrogen (ppm)[c]	<0.61		31
BHA (ppm)[d]	<1.0		31
BHT (ppm)[d]	<1.0		31
Aerobic plate count (CFU/g)	25 ± 64	10 – 360	31
Coliform (MPN/g)	3.0 ± 0.1	3.0 – 3.6	31
Escherichia coli (MPN/g)	<10		31
Salmonella (MPN/g)	Negative		31
Total nitrosoamines (ppb)[e]	4.1 ± 1.54	2.3 – 8.4	31
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 1.35	1.1 – 6.9	31
N-Nitrosopyrrolidine (ppb)[e]	1.6 ± 0.64	0.9 – 3.1	31
Pesticides (ppm)			
α-BHC	<0.01		31
β-BHC	<0.02		31
γ-BHC	<0.01		31
δ-BHC	<0.01		31
Heptachlor	<0.01		31
Aldrin	<0.01		31
Heptachlor epoxide	<0.01		31
DDE	<0.01		31
DDD	<0.01		31
DDT	<0.01		31
HCB	<0.01		31
Mirex	<0.01		31
Methoxychlor	<0.05		31
Dieldrin	<0.01		31
Endrin	<0.01		31
Telodrin	<0.01		31
Chlordane	<0.05		31
Toxaphene	<0.10		31
Estimated PCBs	<0.20		31
Ronnel	<0.01		31
Ethion	<0.02		31
Trithion	<0.05		31
Diazinon	<0.10		31
Methyl chlorpyrifos	0.097 ± 0.066	0.020 – 0.259	31
Methyl parathion	<0.02		31
Ethyl parathion	<0.02		31
Malathion	0.283 ± 0.436	0.020 – 1.850	31
Endosulfan I	<0.01		31
Endosulfan II	<0.01		31
Endosulfan sulfate	<0.03		31

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

During the 3-month studies, serum samples were collected by the study laboratory from five male and five female sentinel rats and mice during week 4 and from these same animals at the end of the study. During the 2-year studies, serum samples were collected by the study laboratory from five male and five female sentinel rats and mice during week 4 and at 6, 12, and 18 months (only three surviving female rats and female mice were available for sampling at this time point) and from 750 mg/kg rats and mice at study termination. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. Fecal samples from mice were tested for *Helicobacter* at 18 months. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
3-Month Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	4 weeks, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	4 weeks, study termination
Sendai	4 weeks, study termination
Hemaglutination Inhibition	
H-1 (Toolan's H-1 virus)	4 weeks, study termination
KRV (Kilham rat virus)	4 weeks, study termination
Immunofluorescence Assay	
RCV/SDA	Study termination

Method and Test	Time of Analysis
RATS (continued)	
2-Year Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, 6, 12, and 18 months, study termination
RCV/SDA	4 weeks, 6, 12, and 18 months, study termination
Sendai	4 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
M. arthritidis	Study termination
Parvovirus	4 weeks, 6, 12, and 18 months, study termination
Sendai	Study termination
MICE	
3-Month Study	
ELISA	
Ectromelia virus	4 weeks, study termination
EDIM (epizootic diarrhea of infant mice)	4 weeks, study termination
GDVII (mouse encephalomyelitis virus)	4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	4 weeks, study termination
Mouse adenoma virus	4 weeks, study termination
MHV (mouse hepatitis virus)	4 weeks, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, study termination
Reovirus 3	4 weeks, study termination
Sendai	4 weeks, study termination
Hemaglutination Inhibition	
K (papovavirus)	4 weeks, study termination
MVM (minute virus of mice)	4 weeks, study termination
Polyoma virus	4 weeks, study termination
Immunofluorescence Assay	
EDIM	Study termination
MCMV (mouse cytomegalovirus)	Study termination
Reovirus 3	Study termination

Method and Test	Time of Analysis
MICE (continued)	
2-Year Study	
ELISA	
Ectromelia virus	4 weeks, 6, 12, and 18 months, study termination
EDIM	4 weeks, 6, 12, and 18 months, study termination
GDVII	4 weeks, 6, 12, and 18 months, study termination
LCM	4 weeks, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	4 weeks, 6, 12, and 18 months, study termination
MHV	4 weeks, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, 6, 12, and 18 months, study termination
Reovirus 3	4 weeks, 6, 12, and 18 months, study termination
Sendai	4 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	Study termination
GDVII	12 months
Mouse adenoma virus-FL	12 months
MCMV	Study termination
MHV	4 weeks
Parvovirus	4 weeks, 6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

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