NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF FORMAMIDE

(CAS NO. 75-12-7)

IN F344/N RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

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

July 2008

NTP TR 541

NIH Publication No. 08-5884

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

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.

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CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

R.D. Irwin, Ph.D., Study Scientist
S.A. Elmore, D.V.M., M.S., Study Pathologist
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
L.T. Burka, Ph.D.
R.S. Chhabra, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
D.P. Orzech, M.S.
S.D. Peddada, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S.

Battelle Columbus Operations
Conducted studies and evaluated pathology findings

M.R. Hejtmancik, Ph.D., Principal Investigator
M.J. Ryan, D.V.M., Ph.D.
D.M. Sells, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
K.J. Cimon, D.V.M., M.S.
J.C. Peckham, D.V.M., M.S., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.

Dynamac Corporation
Prepared quality assessment audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides and prepared pathology report on rats (June 02, 2005)

S.A. Elmore, D.V.M., M.S., Chairperson
ILS, Inc.
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
National Toxicology Program
K. Kuettler, D.V.M., F.T.A., Observer
BASF, Germany
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
G. Pearse, B.V.M.&S.
National Toxicology Program
C.C. Shackelford, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
A. Veit, Observer
Experimental Pathology Laboratories, Inc.

Evaluated slides and prepared pathology report on mice (May 31, 2005)

M.A. Hanes, D.V.M., Chairperson
ILS, Inc.
M.F. Cesta, D.V.M.
National Toxicology Program
K.J. Cimon, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
National Toxicology Program
K. Kuettler, D.V.M., F.T.A., Observer
BASF, Germany
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
G. Pearse, B.V.M.&S.
National Toxicology Program
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.

Constella Group, Inc.
Provided statistical analyses

D.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
B.F. Hall, M.S.
L.M. Harper, B.S.
J.I. Powers, M.A.P.
D.C. Serbus, Ph.D.

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SUMMARY

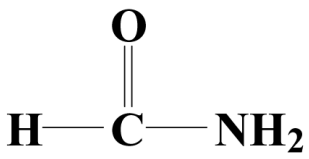
Background
Formamide has a variety of uses, including as a softener for paper, gums, and glues. We studied the effects of formamide on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods
We deposited solutions containing formamide in deionized water directly into the stomachs of male and female rats and mice. Groups of 50 animals received 20, 40, or 80 milligrams of formamide per kilogram body weight five days per week for two years; groups of animals receiving water alone served as the control groups. At the end of the study, tissues from more than 40 sites were examined for every animal.

Results
The only effects observed in male and female rats were lower body weights in animals receiving the highest dose of formamide and a slight increase in bone marrow hyperplasia in male rats. There was an increased occurrence of liver hemangiosarcomas in all three groups of male mice receiving formamide and a less pronounced increase in hepatocellular adenomas or carcinomas of the liver in female mice. In male mice receiving 80 mg/kg of formamide, there was mineralization in the testes.

Conclusions
We conclude that exposure to formamide did not cause cancer in male or female rats. Exposure to formamide did produce hemangiosarcomas in the liver of male mice and may have been related to an increase in liver tumors in female mice. Increased incidences of bone marrow hyperplasia in male rats and testicular mineralization in male mice were also associated with formamide exposure.

ABSTRACT



FORMAMIDE

CAS No. 75-12-7

Chemical Formula: CH_3NO Molecular Weight: 45.04

Synonyms: Carbamaldehyde; formic acid, amide; formimidic acid; methanamide

Formamide is used as a softener for paper, gums, and animal glues; as an ionizing solvent; and in the manufacture of formic esters and hydrocyanic acid. Formamide was nominated for reproductive and genetic toxicity evaluation by the Environmental Defense Fund and for carcinogenicity evaluation by the National Cancer Institute because of the potential for human exposure associated with its widespread industrial use, the absence of data adequately characterizing its potential for reproductive and genetic toxicity, and the fact that acetamide, a compound structurally related to formamide, is hepatocarcinogenic in rats when administered in feed. Male and female F344/N rats and B6C3F1 mice were administered formamide (approximately 100% pure) in deionized water by gavage for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli, Drosophila melanogaster,* and mouse peripheral blood erythrocytes.

3-Month Study in Rats

Groups of 10 male and 10 female rats were administered 0, 10, 20, 40, 80, or 160 mg formamide/kg body weight in deionized water by gavage, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats (clinical pathology study) and five male and five female rats (plasma concentration study) were administered the same doses, 5 days per week for up to 14 weeks. All core study rats survived to the end of the study. Mean body weights of females in the 40 mg/kg group and males and females in the 80 and 160 mg/kg groups were significantly less than those of the vehicle controls. On day 23 and at week 14, there was a dose-related increase in the erythron, evidenced by increases in hematocrit values, hemoglobin c oncentrations, and erythrocyte counts. The incidences of degeneration of the germinal epithelium of the testes and epididymis were significantly increased in 160 mg/kg males.

3-Month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 10, 20, 40, 80, or 160 mg formamide/kg body weight in deionized water by gavage, 5 days per week for 14 weeks. Additional groups of five male and five female mice (plasma concentration study) were administered the same doses, 5 days per week for 14 weeks. All mice survived to the end of the study. Final mean body weights of the 80 and 160 mg/kg males and mean body

weight gains of 40, 80, and 160 mg/kg males were significantly less than those of the vehicle controls. Dosed females differed significantly from vehicle controls in the relative amount of time spent in the estrous stages. All 160 mg/kg males had abnormal residual bodies in the testes.

2-Year Study in Rats

Groups of 50 male and 50 female rats were administered 0, 20, 40, or 80 mg formamide/kg body weight, 5 days per week for 104 to 105 weeks in deionized water by gavage. Survival of all dosed groups of rats was similar to that of the vehicle controls. Mean body weights of 80 mg/kg males were less than those of the vehicle controls throughout most of the study. Mean body weights of 40 and 80 mg/kg females were somewhat less than those of the vehicle controls during the second year of the study. A significant increase in the incidence of bone marrow hyperplasia occurred in 80 mg/kg males. No neoplasms were attributed to exposure to formamide.

2-Year Study in Mice

Groups of 50 male and 50 female mice were administered 0, 20, 40, or 80 mg formamide/kg body weight, 5 days per week for 104 to 105 weeks in deionized water by gavage. Survival of all dosed groups of mice was similar to that of the vehicle controls. Mean body weights of 80 mg/kg males and females were generally less than those of the vehicle controls throughout the study; mean body weights of 40 mg/kg females were generally less after week 13 of the study. The incidences of hemangiosarcoma of the liver occurred with a positive trend in males, and the incidences were significantly increased in the 40 and 80 mg/kg groups. The incidence of hepatocellular adenoma or carcinoma (combined) in 80 mg/kg females was significantly increased. The incidences of mineralization of the testicular arteries and testicular tunic were significantly increased in 80 mg/kg males. The incidence of hematopoietic cell proliferation of the spleen was significantly increased in 80 mg/kg males.

Genetic Toxicology

Formamide gave no evidence for mutagenicity in a series of short-term assays. In three independent Ames assays, formamide was not mutagenic in any of several strains of *S. typhimurium* tested with and without rat or hamster liver S9 activation enzymes or in *E. coli* strain WP *uvrA* pKM101 tested with and without 10% rat liver S9. Negative results were obtained in a test for induction of sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* treated with formamide either by feeding or injection. Formamide did not induce increases in micronucleated erythrocytes in male or female mice treated by gavage for 3 months.

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of form-amide in male or female F344/N rats administered 20, 40, or 80 mg/kg. There was *clear evidence of carcinogenic activity* of formamide in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver. There was *equivocal evidence of carcinogenic activity* of formamide in female B6C3F1 mice based on increased incidences of hepatocellular adenoma or carcinoma (combined).

An increased incidence of bone marrow hyperplasia occurred in male rats. Mineralization of the testicular arteries and tunic and hematopoietic cell proliferation of the spleen in male mice were also associated with administration of formamide.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in deionized water by gavage	0, 20, 40, or 80 mg/kg	0, 20, 40, or 80 mg/kg	0, 20, 40, or 80 mg/kg	0, 20, 40, or 80 mg/kg
Body weights	80 mg/kg group less than the vehicle control group	40 and 80 mg/kg groups less than the vehicle control group	80 mg/kg group less than the vehicle control group	40 and 80 mg/kg groups less than the vehicle control group
Survival rates	26/50, 27/50, 26/50, 29/50	38/50, 30/50, 34/50, 32/50	39/50, 42/50, 36/50, 33/50	38/50, 39/50, 31/50, 39/50
Nonneoplastic effects	Bone Marrow: hyperplasia (19/50, 24/50, 22/50, 29/50)	None	Testis: mineralization of artery (0/50, 2/50, 5/50, 35/50); mineralization of tunic (1/50, 0/50, 5/50, 27/50) Spleen: hematopoietic cell proliferation (14/50, 14/50, 20/50, 28/50)	None
Neoplastic effects	None	None	Liver: hemangiosarcoma (1/50, 5/50, 7/50, 8/50)	None
Equivocal findings	None	None	None	Liver: hepatocellular adenoma or carcinoma (9/50, 15/50, 13/50, 18/50)
Level of evidence of carcinogenic activity	No evidence	No evidence	Clear evidence	Equivocal evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9; negative in *Escherichia coli* WP *uvrA* pKM101 with and without S9			
Sex-linked recessive lethal mutations *Drosophila melanogaster:*	No induction of sex-linked recessive lethal mutations			
Micronucleated erythrocytes Mouse peripheral blood *in vivo:*	Negative			

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 pre-neoplastic 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 formamide on May 16, 2007, 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.

Nancy Kerkvliet, Ph.D., Chairperson
Department of Environmental and Molecular Toxicology
Oregon State University
Corvallis, OR

Christopher Bradfield, Ph.D.*
McArdle Laboratory for Cancer Research
University of Wisconsin
Madison, WI

Kenny Crump, Ph.D.
Environ International
Ruston, LA

Prescott Deininger, Ph.D., Principal Reviewer
Tulane University Medical Center
New Orleans, LA

Jon Mirsalis, Ph.D., Principal Reviewer
SRI International
Menlo Park, CA

Harish Sikka, Ph.D., Principal Reviewer
Environmental Toxicology and Chemistry Laboratory
State University of New York College at Buffalo
Buffalo, NY

Keith Soper, Ph.D.
Merck Research Laboratories
West Point, PA

Vernon Walker, D.V.M., Ph.D.
Lovelace Respiratory Institute
Albuquerque, NM

Special Ad Hoc Reviewers

Russell C. Cattley, V.M.D., Ph.D.
Amgen
Thousand Oaks, CA

Raymond F. Novak, Ph.D.
Institute of Environmental Health Sciences
Wayne State University
Detroit, MI

Michael V. Pino, D.V.M., Ph.D.
Drug Safety Evaluation
Sanofi-aventis
Bridgewater, NJ

---

* Did not attend.

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On May 16, 2007, the draft Technical Report on the toxicology and carcinogenesis studies of formamide 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 formamide discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* in male and female F344/N rats, *clear evidence of carcinogenic activity* in male B6C3F1 mice, and *equivocal evidence of carcinogenic activity* in female B6C3F1 mice.

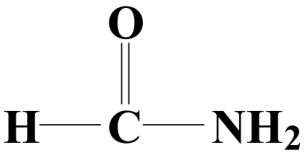
Dr. Deininger, the first principal reviewer, noted that the results for formamide were different from those for *N*,*N*-dimethylformamide. He inquired if there was any insight into the cause of the erythrocyte damage. Dr. Irwin replied that there was little knowledge of the metabolites of formamide. He noted some suggestion in the literature of possible effects on membrane damage. The slight changes in the erythron and hematopoiesis were the only indications of red cell involvement. Dr. Kerkvliet inquired if there could also be a connection with the bone marrow hyperplasia. Dr. S.A. Elmore, NIEHS, stated that one cause of the red blood cell damage could be physical shearing as the cells passed through hemangiosarcomas, and thus the hyperplasia was secondary to the tumors and not vice-versa. Dr. Walker said that the most appropriate statement would be that causes of the hematopoiesis were unknown. He also noted that studies involving immunohistologic staining for *p53* stabilization might help provide information on mechanism for these and other studies in which hemangiosarcomas were observed.

Dr. Mirsalis, the second principal reviewer, agreed in general with the conclusions. He inquired about further discussion of the possible causes of the erythron changes. He asked for comparison with a previous inhalation study that did show a liver tumor response. Dr. Irwin noted that the inhalation studies were on *N*,*N*-dimethylformamide.

Dr. Sikka, the third principal reviewer, agreed with the conclusions. He inquired if discussion could be added regarding the possible mechanism of carcinogenicity of this non-genotoxic chemical and also about the difference in response between rats and mice. Dr. Irwin replied that mechanistic speculation would be difficult given the lack of knowledge of the metabolites; he said that more information would be included about the effects of *N*,*N*-dimethylformamide and *N*-methylformamide.

Dr. Pino suggested that hyperplasia of the bone marrow in male rats be included in the conclusion statement. Dr. Mirsalis moved and Dr. Deininger seconded that, with the addition, the conclusions be accepted as written. The motion was approved unanimously with six votes.

INTRODUCTION



FORMAMIDE
CAS No. 75-12-7

Chemical Formula: CH_3NO Molecular Weight: 45.04

Synonyms: Carbamaldehyde; formic acid, amide; formimidic acid; methanamide

Chemical and Physical Properties

Formamide is a slightly viscous, odorless, colorless liquid (*Merck*, 1996). It has a melting point of 2.55° C, a boiling point of 210.5° C, and a specific gravity of 1.1334. Formamide is soluble in water, miscible in methanol, ethanol, acetone, acetic acid, dioxane, ethylene glycol, glycerol, and phenol, and very slightly soluble in ether and benzene. At atmospheric pressure, formamide begins to decompose at 180° C, producing carbon monoxide and ammonia.

Production, Use, and Human Exposure

Formamide is prepared from carbon monoxide and ammonia at high pressure and temperature (*Merck*, 1996). Formamide is used as a softener for paper, gums, and animal glues; as an ionizing solvent; and in the manufacture of formic esters and hydrocyanic acid. Formamide is classified as a high production volume chemical by the United States Environmental Protection Agency (2003).

Human exposure occurs in industrial and laboratory settings, especially in the pharmaceutical industry, in plastics manufacturing, and in extraction and processing of butadiene. The 8-hour time-weighted average threshold limit value is 10 ppm (ACGIH, 2005).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

The metabolism of formamide is essentially unexplored. In a few early reports dating back to the late 1800s and reviewed by Bray *et al*. (1949), formamide administered to dogs, rabbits, or sheep was reported to be excreted in the urine either unchanged or as formate. This result was confirmed by Bray *et al*. (1949) who found approximately 60% of a 2 to 4 g dose of orally administered formamide was excreted as formate and 40% as unchanged formamide during the 24 hours after administration. In addition, formamide was slowly hydrolyzed by rabbit liver extracts. However, no other publications dealing with the metabolism of formamide were found in a review of the literature.

The metabolism of the structurally related compounds *N*,*N*-dimethylformamide and *N*-methylformamide has been reviewed by Gescher (1993) and Mraz *et al*.

(1993), and the consensus pathways are illustrated in Figure 1. The quantitatively primary metabolite of *N*,*N*-dimethylformamide in rodents and humans is *N*-hydroxy-methyl-*N*-methylformamide, formed by hydroxylation (oxidation) of an *N*-methyl group by cytochrome P4502E1. *N*-hydroxymethyl-*N*-methylformamide can then form *N*-methylformamide non-enzymatically. Both *N*-hydroxymethyl-*N*-methylformamide and *N*-methylformamide are further metabolized by CYP2E1. The immediate product of these reactions has the properties of a reactive carbamoylating agent, such as methyl isocyanate or methyl carbamic acid, and reacts with glutathione to produce *S*-(*N*-methylcarbamoyl)glutathione, which is subsequently processed to *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine that is excreted in the urine. *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine has also been shown to be a metabolite of methylisocyanate in rats. *N*-hydroxymethyl-*N*-methylformamide, formamide, and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine are present in the urine of human volunteers undergoing inhalation and/or dermal exposure to *N*,*N*-dimethylformamide and in workers occupationally exposed to *N*,*N*-dimethylformamide (IARC, 1999).

Humans

No absorption, distribution, metabolism, or excretion studies of formamide in humans were found in a review of the literature.

TOXICITY

Experimental Animals

Little information is available characterizing the toxicity of formamide. Kennedy (1986) has reviewed the few early reports dealing with formamide toxicity; all involve intraperitoneal or intravenous administration of rather large doses (1 g formamide/kg body weight or greater) and reveal a low toxicity for formamide with no organ-specific response. Most of the reports lacked systematic histopathologic evaluation. LD_{50} values reported for intraperitoneal injection were 4.6 g/kg for rats and 7.4 g/kg for mice; for intravenous injection, LD_{50} values were 5.6 g/kg for rats and 5.1 g/kg for mice.

Warheit *et al*. (1989) conducted a 14-day inhalation study in male CD rats exposed to 100, 500, or 1,500 ppm formamide. Three 1,500 ppm animals died during the study, and the most significant chemical-related effect was minimal to severe necrosis of renal tubule epithelial cells that occurred in animals in this exposure group.

Humans

No toxicity studies of formamide in humans were found in a review of the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

During a continuous breeding study in Swiss mice, formamide administered in drinking water at 0, 100, 350, or 750 ppm (20 to 200 mg/kg per day) reduced fertility and litter size in F_0 animals without causing generalized toxicity at 750 ppm (Fail *et al*., 1998). Crossover matings indicated that females were the affected sex. After F_1 mating, exposure to 750 ppm reduced F_2 litter size, increased days to litter, reduced relative ovarian weight, and lengthened estrous cycles. The no-observed-adverse-effect levels for generalized toxicity were 750 and 350 ppm for the F_0 and F_1 generations, respectively. Reproductive performance was normal at 350 ppm for both F_0 and F_1 mice.

Humans

No reproductive or developmental toxicity studies in humans were found in a review of the literature.

CARCINOGENICITY

The carcinogenic potential of formamide has not been evaluated in animal studies, and there have been no epidemiology studies that have examined human risk associated with formamide exposure. Two structurally similar compounds, acetamide and dimethylformamide, have been evaluated in animal carcinogenicity studies.

A significant increase in the incidence of malignant lymphomas occurred in male B6C3F1 mice that received acetamide in the diet for 12 months; however, no treatment-related increases in neoplasm incidences were observed in female mice (Fleischman *et al*., 1980). In groups of F344/N rats that received acetamide in the diet for 12 months, the incidences of hepatocellular carcinomas were significantly increased in both males and

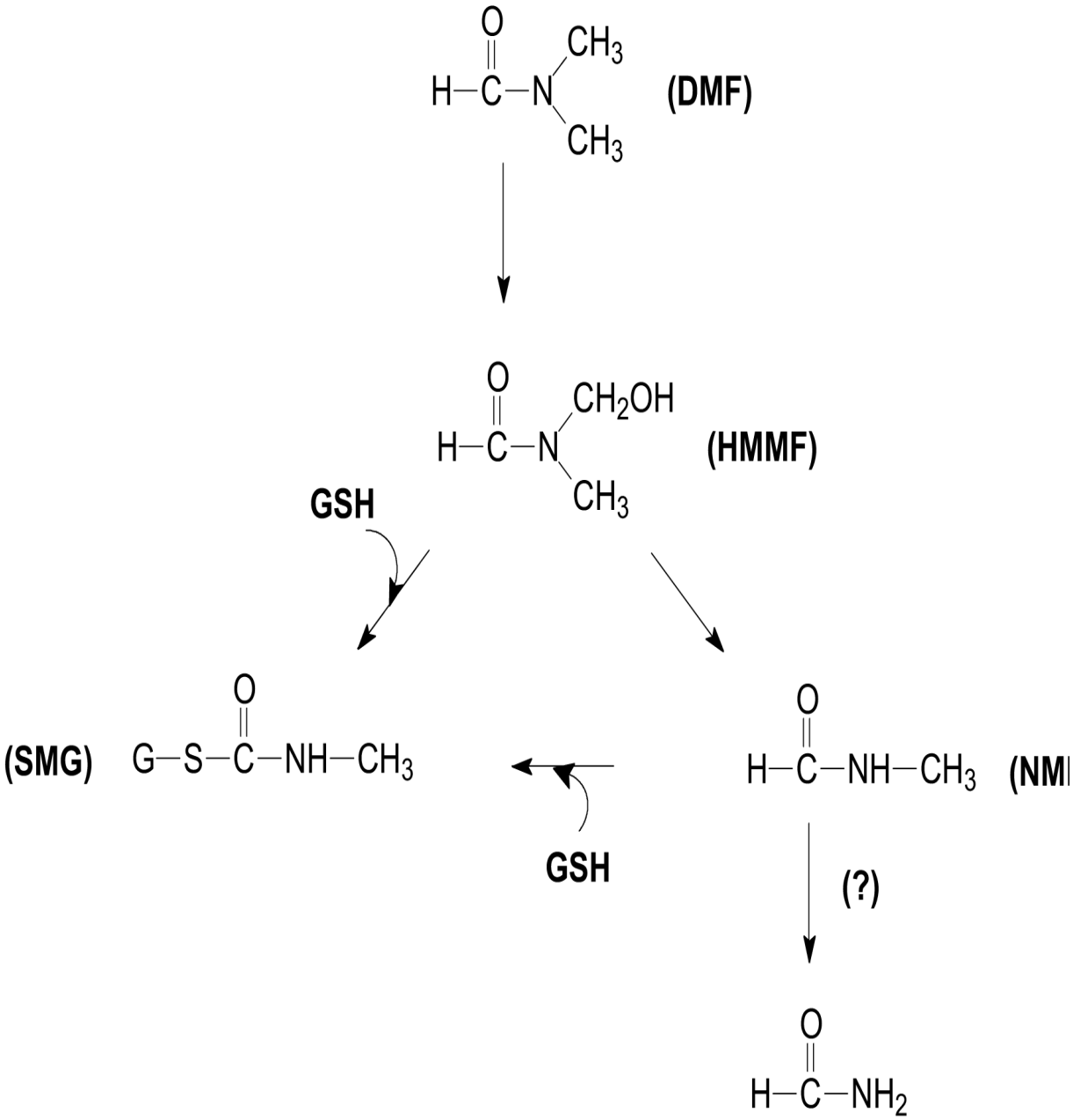


Figure 1
Metabolism of *N,N*-Dimethylformamide and *N*-Methylformamide
DMF=*N,N*-dimethylformamide; GSH=glutathione; HMMF=*N*-hydroxymethyl-*N*-methylformamide; NMF=*N*-methylformamide; SMG=*S*-(*N*-methylcarbamoyl)glutathione

females (Fleischman *et al*., 1980), and hepatocellular carcinomas occurred in Leeds strain rats administered diets containing acetamide for 35 weeks (Flaks *et al*., 1983).

The carcinogenic potential of dimethylformamide has been examined in two inhalation studies in rats and mice. Malley *et al*. (1994) exposed groups of male and female Crl:CD rats and mice to 0, 25, 100, or 400 ppm dimethylformamide, 6 hours per day, 5 days per week for 18 months (mice) or 2 years (rats). Although hepatotoxicity (increased liver weight, hepatocellular hypertrophy, and centrilobular necrosis) occurred in the 100 and 400 ppm groups of rats and mice, there were no increases in the incidences of neoplasms in any of the exposed groups. In a second inhalation study (Senoh *et al*., 2004), groups of male and female F344/DuCrj rats and Crj:BDF1 mice were exposed to 0, 200, 400, or 800 ppm dimethylformamide 6 hours per day, 5 days per week for 104 weeks. The incidences of hepatocellular adenomas and carcinomas were significantly increased in the 400 and 800 ppm groups of rats and in all groups of mice exposed to dimethylformamide. In addition, the incidences of hepatoblastoma were significantly increased in the 200 and 400 ppm groups of male mice.

A number of epidemiology studies of human occupational exposure to dimethylformamide have failed to find a significant association between dimethylformamide exposure and cancer (IARC, 1999).

Genetic Toxicity

Formamides are generally not active in mutagenicity test systems (Kennedy, 1986). Dimethylformamide is a recommended solvent for use in bacterial mutagenicity test systems with chemicals that are not soluble in traditional solvents such as water or dimethylsulfoxide. Only one publication reported mutagenicity test results with formamide; two independent *Salmonella* mutagenicity assays using a number of different tester strains were negative with and without metabolic activation (Mortelmans *et al*., 1986; Appendix E).

Study Rationale

Formamide was nominated for reproductive and genetic toxicity evaluation by the Environmental Defense Fund and for carcinogenicity evaluation by the National Cancer Institute. These nominations were based on the potential for human exposure associated with the widespread industrial use of formamide primarily as a solvent, the absence of data adequately characterizing its potential for reproductive and genetic toxicity, and the fact that acetamide, a compound structurally related to formamide, is hepatocarcinogenic in rats.

MATERIALS AND METHODS

Procurement and Characterization of Formamide

Formamide was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI; lot 08003HQ), and Acros Organics/Fisher Scientific (Fair Lawn, NJ; lot A012538501). Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory, Battelle Columbus Operations (Columbus, OH); the analytical chemistry laboratory also conducted stability studies (Appendix I). Reports on analyses performed in support of the formamide studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless liquid, was identified as formamide by infrared, ultraviolet/visible, and proton nuclear magnetic resonance spectroscopy, and by boiling point and relative density determinations. The purity of lot 08003HQ was determined by gas chromatography and high-performance liquid chromatography (HPLC). Gas chromatography indicated one major peak with no impurities greater than 0.05% relative to the major peak area; HPLC indicated a relative purity of 100%. The overall purity of lot 08003HQ was determined to be approximately 100%. For lot A012538501, HPLC indicated one major peak with no impurities greater than 0.05% relative to the major peak area; HPLC by another system indicated an average relative purity of 101%. Thin-layer chromatography indicated one major spot and three minor spots. Karl Fischer titration indicated 0.06% water. The overall purity of lot A012538501 was determined to be approximately 100%.

Stability studies of the bulk chemical were performed using HPLC. These studies indicated that formamide was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at 25° C or 5° C in sealed amber glass bottles. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing formamide with deionized water to give the required concentration (Table I2). Because the dose formulations were solutions, no homogeneity studies were performed. Stability studies of a 0.6 mg/mL dose formulation of a lot not used in the animal studies were performed using HPLC. Stability was confirmed for at least 50 days for dose formulations stored in the dark in glass containers under ambient and refrigerated conditions, and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of formamide were conducted by the study laboratory using HPLC. During the 2-week studies, the dose formulations were analyzed once; all five dose formulations for rats and mice were within 10% of the target concentrations (Table I3). During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies (Table I4). All 15 dose formulations analyzed for rats and mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months (Table I5). All dose formulations analyzed for rats (27) and mice (30) were within 10% of the target concentrations.

2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 (rats) or 12 (mice) days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice received formamide in deionized water by gavage at doses of 0, 160, 312, 625, 1,250, or 2,500 mg/kg body weight, 5 days per week for a total of 12 doses; vehicle control animals received deionized water only. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed

individually. Clinical findings were recorded daily and at necropsy for rats and mice. The animals were weighed initially, on day 8, and at the end of the studies. At the beginning of the studies, serologic analyses were performed on two male and two female rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K). Details of the study design and animal maintenance are summarized in Table 1. Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were not performed because no gross lesions were observed at necropsy.

In the rat study, all males and females administered 312, 625, 1,250, or 2,500 mg/kg died or were killed moribund before the end of the study. Final mean body weights and mean body weight gains of 160 mg/kg males and females were significantly less than those of the vehicle controls. The absolute right kidney and liver weights of 160 mg/kg females were significantly less than those of the vehicle controls (Table G1).

In the mouse study, all 2,500 mg/kg males and females and 1,250 mg/kg females were sacrificed moribund on day 2 of the study. Final mean body weights and mean body weight gains of 160 mg/kg males and females were significantly less than those of the vehicle controls. The absolute and relative thymus weights of 160 mg/kg females were significantly less than those of the vehicle controls.

3-Month Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Rats were quarantined for 11 (females) or 12 (males) days and mice for 13 (females) or 14 (males) days; animals were 5 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 for evidence of disease. Serological analyses were performed on five male and four or five female rats and mice at the beginning of the 3-month studies, 4 weeks after study start, and at the end of the studies using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 25 male and 25 female rats and 15 male and 15 female mice received formamide in deionized water by gavage at doses of 0, 10, 20, 40, 80, or 160 mg/kg, 5 days per week for up to 14 weeks; control animals received the deionized water vehicle only. Ten male and 10 female rats and mice were used for core studies, five male and five female rats and mice for plasma concentration studies, and 10 male and 10 female rats for clinical pathology studies. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded and animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Hematology and clinical chemistry analyses were performed on clinical pathology study rats on days 4 and 23 and on all core study rats at study termination; clinical pathology study rats were discarded after day 23. At the end of the 3-month study, hematology analyses were performed on core study mice. At all time points, rats and mice were anesthetized with a CO_2/O_2 mixture, and blood was drawn from the retroorbital sinus. The parameters measured are listed in Table 1. Blood samples for hematology were placed in tubes containing potassium EDTA as the anticoagulant. Erythrocyte, platelet, and leukocyte counts; hematocrit value; hemoglobin concentration; and mean cell volume, hemoglobin, and hemoglobin concentration were measured using a Cell-Dyn® automated cell counter (Abbott Diagnostics, Santa Clara, CA). Leukocyte differentials, nucleated erythrocyte counts, and morphological evaluation of blood cells 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). Smears made from preparations of equal volumes of new methylene blue (Sigma Chemical Company, St. Louis, MO) and whole blood were examined microscopically using the Miller disc method for the quantitative determination of reticulocytes. Samples for clinical chemistry analyses were collected into microcollection serum separator tubes, allowed to clot at room temperature, and the serum was obtained by centrifugation. Samples were analyzed using a Hitachi 911® chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) with reagents obtained from the manufacturer.

After dosing on days 9 (rats only), 23, and 93, five male and five female plasma study rats and mice were anesthetized with a CO_2/O_2 mixture, blood was drawn from the retroorbital sinus and placed into heparinized tubes, and the tubes were centrifuged. The plasma was shipped

to Midwest Research Institute (Kansas City, MO) for analysis. Gas chromatography using thermionic specific detection and an acetone extraction method was used to assay the concentration of formamide in the fortified plasma samples.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on core study rats and mice administered 0, 20, 40, or 80 mg/kg. 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 spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulf-oxide. 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 160 mg/kg animals at the end of the studies. The epididymis, kidney, and testis of male rats, testis of male mice, and gallbladder, pancreas, and spleen, of male and female mice were examined in the remaining dosed groups. Table 1 lists the tissues and organs routinely examined.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were administered formamide in deionized water by gavage at doses of 0, 20, 40, or 80 mg/kg, 5 days per week for 104 to 105 weeks. Formulations were administered at a volume of 5 mL/kg (rats) or 10 mL/kg (mice) and were calculated based on each animal's most recent body weight.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats were quarantined for 12 (females) or 13 (males) days, and mice were quarantined for 20 (females) or 21 (males) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 6 to 7 weeks old at the beginning of the studies, and mice were 7 to 8 weeks old. Serum samples were obtained from four or five male and female sentinel rats and mice at 6, 12, and 18 months and from five male and female 80 mg/kg rats and mice at the end of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

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

Clinical Examinations and Pathology

Animals were observed twice daily. Clinical findings were recorded every 4 weeks beginning with week 5 and

at the end of the studies. Body weights were recorded on day 1, weekly for the first 13 weeks, at 4-week intervals thereafter, and at terminal sacrifice.

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 (except for the eyes, which were fixed in Davidson's solution before being transferred to 10% neutral buffered formalin), processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

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

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

Table 1
Experimental Design and Materials and Methods in the Gavage Studies of Formamide

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
Rats: 11 days Mice: 12 days	Rats: 11 (females) or 12 (males) days Mice: 13 (females) or 14 (males) days	Rats: 12 (females) or 13 (males) days Mice: 20 (females) or 21 (males) days
Average Age When Studies Began		
6 weeks	5 to 7 weeks	Rats: 6 to 7 weeks Mice: 7 to 8 weeks
Date of First Dose		
Rats: June 9, 1997 Mice: June 10, 1997	Rats: October 13 (females) or 14 (males), 1997 Mice: October 15 (females) or 16 (males), 1997	Rats: March 20 (females) or 21 (males), 2001 Mice: October 3 (females) or 4 (males), 2001
Duration of Dosing		
5 days/week for 16 days	5 days/week for 14 weeks	Rats: 5 days/week for 104 (males) or 105 (females) weeks Mice: 5 days/week for 104 (females) or 105 (males) weeks
Date of Last Dose		
Rats: June 24, 1997 Mice: June 25, 1997	Rats: January 12 (females) or 13 (males), 1998 Mice: January 14 (females) or 15 (males), 1998	Rats: March 17 (males) or 20 (females), 2003 Mice: September 30 (females) or October 2 (males), 2003
Necropsy Dates		
Rats: June 25, 1997 Mice: June 26, 1997	Rats: January 13 (females) or 14 (males), 1998 Mice: January 15 (females) or 16 (males), 1998	Rats: March 17-18 (males) or 19-21 (females), 2003 Mice: September 29 to October 1 (females) or October 1-3 (males), 2003
Average Age at Necropsy		
8 weeks	19 to 20 weeks	Rats: 109-111 weeks Mice: 110-112 weeks
Size of Study Groups		
5 males and 5 females	Core: 10 male and 10 female rats and mice Plasma concentration: 5 male and 5 female rats and mice Clinical pathology: 10 male and 10 female rats	50 males and 50 females

Table 1
Experimental Design and Materials and Methods in the Gavage Studies of Formamide

2-Week Studies	3-Month Studies	2-Year Studies
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage		
Rats: 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)
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 2-week studies	Same as 2-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 2-week studies	Same as 2-week studies
Cages		
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed twice weekly for rats and female mice and weekly for male mice	Same as 2-week studies	Same as 2-week studies, except manufactured in Seaford, DE
Bedding		
Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products, Corp., Montville, NJ), changed twice weekly for rats and female mice and weekly for male mice	Same as 2-week studies	Same as 2-week studies
Cage Filters		
Spun-bonded DuPont 2024 polyester (Snow Filtration, Co., Cincinnati, OH)	Same as 2-week studies, changed every 2 weeks	Same as 3-month studies
Racks		
Stainless steel (Lab Products, Seaford, DE)	Same as 2-week studies, changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 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, 160, 312, 625, 1,250, or 2,500 mg/kg in deionized water by gavage (dosing volume=5 mL/kg body weight for rats or 10 mL/kg for mice)	0, 10, 20, 40, 80, or 160 mg/kg in deionized water by gavage (dosing volume=5 mL/kg for rats or 10 mL/kg for mice)	0, 20, 40, or 80 mg/kg in deionized water by gavage (dosing volume=5 mL/kg for rats or 10 mL/kg for mice)

Table 1
Experimental Design and Materials and Methods in the Gavage Studies of Formamide

2-Week Studies	3-Month Studies	2-Year Studies
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for the first 13 weeks, at 4-week intervals thereafter, and at the end of the studies; clinical findings were recorded every four weeks beginning with week 5 and at the end of the studies.
Method of Sacrifice		
CO_2 asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all 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 clinical pathology 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 core study mice at the end of the study for hematology. ***Hematology:*** automated hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None

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

2-Week Studies	3-Month Studies	2-Year Studies
Histopathology		
None	Complete histopathology was performed on core study vehicle control and 160 mg/kg animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone (including marrow), brain, clitoral gland, esophagus, gallbladder (mice), heart (including aorta), large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung (and mainstem bronchi), 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. The epididymis, kidney, and testis of male rats, testis of male mice, and gallbladder, pancreas, and spleen of male and female mice were examined in the remaining dosed groups.	Complete histopathology was performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone (including marrow), brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart (including aorta), large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes [mandibular (mice only) and mesenteric], mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from core study males in the 0, 20, 40, and 80 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from core study females in the 0, 20, 40, and 80 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
Determinations of Formamide in Plasma		
None	On days 9 (rats only), 23, and 93, five male and five female plasma study rats and mice were anesthetized with a CO_2/O_2 mixture, and blood was drawn from the retroorbital sinus for determination of formamide concentrations in plasma.	None

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored 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, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., 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 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, plasma concentration, 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.

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 formamide was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, sex-linked recessive lethal mutations in *Drosophila melanogaster*, 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.

An analysis of the association between results in the *Drosophila* sex-linked recessive lethal (SLRL) assay and the results in rodent cancer bioassays revealed that a positive response in the *Drosophila* assay is highly predictive of rodent carcinogenicity, but only a small portion of demonstrated rodent carcinogens, and chemicals that are identified as mutagens in other assays, are detected in the *Drosophila* assay; thus, the SLRL assay demonstrates high specificity but poor sensitivity for both potential rodent carcinogens and known mutagens (Foureman *et al.*, 1994).

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

RESULTS

RATS

3-MONTH STUDY

Based on the acute toxic effects observed in male and female rats administered 312 mg/kg or greater for 2 weeks, 10, 20, 40, 80, and 160 mg/kg were selected as the doses for the 3-month study. All core study rats survived to the end of the study (Table 2). Final mean body weights of 80 and 160 mg/kg males and females and 40 mg/kg females and mean body weight gains of 80 and 160 mg/kg males and females were significantly less than those of the vehicle controls. By day 29 of the study, thinness was observed in 160 mg/kg males and 80 and 160 mg/kg females. No other clinical findings were noted.

TABLE 2
Survival and Body Weights of Rats in the 3-Month Gavage Study of Formamide

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	107 ± 3	343 ± 5	236 ± 7	
10	10/10	108 ± 3	348 ± 6	240 ± 4	101
20	10/10	107 ± 4	343 ± 6	237 ± 3	100
40	10/10	105 ± 5	337 ± 8	232 ± 4	98
80	10/10	105 ± 3	317 ± 7**	213 ± 5**	93
160	10/10	104 ± 4	259 ± 5**	155 ± 5**	76
Female					
0	10/10	92 ± 3	188 ± 3	96 ± 3	
10	10/10	91 ± 3	191 ± 4	100 ± 3	101
20	10/10	92 ± 3	184 ± 3	92 ± 3	98
40	10/10	90 ± 3	177 ± 3*	87 ± 4	94
80	10/10	92 ± 4	175 ± 4*	83 ± 3**	93
160	10/10	91 ± 3	150 ± 5**	59 ± 3**	80

* 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 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

Hematology and clinical chemistry data for rats in the 3-month study are shown in Tables 3 and F1. On day 23 and at week 14, there was a dose-related increase in the erythron, evidenced by increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. On day 23, the erythron increase occurred in 80 and 160 mg/kg females and 160 mg/kg males, but at week 14, groups administered 40 mg/kg or greater were affected. The greatest magnitude increase occurred in hematocrit values (approximately 12%) and hemoglobin concentrations (approximately 15%) at week 14 in the 160 mg/kg groups. At week 14, the increase in the erythron was accompanied by increases in erythrocyte size, evidenced by 6% or less increases in mean cell volume in 40 mg/kg females and 80 and 160 mg/kg males and females. The erythron increase was not accompanied by any alteration in reticulocyte counts. However, there were apparent, albeit inconsistent, increases in nucleated erythrocyte counts in the 80 and 160 mg/kg groups. On day 23 and at week 14, there were apparent increases in segmented neutrophil counts in the 80 and 160 mg/kg groups that could suggest an inflammatory response or an alteration in the distribution of the neutrophils from the marginal pool to the circulating neutrophil pool. Platelet counts demonstrated small decreases in various higher dosed groups at all time points. The platelet count decreases are not considered to be clinically significant and may also indicate an alteration in peripheral distribution from circulating to marginal platelet pools. On day 23 (females) and at week 14 (males and females), there were small (<10%) decreases in albumin and total protein concentrations in the 160 mg/kg groups that were probably related to the marked decrease in body weight gain for the 160 mg/kg groups.

TABLE 3
Selected Hematology Data for Rats in the 3-Month Gavage Study of Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Male						
n						
Day 4	9	9	10	10	10	10
Day 23	8	10	10	10	10	9
Week 14	10	10	9	10	10	10
Automated hematocrit (%)						
Day 4	40.6 ± 0.3	41.7 ± 0.6	41.2 ± 0.6	40.2 ± 0.6	40.5 ± 0.8	38.9 ± 0.4
Day 23	44.2 ± 0.9	44.1 ± 0.7	45.7 ± 0.6	45.4 ± 0.5	45.4 ± 0.4	48.2 ± 0.4**
Week 14	45.0 ± 0.4	44.7 ± 0.2	44.9 ± 0.5	46.5 ± 0.3*	47.4 ± 0.3**	50.1 ± 0.4**
Hemoglobin (g/dL)						
Day 4	13.6 ± 0.1	13.9 ± 0.2	13.8 ± 0.3	13.5 ± 0.2	13.6 ± 0.3	13.3 ± 0.2
Day 23	14.9 ± 0.2	14.9 ± 0.2	15.2 ± 0.2	15.4 ± 0.2	15.3 ± 0.2	16.5 ± 0.2**
Week 14	15.0 ± 0.1	15.0 ± 0.1	15.0 ± 0.2	15.5 ± 0.1**	15.9 ± 0.1**	17.1 ± 0.1**
Erythrocytes (10^6/μL)						
Day 4	6.94 ± 0.05	7.20 ± 0.13	7.09 ± 0.13	6.88 ± 0.12	7.00 ± 0.16	6.80 ± 0.08
Day 23	7.67 ± 0.15	7.62 ± 0.15	7.87 ± 0.13	7.84 ± 0.09	7.77 ± 0.09	8.48 ± 0.07**
Week 14	8.50 ± 0.06	8.40 ± 0.05	8.52 ± 0.09	8.77 ± 0.06*	8.81 ± 0.08*	8.91 ± 0.06**
Reticulocytes (10^6/μL)						
Day 4	0.22 ± 0.02	0.25 ± 0.04	0.26 ± 0.04	0.25 ± 0.03	0.27 ± 0.03	0.23 ± 0.02
Day 23	0.13 ± 0.02	0.17 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	0.16 ± 0.01
Week 14	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 4	0.05 ± 0.02	0.09 ± 0.03	0.08 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.17 ± 0.05
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0.03 ± 0.02
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.01**	0.03 ± 0.01*
Mean cell volume (fL)						
Day 4	58.5 ± 0.1	57.9 ± 0.5	58.1 ± 0.2	58.5 ± 0.3	57.9 ± 0.2*	57.2 ± 0.3**
Day 23	57.7 ± 0.2	57.9 ± 0.4	58.0 ± 0.3	57.9 ± 0.3	58.5 ± 0.4	56.9 ± 0.4
Week 14	52.9 ± 0.3	53.2 ± 0.1	52.7 ± 0.2	53.1 ± 0.2	53.8 ± 0.2**	56.2 ± 0.2**

TABLE 3
Hematology Data for Rats in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Female						
n						
Day 4	8	10	10	10	10	10
Day 23	10	8	9	10	10	8
Week 14	10	10	10	10	10	10
Automated hematocrit (%)						
Day 4	42.1 ± 0.8	41.4 ± 0.5	40.7 ± 0.8	40.4 ± 0.5	40.9 ± 0.4	40.4 ± 0.3
Day 23	44.1 ± 0.5	44.2 ± 0.7	44.8 ± 0.9	45.7 ± 0.5	46.5 ± 0.5**	48.7 ± 0.4**
Week 14	43.2 ± 0.4	43.8 ± 0.3	43.6 ± 0.4	45.1 ± 0.3**	46.8 ± 0.6**	48.8 ± 0.4**
Hemoglobin (g/dL)						
Day 4	14.1 ± 0.3	14.0 ± 0.2	13.7 ± 0.3	13.7 ± 0.2	13.9 ± 0.1	13.9 ± 0.1
Day 23	15.1 ± 0.1	15.1 ± 0.2	15.2 ± 0.3	15.5 ± 0.1	16.0 ± 0.2**	16.8 ± 0.1**
Week 14	14.4 ± 0.1	14.6 ± 0.1	14.6 ± 0.2	15.1 ± 0.1**	15.5 ± 0.2**	16.6 ± 0.2**
Erythrocytes (10^6/µL)						
Day 4	7.23 ± 0.13	7.09 ± 0.10	6.93 ± 0.15	6.86 ± 0.09	7.05 ± 0.06	7.09 ± 0.07
Day 23	7.58 ± 0.10	7.68 ± 0.14	7.65 ± 0.18	7.88 ± 0.09	8.05 ± 0.11**	8.62 ± 0.08**
Week 14	7.64 ± 0.07	7.73 ± 0.06	7.71 ± 0.06	7.85 ± 0.05*	8.07 ± 0.09**	8.27 ± 0.10**
Reticulocytes (10^6/µL)						
Day 4	0.25 ± 0.02	0.26 ± 0.01	0.29 ± 0.02	0.27 ± 0.01	0.28 ± 0.02	0.22 ± 0.02
Day 23	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.12 ± 0.02	0.11 ± 0.02
Week 14	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Nucleated erythrocytes (10^3/µL)						
Day 4	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.07 ± 0.02	0.02 ± 0.01	0.15 ± 0.03*
Day 23	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.11 ± 0.02**	0.09 ± 0.04**
Week 14	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02
Mean cell volume (fL)						
Day 4	58.3 ± 0.4	58.4 ± 0.2	58.7 ± 0.2	58.9 ± 0.3	58.1 ± 0.5	57.1 ± 0.3
Day 23	58.3 ± 0.3	57.6 ± 0.5	58.6 ± 0.2	57.9 ± 0.2	57.8 ± 0.3	56.5 ± 0.4**
Week 14	56.5 ± 0.1	56.7 ± 0.1	56.6 ± 0.1	57.4 ± 0.1**	58.0 ± 0.1**	59.0 ± 0.3**

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

The concentration of formamide in the plasma of male and female rats was monitored on days 9, 23, and 90 (Table 4). Plasma concentrations increased linearly with increasing dose at each time point (Figure 2). However, at 40 mg/kg or greater, there was a trend of increasing plasma concentration with duration of dosing (Figure 3).

No biologically significant organ weight changes were observed (Table G2). The number of spermatid heads per gram of cauda in 20 mg/kg males was significantly less than that of the vehicle controls (Table H1). Females administered 40 mg/kg differed significantly from the vehicle controls in the relative time spent in the estrous stages (Table H2).

TABLE 4
Formamide Concentrations in Plasma of Rats in the 3-Month Gavage Study of Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
n	5	5	5	5	5	5
Male						
Day 9	1.120 ± 0.203	15.240 ± 1.087**	31.200 ± 1.991**	49.420 ± 2.775**	113.460 ± 3.504**	243.000 ± 9.848**
Day 23	0.920 ± 0.460	16.020 ± 1.376**	33.420 ± 2.053**	59.440 ± 2.846**	147.820 ± 3.093**	253.340 ± 10.555**
Week 14	0.680 ± 0.218	20.460 ± 0.857**	39.940 ± 0.700**	82.480 ± 3.358**	182.200 ± 8.504**	293.900 ± 19.395**
Female						
Day 9	0.660 ± 0.206	15.940 ± 1.142**	28.400 ± 1.048**	58.180 ± 5.970**	112.560 ± 4.261**	240.380 ± 10.283**
Day 23	1.840 ± 0.262	17.825 ± 0.457*	26.420 ± 0.591**	49.700 ± 2.804**	119.600 ± 3.265**	223.200 ± 23.245**
Week 14	1.200 ± 0.045	19.520 ± 1.384**	35.340 ± 0.725**	76.660 ± 2.867**	146.650 ± 5.812**	297.960 ± 12.037**

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

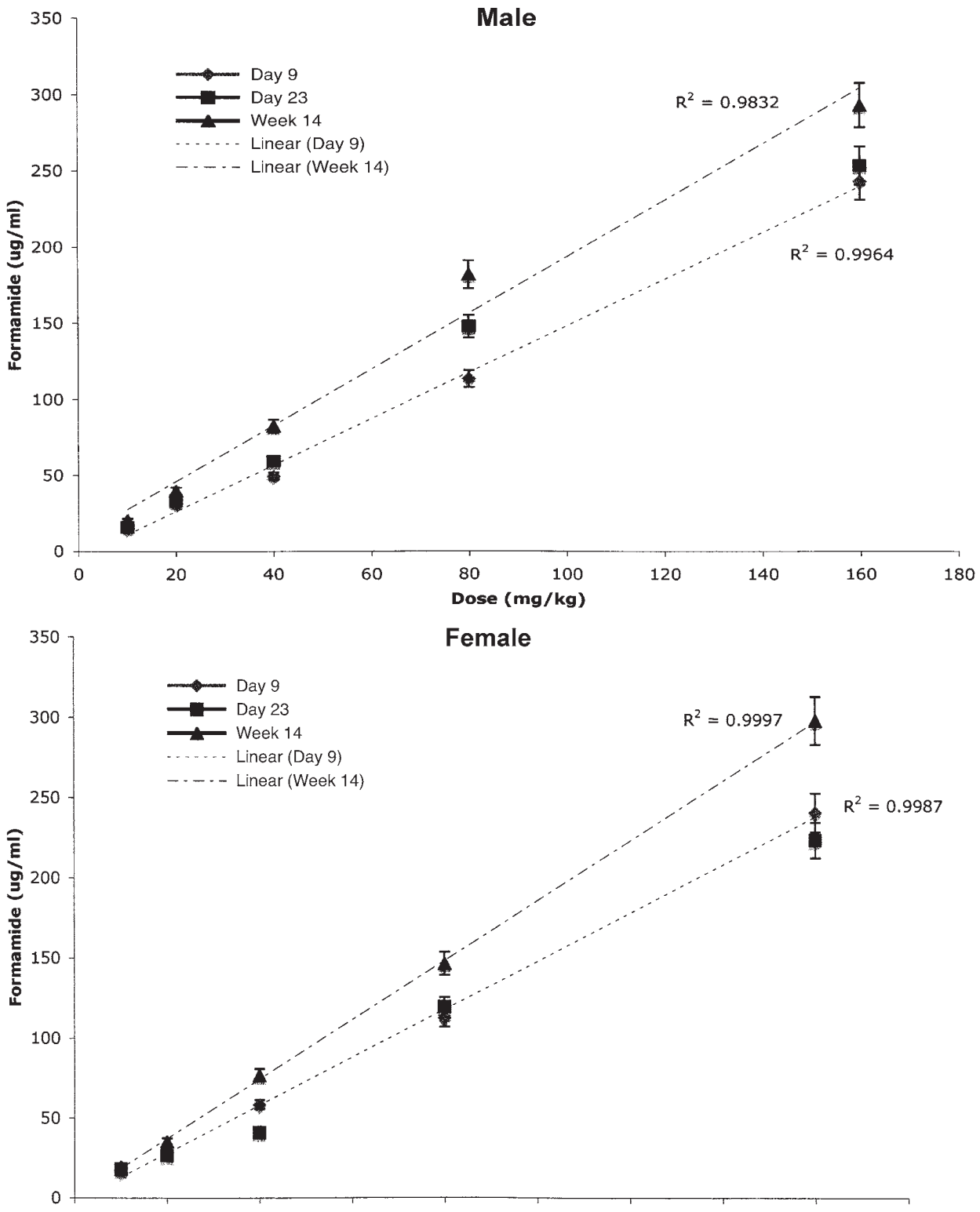


FIGURE 2
Plasma Concentrations of Formamide by Dose in Rats Administered Formamide by Gavage for 3 Months

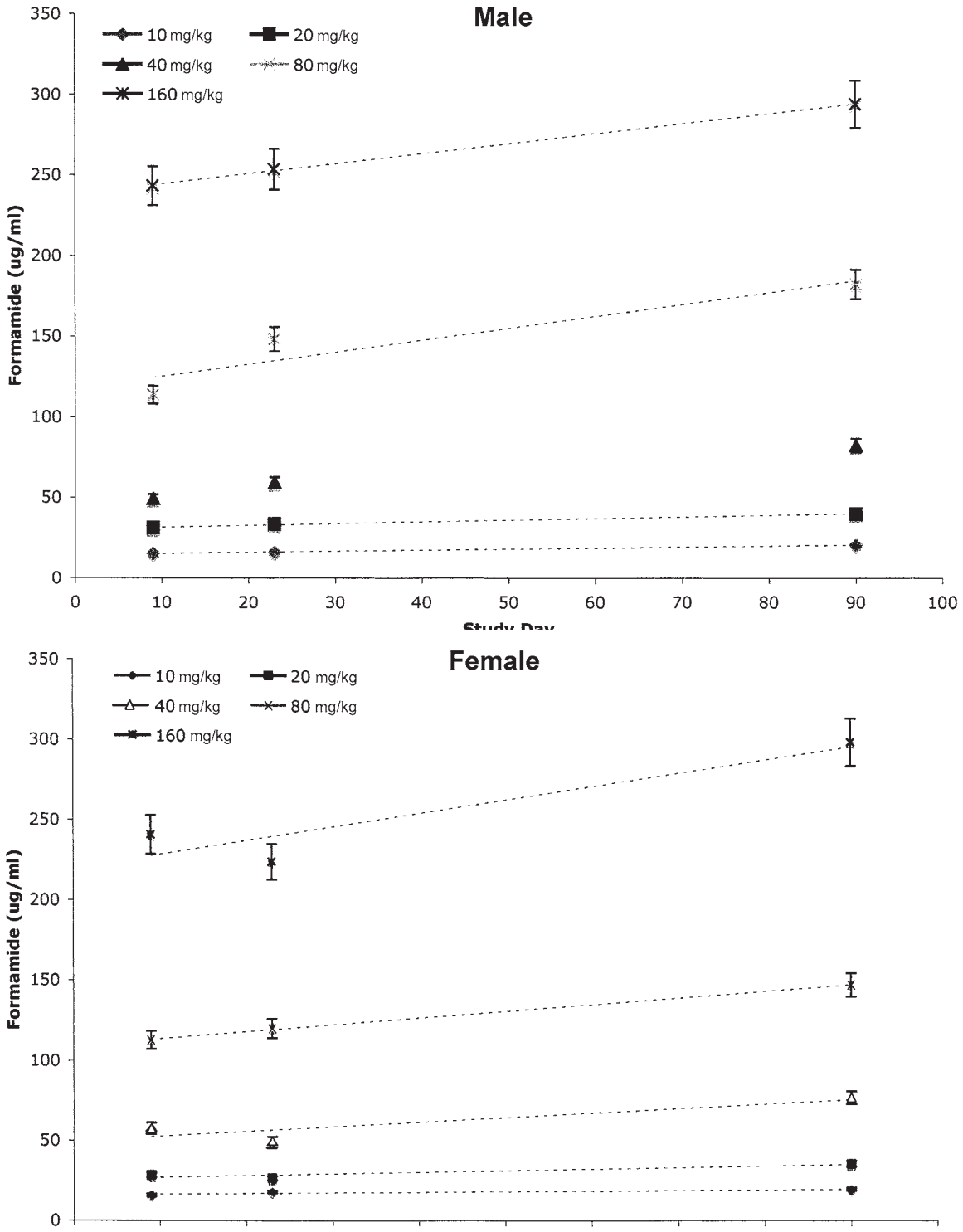


Figure 3
Plasma Concentrations of Formamide by Study Day in Rats Administered Formamide by Gavage for 3 Months

The only microscopic findings attributed to treatment occurred in the testis and epididymis (Table 5). There was minimal to mild degeneration of the germinal epithelium in the seminiferous tubules of the testis in seven 160 mg/kg males. This lesion was characterized by several changes including vacuolization, degeneration, and necrosis of spermatocytes and spermatids; disruption of the orderly appearance of the germinal epithelium; and retention of elongated spermatids. These lesions were observed in scattered individual or small clusters of seminiferous tubules. The epididymides of nine 160 mg/kg males contained a minimal increase above background levels of degenerate cells. These cells appeared to be degenerative (exfoliated) germinal epithelial cells (from the testes) often fused into multinucleated forms within the lumen of the epididymal tubules.

Dose Selection Rationale: Based on the decreased body weights in males and females administered 160 mg/kg, the highest dose selected for the 2-year study in rats was 80 mg/kg.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Male Rats in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Testis[a]	10	10	10	10	10	10
Germinal Epithelium Degeneration[b]	0	0	0	0	0	7** (1.3)[c]
Epididymis	10	10	10	10	10	10
Germinal Epithelium Degeneration	0	1 (1.0)	0	0	0	9** (1.0)

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

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 4). Survival of all dosed groups of rats was similar to that of the vehicle controls.

Body Weights and Clinical Findings

Mean body weights of 80 mg/kg males were generally less than those of the vehicle controls after week 17, and mean body weights of 40 and 80 mg/kg females were less than those of the vehicle controls after weeks 65 and 45, respectively (Tables 7 and 8; Figure 5).

Table 6
Survival of Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	24	15	22	18
Natural deaths	0	8	2	3
Animals surviving to study termination	26	27	26	29
Percent probability of survival at end of study[a]	52	54	52	58
Mean survival (days)[b]	673	675	678	684
Survival analysis[c]	P=0.609N	P=1.000	P=1.000N	P=0.690N
Female				
Animals initially in study	50	50	50	50
Moribund	10	16	14	14
Natural deaths	2	4	2	4
Animals surviving to study termination	38	30[d]	34	32
Percent probability of survival at end of study	76	60	68	64
Mean survival (days)	703	670	692	684
Survival analysis	P=0.467	P=0.098	P=0.491	P=0.261

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] 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**.
[d] Includes one animal that died during the last week of the study

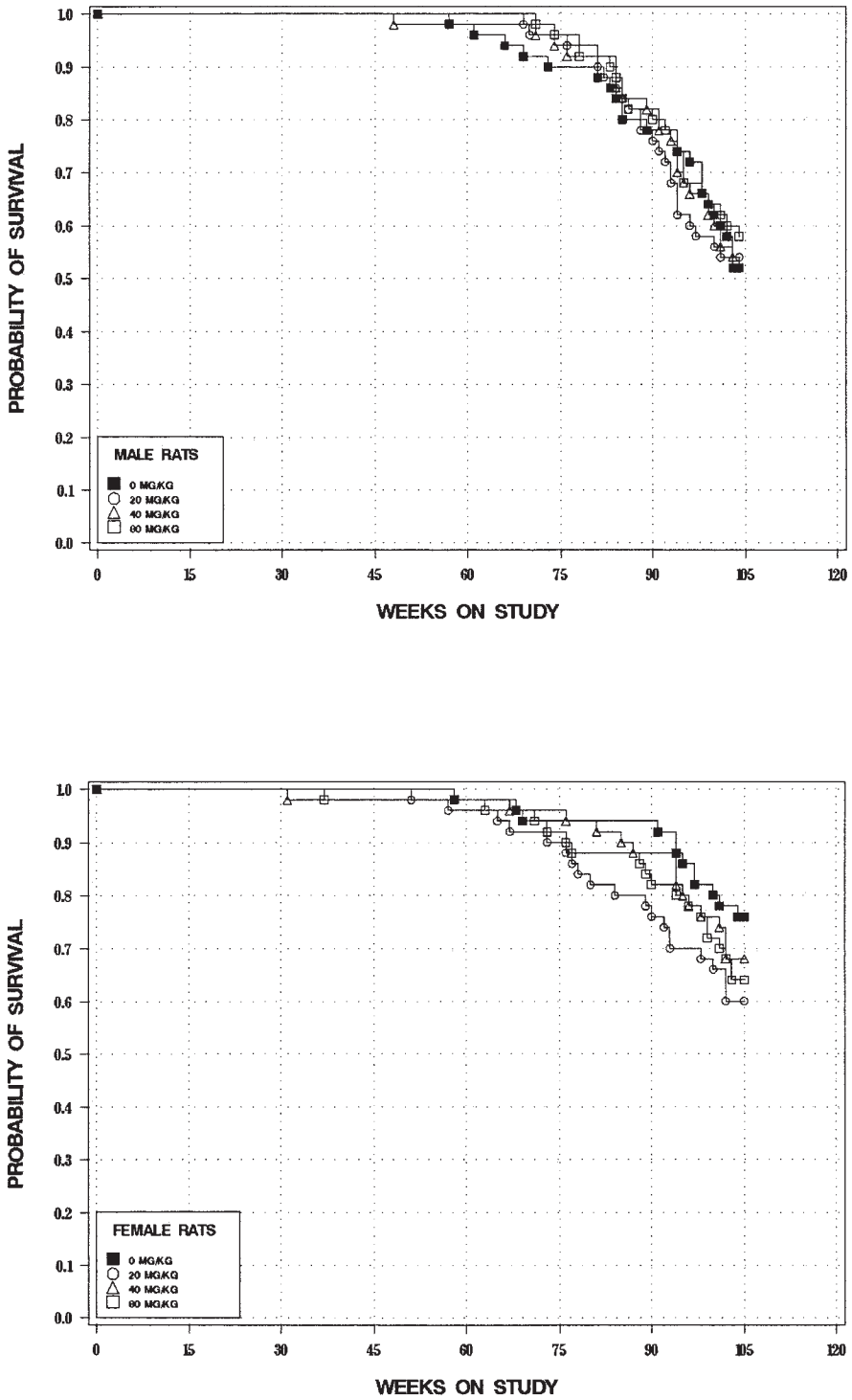


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Rats Administered Formamide by Gavage for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Formamide

Weeks on Study	Vehicle Control		20 mg/kg			40 mg/kg			80 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	122	50	123	100	50	124	101	50	123	101	50
2	153	50	153	100	50	154	101	50	151	99	50
3	189	50	189	100	50	190	101	50	185	98	50
4	216	50	215	100	50	216	100	50	210	97	50
5	241	50	240	100	50	241	100	50	233	97	50
6	261	50	259	99	50	260	100	50	252	96	50
7	272	50	275	101	50	276	101	50	267	98	50
8	288	50	291	101	50	293	102	50	281	98	50
9	303	50	304	100	50	307	101	50	293	97	50
10	314	50	316	100	50	316	100	50	302	96	50
11	325	50	328	101	50	327	101	50	313	96	50
12	335	50	338	101	50	336	100	50	321	96	50
13	343	50	346	101	50	345	100	50	329	96	50
17	379	50	378	100	50	376	99	50	359	95	50
21	400	50	401	100	50	400	100	50	377	94	50
25	423	50	424	100	50	423	100	50	398	94	50
29	437	50	438	100	50	437	100	50	413	94	50
33	450	50	455	101	50	454	101	50	430	96	50
37	465	50	469	101	50	466	100	50	442	95	50
41	474	50	480	101	50	478	101	50	455	96	50
45	482	50	487	101	50	483	100	50	459	95	50
49	487	50	492	101	50	490	101	49	464	95	50
53	493	50	500	101	50	497	101	49	468	95	50
57	505	50	509	101	50	501	99	49	473	94	50
61	509	49	512	101	50	510	100	49	481	95	50
65	502	48	505	101	50	501	100	49	472	94	50
69	505	47	504	100	50	504	100	49	472	94	50
73	510	46	509	100	48	509	100	48	474	93	49
77	508	45	502	99	47	501	99	46	468	92	48
81	506	45	499	99	46	499	99	46	463	92	46
85	501	42	502	100	43	501	100	43	458	92	44
89	505	40	503	100	39	504	100	42	452	90	41
93	498	39	499	100	36	500	100	39	445	89	39
97	504	36	498	99	30	503	100	33	440	87	34
101	504	31	498	99	28	499	99	30	431	86	32
105	504	26	492	98	27	500	99	26	416	83	29
Mean for weeks											
1-13	259		260	100		260	101		251	97	
14-52	444		447	101		445	100		422	95	
53-105	504		502	100		502	100		458	91	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Formamide

Weeks on Study	Vehicle Control		20 mg/kg			40 mg/kg			80 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	95	50	96	101	50	94	99	50	96	101	50
2	114	50	115	101	50	113	99	50	114	100	50
3	131	50	131	100	50	127	97	50	128	98	50
4	144	50	144	100	50	140	97	50	139	97	50
5	155	50	155	100	50	150	97	50	149	96	50
6	167	50	165	99	50	160	96	50	158	95	50
7	175	50	173	99	50	167	96	50	166	95	50
8	179	50	178	100	50	173	97	50	171	96	50
9	183	50	182	100	50	177	97	50	174	95	50
10	187	50	185	99	50	181	97	50	178	95	50
11	191	50	189	99	50	184	97	50	180	95	50
12	194	50	191	99	50	187	97	50	184	95	50
13	197	50	194	98	50	189	96	50	187	95	50
17	208	50	206	99	50	201	97	50	196	94	50
21	218	50	217	99	50	211	97	50	206	94	50
25	226	50	225	100	50	220	97	50	216	95	50
29	235	50	234	100	50	228	97	50	222	95	50
33	243	50	242	100	50	235	96	49	232	95	50
37	254	50	254	100	50	248	98	49	242	95	50
41	262	50	260	99	50	254	97	49	248	95	49
45	271	50	268	99	50	261	97	49	256	95	49
49	281	50	277	99	50	267	95	49	262	93	49
53	293	50	287	98	49	277	95	49	271	92	49
57	310	50	300	97	49	290	94	49	279	90	49
61	319	49	310	97	48	299	94	49	288	90	49
65	318	49	310	98	48	303	96	49	289	91	48
69	326	48	315	97	46	303	93	48	291	89	48
73	333	47	320	96	46	310	93	48	298	89	47
77	337	47	322	96	44	317	94	47	302	90	44
81	341	47	328	96	41	319	94	47	304	89	44
85	345	47	331	96	40	321	93	46	308	89	44
89	349	47	335	96	40	327	94	44	306	88	43
93	347	46	334	96	37	326	94	44	306	88	41
97	352	42	338	96	35	330	94	39	305	87	39
101	358	40	341	95	33	329	92	37	309	86	36
105	352	38	332	94	29	324	92	34	301	85	32
Mean for weeks											
1-13	163		161	100		157	97		156	96	
14-52	244		243	99		236	97		231	95	
53-105	334		322	96		313	94		297	89	

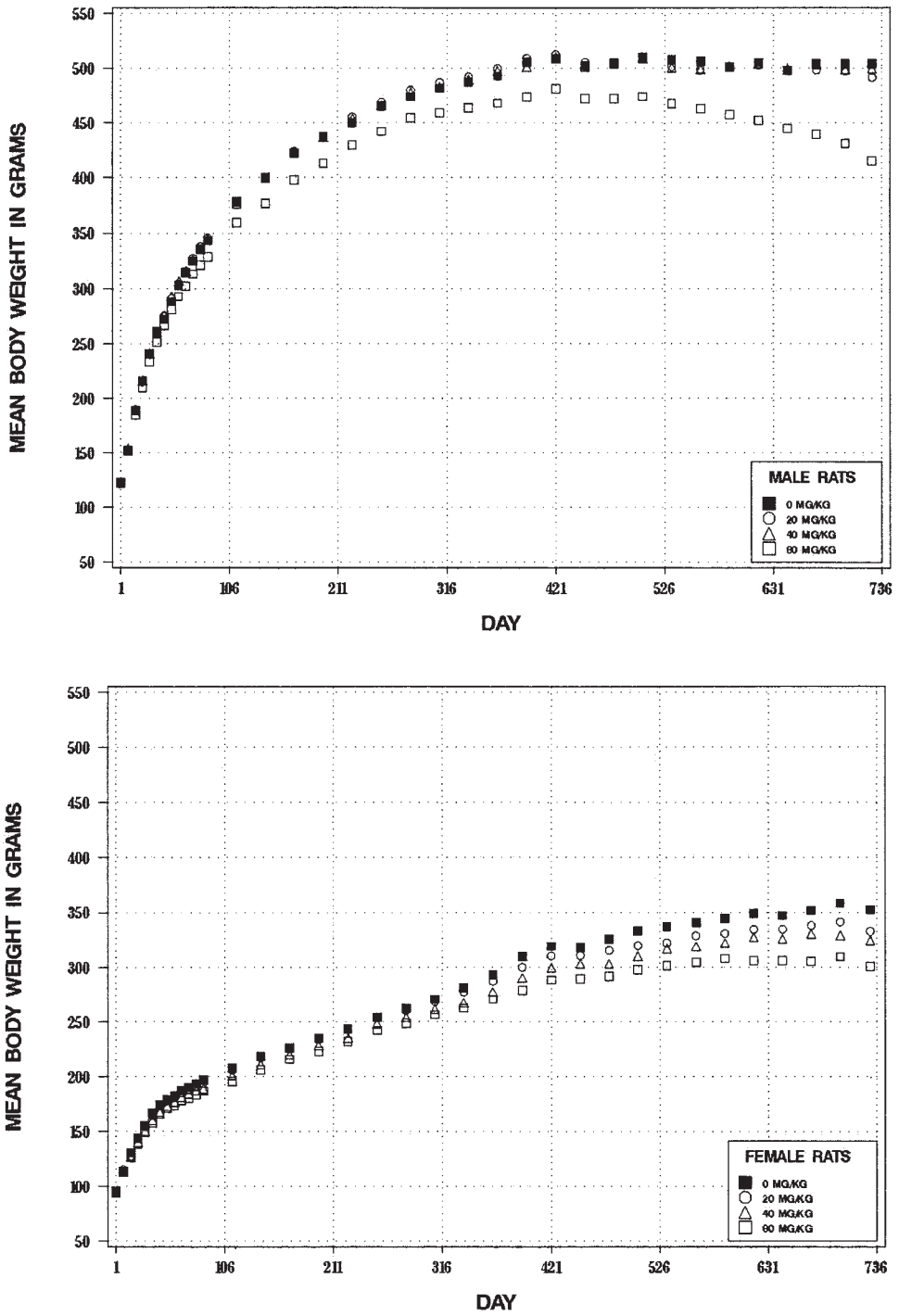


FIGURE 5
Growth Curves for Male and Female Rats Administered Formamide by Gavage for 2 Years

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 pituitary gland, skin, bone marrow, pancreas, and liver. 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.

Pituitary gland: There were increases in the incidences of pituitary gland (pars distalis) adenoma in dosed males (vehicle control, 11/50; 20 mg/kg, 15/50; 40 mg/kg, 21/50; 80 mg/kg, 18/50; Tables A1 and A2); however, the increase was significant only in the 40 mg/kg group, and the vehicle control incidence was lower than the mean NTP historical control incidence for all routes of administration [647/1,439 (46% ± 20%), range 12%-76%]. There were decreases in the incidences of pituitary gland (pars distalis) adenoma or carcinoma (combined) in dosed female rats (24/50, 21/50, 16/50, 15/50; Tables B1 and B2). The biological significance of the increased incidence of pituitary gland adenoma in males is uncertain, and the increase is not considered to be treatment related due to the low incidence in the controls and the decreased incidences of pituitary gland neoplasms in females.

Skin: There were decreases in the incidences of skin keratoacanthomas (8/50, 3/50, 4/50, 2/50) and skin fibroma or multiple fibroma (6/50, 4/50, 3/50, 2/50) in dosed males (Tables A1 and A2). The vehicle control incidences were relatively high compared to mean NTP historical control incidences for all routes of administration [keratoacanthoma: 81/1,449 (6% ± 5%), range 0%-20%; fibroma: 126/1,449 (9% ± 4%), range 2%-18%]. The biological significance of the minimal decreases in these skin lesions in males is uncertain but is not considered to be treatment related.

Other organs: The incidences of bone marrow hyperplasia in 80 mg/kg males (19/50, 24/50, 22/50, 29/50; Table A3) and pancreatic acinus atrophy in 80 mg/kg females (7/50, 9/50, 10/50, 14/50; Table B3) were significantly greater than those in the vehicle control groups. There were decreases in the incidences of oval cell hyperplasia of the liver in dosed males (23/50, 15/50, 20/50, 12/50; Table A3) and bile duct hyperplasia of the liver in dosed females (27/50, 28/50, 15/50, 16/50; Table B3). Other than the bone marrow hyperplasia, the biological significance of these slight increases and decreases is uncertain, and they are not considered to be treatment related. The bone marrow hyperplasia may have been a response to erythrocyte damage.

Mice

3-Month Study

Based on the acute toxic effects observed in male and female mice administered 312 mg/kg or greater for 2 weeks, 10, 20, 40, 80, and 160 mg/kg were selected as the doses for the 3-month study. All mice survived to the end of the study (Table 9). Final mean body weights of 80 and 160 mg/kg males and mean body weight gains of 40, 80, and 160 mg/kg males were significantly less than those of the vehicle controls. There were no clinical findings related to formamide administration.

Table 9
Survival and Body Weights of Mice in the 3-Month Gavage Study of Formamide

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	22.8 ± 0.4	38.4 ± 0.9	15.6 ± 0.7	
10	10/10	22.8 ± 0.6	38.3 ± 0.9	15.5 ± 0.7	100
20	10/10	22.7 ± 0.5	36.8 ± 1.5	14.1 ± 1.1	96
40	10/10	23.1 ± 0.5	35.8 ± 1.4	12.7 ± 1.1*	93
80	10/10	22.7 ± 0.4	34.4 ± 0.9*	11.7 ± 0.7**	90
160	10/10	23.0 ± 0.5	32.6 ± 0.8**	9.6 ± 0.5**	85
Female					
0	10/10	18.6 ± 0.2	29.1 ± 0.7	10.5 ± 0.5	
10	10/10	18.5 ± 0.3	30.4 ± 1.4	12.0 ± 1.2	105
20	10/10	18.4 ± 0.3	29.0 ± 1.2	10.6 ± 1.0	100
40	10/10	18.0 ± 0.4	28.3 ± 1.4	10.3 ± 1.2	97
80	10/10	17.7 ± 0.5	28.3 ± 0.8	10.6 ± 0.5	97
160	10/10	18.9 ± 0.3	26.5 ± 0.4	7.6 ± 0.3	91

* 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 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

The hematology data for mice are shown in Table F2. Similar to the rat study, apparent increases in segmented neutrophil counts occurred in 160 mg/kg males and females. Unlike the rat study, however, there were apparent small (<10%) decreases in hematocrit values and erythrocyte counts but not hemoglobin concentrations in 160 mg/kg males. Minimal (≤7%) increases in mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration values occurred in 80 and 160 mg/kg males and females. The mechanism for this apparent change is unknown, but the minimal nature would suggest that the clinical significance is questionable.

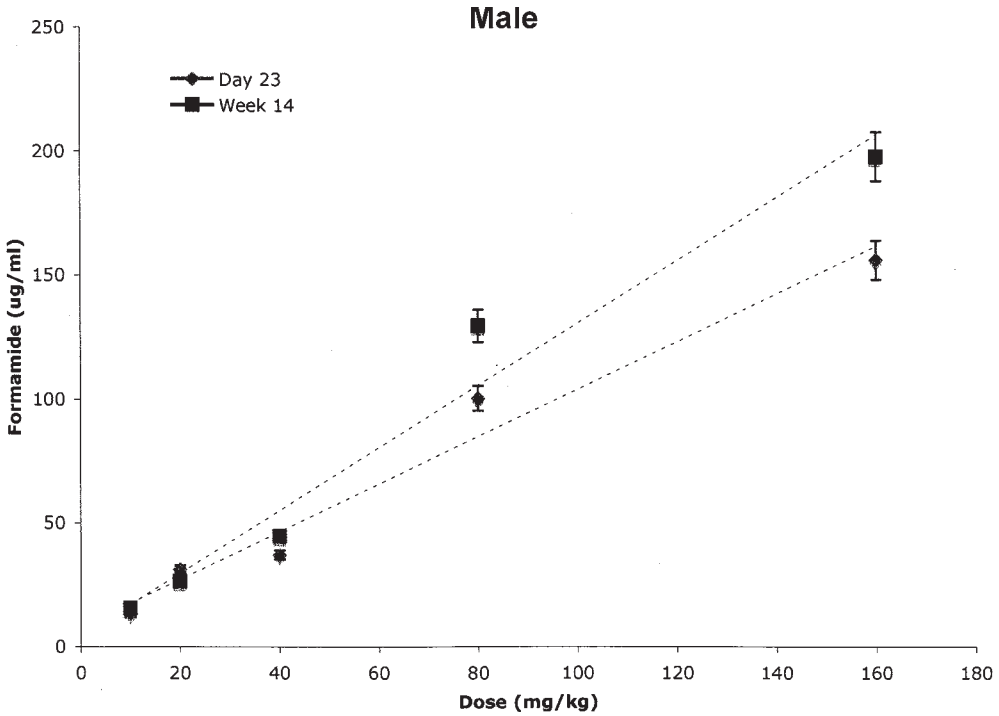
No biologically significant organ weight changes were observed (Table G4). No significant differences in reproductive parameters were observed between dosed and vehicle control groups of males (Table H3). Dosed females differed significantly from the vehicle controls in the relative time spent in the estrous stages (Table H4).

As observed with rats, formamide concentrations in mouse plasma increased linearly with increasing dose in males and females at both time points with a tendency for plasma concentrations to be higher in animals dosed the full 14 weeks (Table 10; Figure 6).

TABLE 10
Formamide Concentrations in Plasma of Mice in the 3-Month Gavage Study of Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
n	5	5	5	5	5	5
Male						
Day 23	1.340 ± 0.223	13.350 ± 0.574**[b]	31.400 ± 3.841**	37.125 ± 3.107**[b]	100.212 ± 3.066**	155.866 ± 6.209**
Week 14	0.220 ± 0.143	15.600 ± 0.547**	26.420 ± 2.000**	44.720 ± 1.788**	129.406 ± 29.825**	197.420 ± 28.220**
Female						
Day 23	0.860 ± 0.397	11.240 ± 1.223**	21.180 ± 0.767**	40.340 ± 0.815**	96.280 ± 4.228**	166.120 ± 5.367**
Week 14	0.060 ± 0.060	18.800 ± 0.367**	24.840 ± 0.519**	52.400 ± 2.464**	113.620 ± 3.764**	202.840 ± 8.903**

** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are given as mean (µg/mL) ± standard error. Statistical tests were performed on unrounded data.
[b] n=4



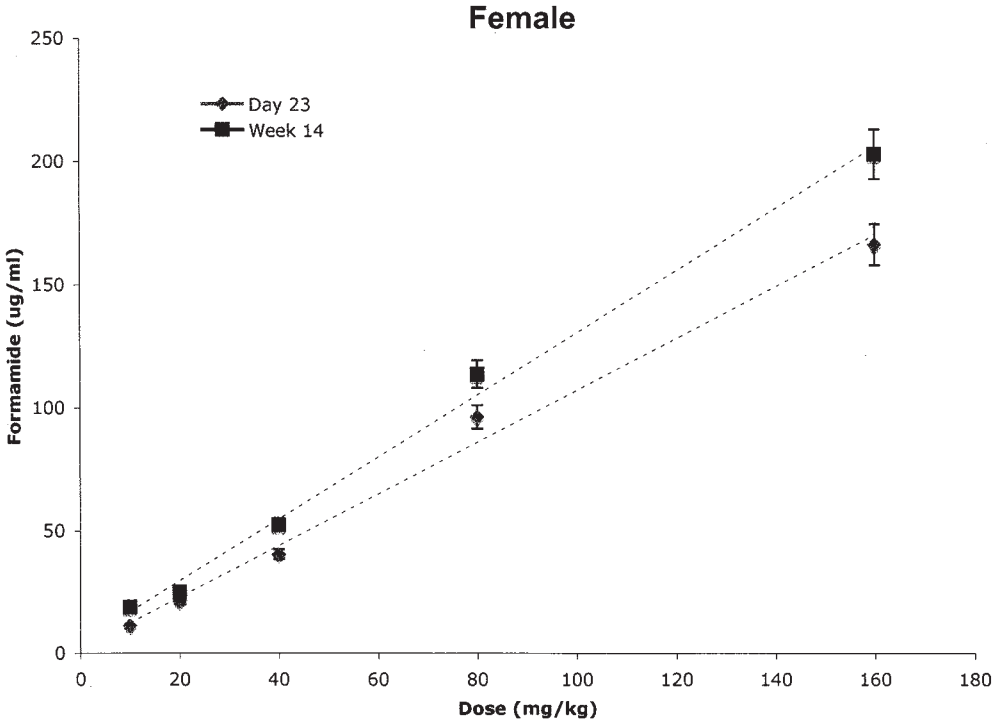


FIGURE 6
Plasma Concentrations of Formamide by Dose in Mice Administered Formamide by Gavage for 3 Months

All 160 mg/kg males had abnormal residual bodies within scattered sections of the testicular seminiferous tubules (Table 11; Plates 1 through 3). Residual bodies are the result of a separation of a cytoplasmic package from the developing spermatid, resulting in an approximately 25% reduction in spermatid volume. Residual bodies are formed at the time of sperm release as a lobe of cytoplasm is pinched off from the spermatid. The residual bodies contain packed RNA and other organelles and inclusions that were once used by the spermatid during development but are no longer necessary for sperm survival after the sperm are released. The residual bodies are phagocytized by the Sertoli cells and transported to the base of the tubule where they are digested and eliminated by the Sertoli cells. The abnormal appearance of the residual bodies in this study suggests that there may be aberrant cell membrane fusion resulting in larger residual bodies that are too large to be phagocytized by Sertoli cells. These abnormal residual bodies were characterized by a 5- to 20-fold increase in size compared to vehicle controls. Approximately one to eight of these large abnormal residual bodies were present within each stage VIII tubule, and overall, there were fewer residual bodies compared to vehicle controls. These abnormal residual bodies were mostly present within the tubule lumen; however, they were occasionally present within the germinal epithelium.

Lesions of the gallbladder occurred in 160 mg/kg males and females and included hyperplasia and cytoplasmic alteration of the epithelium and chronic active inflammation (Table 11). Hyperplasia was characterized by increased numbers of gallbladder epithelial cells. Increased numbers of mucous glands in the gallbladder tunica propria accompanied the epithelial hyperplasia. The cytoplasmic alteration of gallbladder epithelium consisted of a uniform eosinophilia of the cytoplasm, suggesting the accumulation of protein. Chronic active inflammation of the gallbladder consisted of infiltration of the tunica propria of the gallbladder and common bile duct by neutrophils and mononuclear inflammatory cells.

Pancreatic lesions occurred in 40, 80, and 160 mg/kg males and females (Table 11) and consisted of hyperplasia, cytoplasmic alteration, and chronic active inflammation within the duct epithelium. The appearance of hyperplasia and cytoplasmic alteration in the pancreatic duct epithelium was identical to that in the gallbladder epithelium. Chronic active inflammation was characterized by infiltration of the tunica propria of the duct with polymorphonuclear inflammatory cells, increased numbers of mononuclear inflammatory cells, ulceration of the epithelial lining (in some cases), and fibrosis of the tunica propria.

Dose Selection Rationale: Based on increased incidences of lesions of the gallbladder and pancreas in males and females and decreased body weights in males administered 160 mg/kg, the highest dose selected for the 2-year study in mice was 80 mg/kg.

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg		20 mg/kg	40 mg/kg		80 mg/kg		160 mg/kg	
Male										
Testis[a]	10	10		10	10		10		10	
Abnormal Residual Bodies[b]	0	0		0	0		0		10**	(1.0)[c]
Gallbladder	10	9		9	10		9		10	
Epithelium, Hyperplasia	0	0		0	0		0		1	(2.0)
Epithelium, Cytoplasmic Alteration	0	0		0	0		0		2	(3.5)
Inflammation, Chronic Active	0	0		0	0		0		2	(1.0)
Pancreas	10	10		10	10		10		10	
Duct, Hyperplasia	0	0		0	1	(1.0)	3	(1.0)	6**	(1.5)
Duct, Cytoplasmic Alteration	0	0		0	0		2	(1.5)	3	(2.3)
Duct, Inflammation, Chronic Active	0	1	(1.0)	0	1	(1.0)	3	(1.0)	5*	(1.2)
Female										
Gallbladder	10	10		10	10		9		9	
Epithelium, Hyperplasia	0	0		0	0		0		2	(2.0)
Epithelium, Cytoplasmic Alteration	0	0		0	0		0		2	(2.0)
Inflammation, Chronic Active	0	0		0	0		0		2	(1.5)
Pancreas	10	10		10	10		10		10	
Duct, Hyperplasia	0	0		0	3	(1.0)	4*	(1.0)	5*	(2.0)
Duct, Cytoplasmic Alteration	0	0		0	0		0		5*	(2.6)
Duct, Inflammation, Chronic Active	0	1	(1.0)	0	2	(1.0)	4*	(1.3)	5*	(1.8)

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

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 7). Survival of all dosed groups of mice was similar to that of vehicle controls.

Body Weights and Clinical Findings

Mean body weights of 80 mg/kg males were generally less than those of the vehicle controls throughout the study; mean body weights of 40 and 80 mg/kg females were generally less than those of the vehicle controls after weeks 13 and 6, respectively (Figure 8; Tables 13 and 14). No clinical findings were attributed to the administration of formamide.

Table 12
Survival of Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	4	8	6	14
Natural deaths	7	0	8	3
Animals surviving to study termination	39	42	36	33
Percent probability of survival at end of study[a]	78	84	72	66
Mean survival (days)[b]	698	708	690	695
Survival analysis[c]	P=0.101	P=0.586N	P=0.680	P=0.290
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	0	1	0
Moribund	7	8	8	8
Natural deaths	5	3	10	3
Animals surviving to study termination	38	39[e]	31	39
Percent probability of survival at end of study	76	78	63	78
Mean survival (days)	692	700	670	711
Survival analysis	P=0.977N	P=0.951N	P=0.275	P=0.886N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] 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**.
[d] Censored from survival analyses
[e] Includes one animal that died during the last week of the study

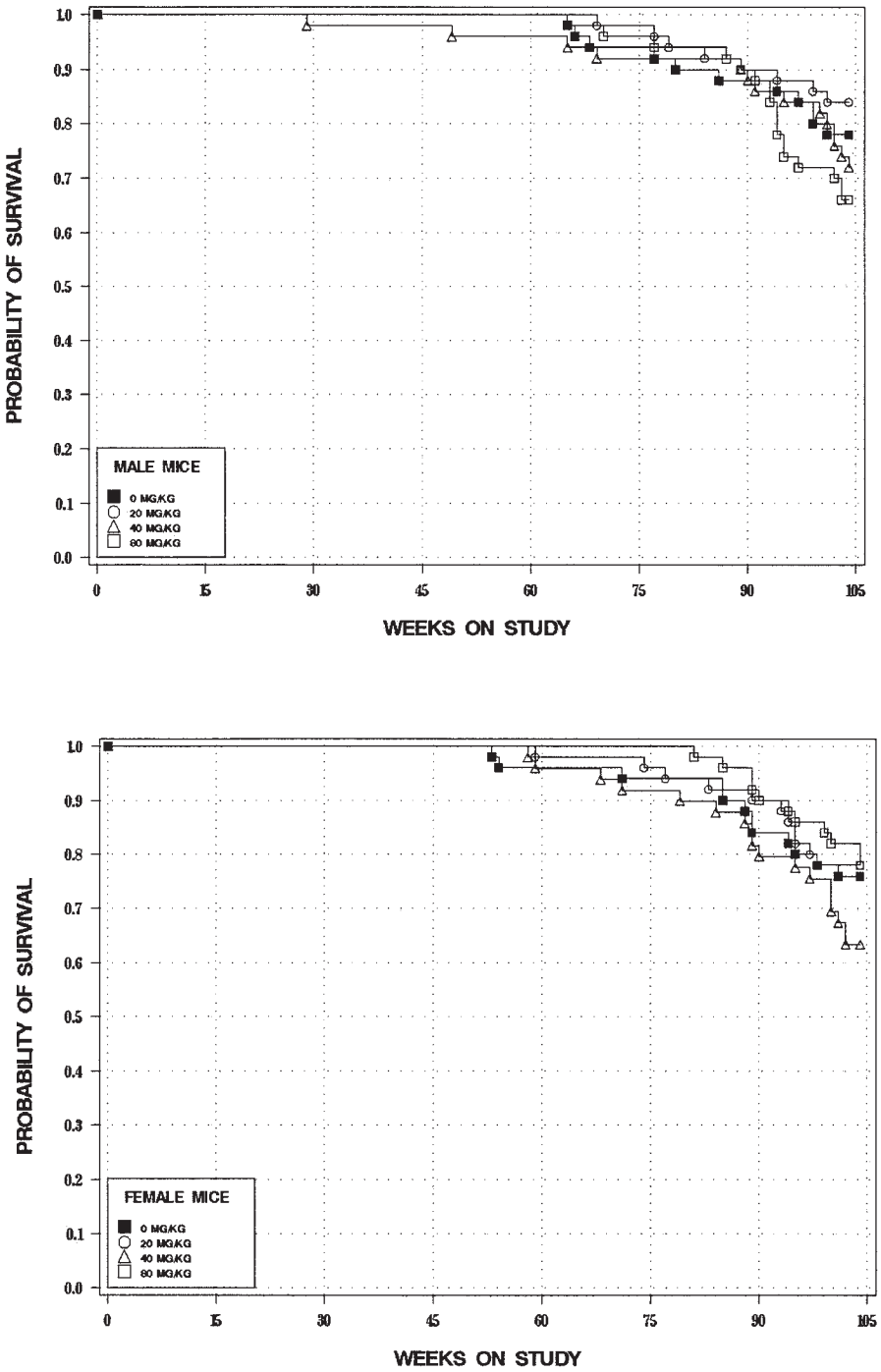


FIGURE 7
Kaplan-Meier Survival Curves for Male and Female Mice Administered Formamide by Gavage for 2 Years

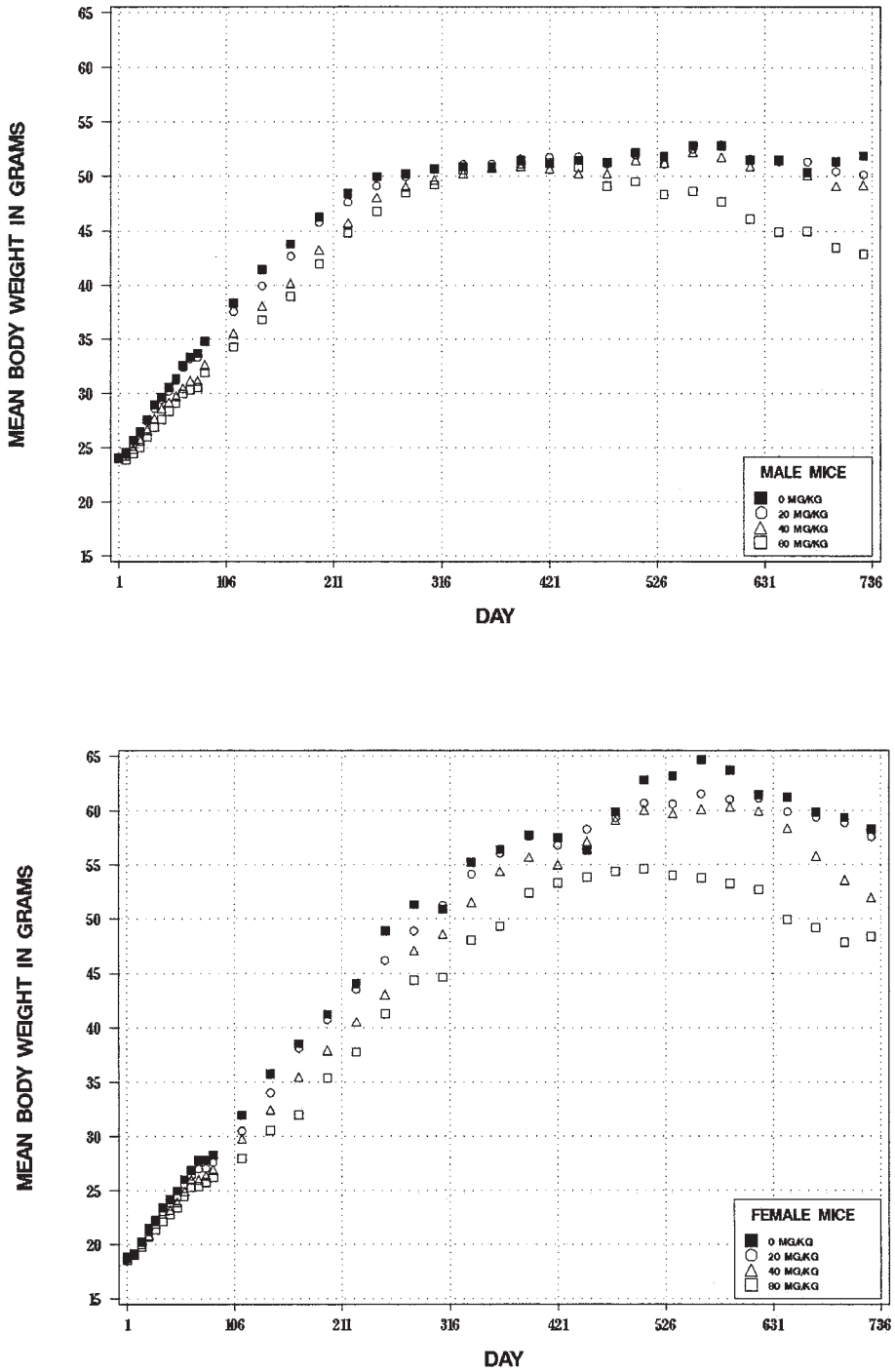


FIGURE 8
Growth Curves for Male and Female Mice Administered Formamide by Gavage for 2 Years

Table 13
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Formamide

Weeks on Study	Vehicle Control		20 mg/kg			40 mg/kg			80 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	24.0	50	24.0	100	50	24.1	101	50	24.1	100	50
2	24.6	50	24.4	99	50	24.3	99	50	23.9	97	50
3	25.7	50	25.3	99	50	24.9	97	50	24.4	95	50
4	26.5	50	26.4	100	50	25.6	97	50	25.0	94	50
5	27.6	50	27.4	99	50	26.6	97	50	26.0	94	50
6	29.0	50	28.6	99	50	27.7	96	50	26.9	93	50
7	29.6	50	29.4	99	50	28.6	97	50	27.6	93	50
8	30.5	50	30.2	99	50	29.2	96	50	28.4	93	50
9	31.3	50	31.2	99	50	29.8	95	50	29.1	93	50
10	32.6	50	32.4	99	50	30.5	94	50	30.0	92	50
11	33.3	50	33.2	100	50	31.2	93	50	30.3	91	50
12	33.7	50	33.3	99	50	31.2	93	50	30.5	91	50
13	34.7	50	34.7	100	50	32.6	94	50	31.9	92	50
17	38.3	50	37.5	98	50	35.5	93	50	34.3	89	50
21	41.4	50	39.9	96	50	38.0	92	50	36.8	89	50
25	43.8	50	42.6	97	50	40.2	92	50	38.9	89	50
29	46.2	50	45.8	99	50	43.2	94	50	41.9	91	50
33	48.5	50	47.6	98	50	45.7	94	49	44.8	92	50
37	50.0	50	49.1	98	50	48.1	96	49	46.8	94	50
41	50.3	50	50.0	99	50	49.1	98	49	48.5	96	50
45	50.7	50	50.7	100	50	49.7	98	49	49.2	97	50
49	50.9	50	51.1	100	50	50.3	99	49	50.6	99	50
53	50.8	50	51.1	101	50	50.8	100	48	50.9	100	50
57	51.5	50	51.6	100	50	51.0	99	48	51.2	99	50
61	51.2	50	51.8	101	50	50.8	99	48	51.3	100	50
65	51.5	50	51.8	101	50	50.3	98	47	50.8	99	49
69	51.3	47	51.2	100	50	50.3	98	47	49.1	96	49
73	52.2	47	51.9	99	49	51.5	99	46	49.5	95	48
77	51.8	47	51.4	99	48	51.3	99	46	48.4	93	48
81	52.9	45	52.5	99	47	52.2	99	46	48.7	92	47
85	52.8	45	52.9	100	46	51.8	98	46	47.7	90	47
89	51.5	44	51.6	100	46	51.0	99	46	46.1	89	46
93	51.5	44	51.4	100	45	51.5	100	43	44.9	87	44
97	50.4	43	51.4	102	44	50.2	100	42	45.0	89	36
101	51.4	40	50.5	98	43	49.1	96	40	43.5	85	36
105	51.9	39	50.2	97	42	49.2	95	36	42.8	83	33
Mean for weeks											
1-13	29.5		29.3	99		28.2	96		27.5	94	
14-52	46.7		46.0	98		44.4	95		43.5	93	
53-105	51.6		51.5	100		50.8	99		47.9	93	

Table 14
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Formamide

Weeks on Study	Vehicle Control		20 mg/kg			40 mg/kg			80 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	18.9	50	18.6	99	50	18.6	99	50	18.7	99	50
2	19.2	50	19.2	100	50	19.0	99	49	19.0	99	50
3	20.3	50	20.2	100	50	20.0	99	49	19.8	98	50
4	21.5	50	21.2	98	50	20.9	97	49	20.7	96	50
5	22.3	50	22.2	99	50	21.9	98	49	21.4	96	50
6	23.4	50	23.1	99	50	23.1	99	49	22.1	95	50
7	24.2	50	23.8	99	50	23.2	96	49	22.8	94	50
8	25.0	50	24.4	98	50	23.9	96	49	23.4	94	50
9	26.0	50	25.6	98	50	24.9	96	49	24.5	94	50
10	26.9	50	26.4	98	50	26.0	97	49	25.3	94	50
11	27.8	50	27.0	97	50	26.0	94	49	25.4	91	50
12	27.8	50	27.0	97	50	26.4	95	49	25.8	93	50
13	28.3	50	27.6	98	50	26.9	95	49	26.2	93	50
17	31.9	50	30.5	95	50	29.8	93	49	28.0	88	50
21	35.8	50	34.0	95	50	32.4	91	49	30.5	85	50
25	38.5	50	38.1	99	50	35.4	92	49	31.9	83	50
29	41.2	50	40.7	99	50	37.9	92	49	35.3	86	50
33	44.0	50	43.5	99	50	40.5	92	49	37.8	86	50
37	48.9	50	46.2	94	50	43.0	88	49	41.3	84	50
41	51.3	50	48.9	95	50	47.1	92	49	44.3	86	50
45	50.9	50	51.3	101	50	48.6	96	49	44.6	88	50
49	55.3	50	54.2	98	50	51.6	93	49	48.0	87	50
53	56.4	50	56.1	99	50	54.4	97	49	49.4	88	50
57	57.7	48	57.6	100	50	55.7	97	49	52.4	91	50
61	57.5	48	56.8	99	49	55.0	96	47	53.4	93	50
65	56.4	48	58.3	103	49	57.2	101	47	53.9	96	50
69	59.9	48	59.3	99	49	59.1	99	46	54.4	91	50
73	62.8	47	60.7	97	49	60.1	96	45	54.6	87	50
77	63.2	47	60.6	96	48	59.7	95	45	54.0	86	50
81	64.7	47	61.5	95	47	60.1	93	44	53.8	83	50
85	63.7	47	61.0	96	46	60.4	95	43	53.3	84	49
89	61.5	44	61.1	99	46	59.9	98	42	52.8	86	47
93	61.2	42	59.9	98	45	58.3	95	39	50.0	82	45
97	59.8	40	59.4	99	41	55.8	93	38	49.2	82	43
101	59.4	39	58.9	99	39	53.6	90	34	47.9	81	41
105	58.3	38	57.6	99	38	52.0	89	31	48.4	83	39
Mean for weeks											
1-13	24.0		23.6	98		23.1	97		22.7	95	
14-52	44.2		43.0	97		40.7	92		38.0	86	
53-105	60.2		59.2	98		57.2	95		52.0	87	

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, testis, lung, spleen, and pancreatic islets. 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 hemangiosarcoma occurred with a positive trend in males, and the incidences were significantly increased at 40 and 80 mg/kg (Tables 15 and C2). The incidences of hemangiosarcoma in all dosed groups of males exceeded the historical control ranges (all routes) (Tables 15 and C3). Microscopically, the hemangiosarcomas were composed of tortuous vascular spaces lined by plump, elongated spindle cells. They were often associated with thrombi and infarction or centrilobular necrosis in the surrounding parenchyma and adjacent liver lobes. Hemangiosarcomas also occurred in the spleen (vehicle control, 2/50; 20 mg/kg, 2/50; 40 mg/kg, 2/50; 80 mg/kg, 3/50; Table C2), but the incidences did not increase in a dose-dependent manner; the vehicle control incidence was at the high end of the historical control ranges, and the incidence in the 80 mg/kg group only slightly exceeded the current ranges of NTP historical control values (Table C3).

The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in 80 mg/kg females but was within the historical control ranges (Tables 15, D2, and D3). Microscopically, the hepatocellular adenomas were well-circumscribed lesions usually occupying an area greater than one liver lobule, usually lacking central veins and portal areas, and with distinct compression of adjacent parenchyma. The hepatocytes were well-differentiated and occurred in irregular plates (one to three layers thick). Mitotic figures and eosinophilic cytoplasmic inclusions were also characteristic features. Microscopically, the hepatocellular carcinomas were large neoplasms that often showed solid or trabecular growth patterns (cords three or more cell layers thick) and cytological atypia.

The incidences of clear cell focus in 80 mg/kg males and fatty change in 40 and 80 mg/kg males were significantly decreased; the biological significance of these decreases is uncertain (Tables 15 and C4).

TABLE 15
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Clear Cell Focus[a]	29	27	21	8**
Fatty Change	31 (1.6)[b]	25 (1.4)	15** (1.3)	1** (1.0)
Hemangiosarcoma[c]				
Overall rate[d]	1/50 (2%)	5/50 (10%)	7/50 (14%)	8/50 (16%)
Adjusted rate[e]	2.2%	10.6%	15.2%	17.5%
Terminal rate[f]	1/39 (3%)	4/42 (10%)	4/36 (11%)	3/33 (9%)
First incidence (days)	728 (T)	655	449	635
Poly-3 test[g]	P=0.018	P=0.110	P=0.032	P=0.016
Female				
Number Examined Microscopically	50	50	50	50
Hepatocellular Adenoma, Multiple	2	3	2	2
Hepatocellular Adenoma (includes multiple)[h]				
Overall rate	6/50 (12%)	12/50 (24%)	13/50 (26%)	12/50 (24%)
Adjusted rate	13.2%	26.2%	29.4%	25.4%
Terminal rate	3/38 (8%)	11/39 (28%)	9/31 (29%)	11/39 (28%)
First incidence (days)	653	674	407	694
Poly-3 test	P=0.149	P=0.096	P=0.051	P=0.109
Hepatocellular Carcinoma, Multiple	0	1	0	1
Hepatocellular Carcinoma (includes multiple)[i]	4	4	0	6
Hepatocellular Adenoma or Carcinoma[j]				
Overall rate	9/50 (18%)	15/50 (30%)	13/50 (26%)	18/50 (36%)
Adjusted rate	19.8%	32.6%	29.4%	37.8%
Terminal rate	6/38 (16%)	13/39 (33%)	9/31 (29%)	14/39 (36%)
First incidence (days)	653	665	407	657
Poly-3 test	P=0.056	P=0.124	P=0.209	P=0.044

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year water gavage studies with vehicle controls given NTP-2000 diet (mean ± standard deviation): 6/200 (3.0% ± 1.2%), range 2%-4%; all routes: 33/1,496 (2.3% ± 1.6%), range 0%-8%
[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 is the P value corresponding to pairwise comparison between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence for 2-year water gavage studies: 41/200 (20.5% ± 8.7%), range 12%-28%; all routes: 402/1,593 (25.8% ± 15.8%), range 2%-62%
[i] Historical incidence for 2-year water gavage studies: 16/200 (8.0% ± 4.3%), range 4%-14%; all routes: 159/1,593 (10.2% ± 6.6%), range 0%-28%
[j] Historical incidence for 2-year water gavage studies: 53/200 (26.5% ± 10.0%), range 18%-40%; all routes: 505/1,593 (32.4% ± 17.5%), range 8%-64%

Testis: There were significant increases in the incidences of minimal to mild mineralization of the testicular arteries in 40 and 80 mg/kg males and the testicular tunic in 80 mg/kg males (Tables 16 and C4; Plates 4 through 6).

Lung: In 80 mg/kg male mice, there was a significant decrease in the incidence of alveolar/bronchiolar adenoma; however, the vehicle control incidence is higher than the NTP historical control mean incidence for all routes of administration (Tables 16, C1, and C2). The biological significance of this decrease in pulmonary alveolar/bronchiolar adenoma is uncertain. The incidence of histiocytic cellular infiltration in the lung alveolus was significantly increased for 80 mg/kg males, but the biological significance of the increase is uncertain (Tables 16 and C4).

Other organs: In 80 mg/kg males, there was a significant increase in the incidence of splenic hematopoietic cell proliferation (Tables 16 and C4). There were significant decreases in the incidences of hyperplasia of the pancreatic islets in all dosed groups of males and in the incidence of splenic hematopoietic cell proliferation in 80 mg/kg females (Tables 16, C4, and D4). The biological significance of the increases and decreases in these nonneoplastic lesions is uncertain. The increased incidence of splenic hematopoietic cell proliferation in males may be due to erythrocyte damage induced by the hepatic hemangiosarcomas that occurred in male mice.

TABLE 16
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control		20 mg/kg		40 mg/kg		80 mg/kg	
Male								
Testis[a]	50		50		50		50	
Artery, Mineralization[b]	0		2	(1.0)[c]	5*	(1.4)	35**	(1.6)
Tunic, Mineralization	1	(1.0)	0		5	(1.0)	27**	(1.8)
Lung	50		50		50		50	
Alveolus, Infiltration Cellular, Histiocyte	1	(1.0)	0		1	(2.0)	8*	(1.5)
Alveolar/bronchiolar Adenoma, Multiple	2		1		1		0	
Alveolar/bronchiolar Adenoma[d] (includes multiple)	10		4		7		1**	
Spleen	50		50		50		50	
Hematopoietic Cell Proliferation	14	(2.1)	14	(2.2)	20	(1.9)	28**	(2.0)
Islets, Pancreatic	50		50		50		50	
Hyperplasia	35	(1.4)	24*	(1.3)	24*	(1.3)	9**	(1.3)
Female								
Spleen	50		49		49		50	
Hematopoietic Cell Proliferation	17	(2.6)	17	(2.2)	15	(2.7)	8*	(2.8)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year water gavage studies with vehicle controls given NTP-2000 diet (mean ± standard deviation): 40/200 (20.0% ± 0.0%), range 20%; all routes: 263/1,498 (17.9% ± 6.1%), range 6%-28%

Genetic Toxicology

In three independent Ames assays, formamide (concentrations up to the maximum of 10,000 µg/plate) did not induce mutagenic activity in any of several strains of *Salmonella typhimurium* tested with and without rat or hamster liver S9 activation enzymes (Table E1; Mortelmans *et al.*, 1986) or in *Escherichia coli* strain WP *uvrA* pKM101 with and without 10% rat liver S9 (Table E1). Negative results were obtained with formamide in a test for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* treated with formamide either in feed (2,500 or 5,000 ppm) or by abdominal injection (21,570 ppm) (Table E2). No increases in micronucleated normochromatic erythrocytes were observed in male or female B6C3F1 mice treated with formamide (up to 160 mg/kg) by gavage for 3 months; no significant effects on the percentage of polychromatic (immature) erythrocytes were seen in either male or female mice, indicating the absence of formamide-induced bone marrow toxicity (Table E3).

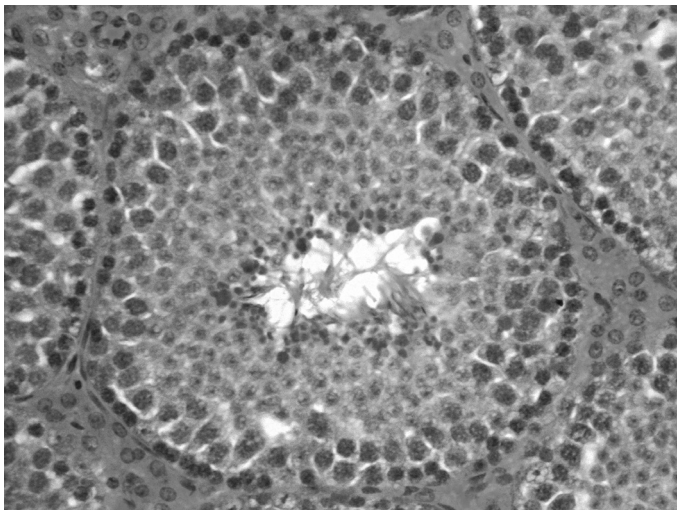


PLATE 1
The range in size of residual bodies from a Stage VIII tubule in a vehicle control B6C3F1 mouse in the 3-month gavage study of formamide

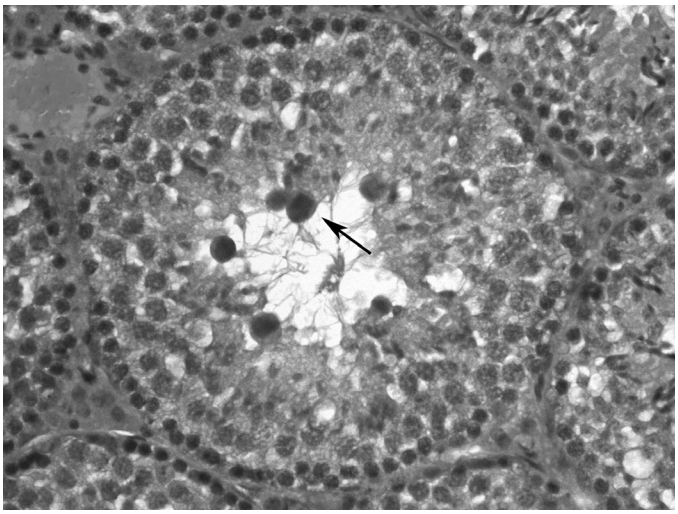


PLATE 2
Large abnormal residual bodies (arrow) present in a male B6C3F1 mouse administered 160 mg/kg formamide by gavage for 3 months

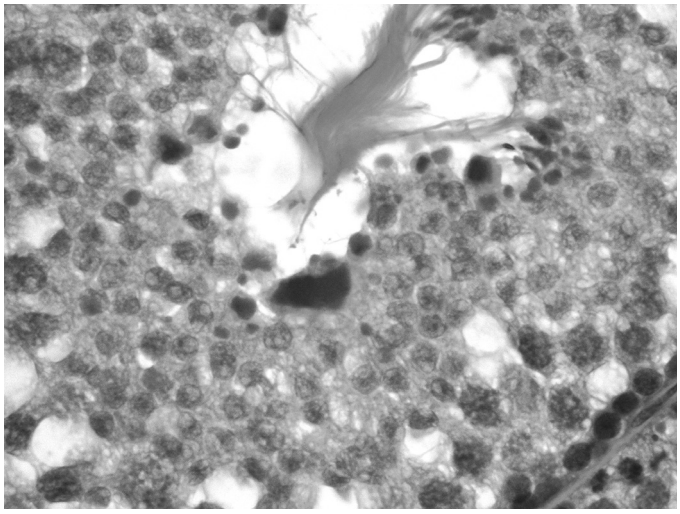


PLATE 3
An atypically large and angular residual body within a Stage VIII tubule in a male B6C3F1 mouse administered 160 mg/kg formamide by gavage for 3 months

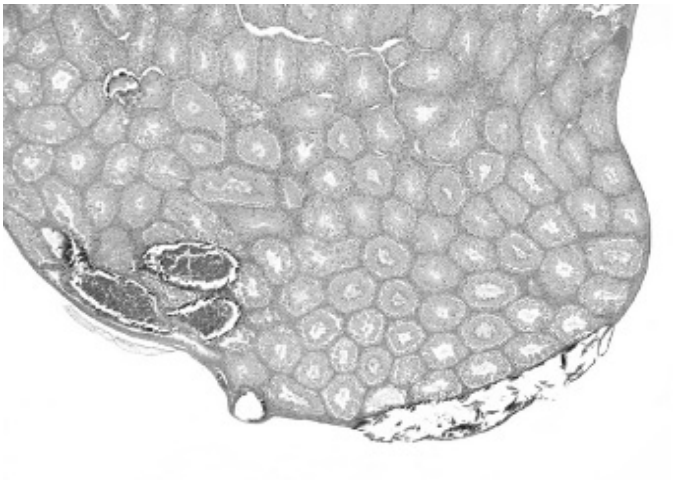


PLATE 4
Arterial and capsular mineralization in the testis of a male B6C3F1 mouse administered 80 mg/kg formamide by gavage for 2 years

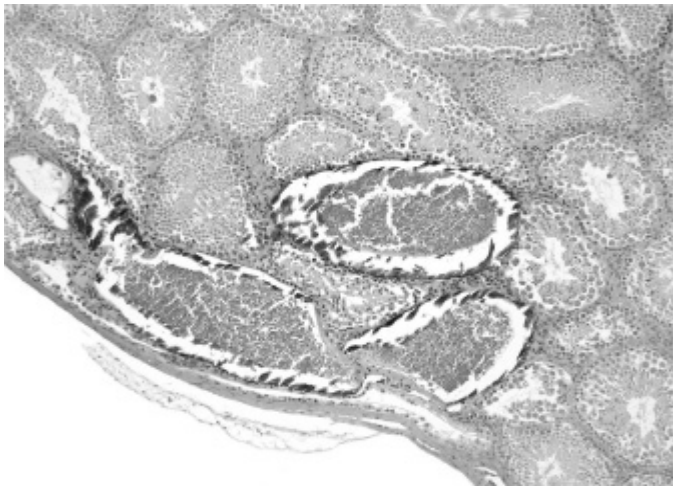


Plate 5
Higher magnification of an area of arterial mineralization in Plate 4

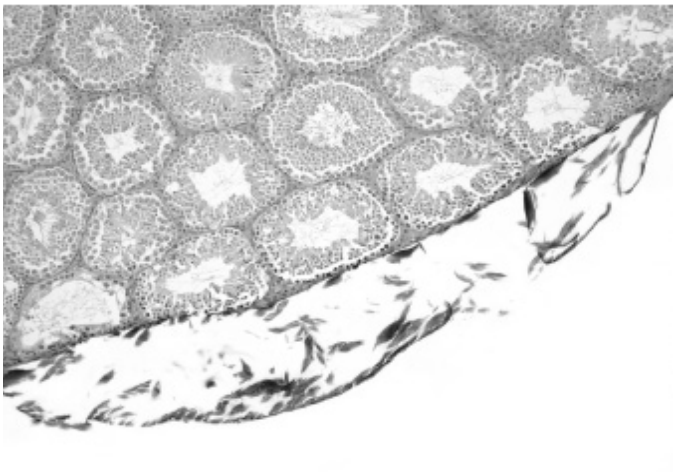


Plate 6
Higher magnification of an area of capsular mineralization in Plate 4

DISCUSSION AND CONCLUSIONS

Formamide is an odorless, colorless liquid that is soluble in water and miscible with methanol, ethanol, acetone, and several other organic solvents. Because of its desirable solvent properties, it is used extensively in manufacturing and is classified as a high production volume chemical by the United States Environmental Protection Agency (2003).

Human exposure occurs primarily by the inhalation or dermal route in industrial and laboratory settings where the 8-hour time-weighted average threshold limit value is 10 ppm (ACGIH, 2005). However, it was not feasible to use the inhalation route for the studies reported here, because the low vapor pressure of formamide made it impossible to achieve a maximum vapor concentration that would allow adequate toxicological evaluation. In addition, the relatively high viscosity of formamide combined with its low vapor pressure made dermal exposure impractical. Formamide was found to be unstable in feed and not palatable when administered in drinking water; therefore, gavage was selected as the route of exposure.

Doses of formamide up to 160 mg/kg for 3 months produced a relatively mild toxic response in rats. Most of the effects were observed in groups that received the highest dose of 160 mg/kg and consisted of 20% to 25% body weight reductions and an increase in the erythron that was evidenced by increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. The only microscopic lesion identified was degeneration of the germinal epithelium in the testis and epididymis of 160 mg/kg male rats. Slight body weight reductions also occurred in groups that received 80 mg/kg and in females that received 40 mg/kg; however, few other changes were noted at these doses.

The 20% to 25% reduction in body weight observed at 160 mg/kg in the 3-month rat study eliminated this dose as a high dose for the 2-year study. Body weights of groups that received 80 mg/kg were 7% less than those of the vehicle controls at the end of the 3-month study and were within the range acceptable for the high dose. Therefore, doses of 20, 40, and 80 mg/kg were selected for the 2-year rat study.

Body weights of male mice administered 160 mg/kg were 15% less than those of the vehicle controls, and body weights of female mice that received 160 mg/kg were 9% less than those of the vehicle controls. Microscopic lesions consisting of hyperplasia and cytoplasmic alteration of the epithelium of the gallbladder and pancreatic duct were present in male and female mice receiving 160 mg/kg. In addition, all 160 mg/kg males had abnormal residual bodies within scattered sections of the testicular seminiferous tubules; however, these lesions were not thought to pose a significant toxic risk over the course of the 2-year study. Therefore, based on reduced body weights at 160 mg/kg, doses of 20, 40, and 80 mg/kg were selected for the 2-year mouse study.

During the 2-year studies, survival of groups of rats and mice administered formamide was comparable to that of the vehicle control groups. Mean body weights of 80 mg/kg male rats and mice were less than those of the vehicle controls throughout most of the 2-year study. Mean body weights of 40 and 80 mg/kg female rats and mice were less than those of the vehicle controls during the second year of the study.

There were no significant changes in the incidences of neoplasms in rats associated with administration of formamide. The incidences of pituitary gland (pars distalis) adenoma were increased in dosed male rats; however, the vehicle control incidence was lower than the mean NTP historical control incidence for all routes of administration, and the incidences of pituitary gland (pars distalis) adenoma or carcinoma (combined) in female rats decreased with dose. Therefore, the biological significance of the increased incidences of pituitary gland adenoma in male rats is uncertain, and the increases are not considered treatment related.

The incidence of bone marrow hyperplasia was increased in male rats that received 80 mg/kg during the 2-year

study. This is consistent with the mild changes noted in the erythron at this dose during the 3-month study and suggests that formamide exposure stimulates a slight increase in hematopoiesis. A similar increase in bone marrow hyperplasia was not observed in females in the 2-year study, although changes in the erythron, similar to those observed in males, occurred during the 3-month study in females.

The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in 80 mg/kg female mice when compared to the concurrent vehicle control group but was within the historical control range for all routes of administration. The incidences of hepatocellular adenoma were increased in all groups of female mice administered formamide, but the incidences were essentially the same in each dosed group. There were four hepatocellular carcinomas in the 20 mg/kg group, six in the 80 mg/kg group, but none in the 40 mg/kg group and four in the vehicle control group. Although the individual incidences of adenoma or carcinoma did not increase with dose, the combined incidence in the 80 mg/kg group was twice the incidence in the vehicle control group. Therefore, the significant increase in the incidence of adenoma or carcinoma (combined) in the 80 mg/kg group was considered an equivocal finding.

Both *N*,*N*-dimethylformamide and *N*-methylformamide are converted to a reactive intermediate by the action of cytochrome P450 in the liver. The reactive intermediate then reacts with glutathione to form the respective carbamoyl glutathione (Gescher, 1993). Both compounds are also hepatotoxic; *N*,*N*-dimethylformamide caused hepatocyte necrosis in rats (NTP, 1992), and *N*-methylformamide caused hepatocellular necrosis in mice (Tulip and Timbrell, 1988). By contrast, formamide was not toxic to hepatocytes in either rats or mice in the 3-month studies. This suggests that the metabolism of formamide may differ from that of *N*,*N*-dimethylformamide or *N*-methylformamide.

In groups of male mice administered formamide for 2 years, the incidences of hepatic hemangiosarcoma occurred with a positive trend and were significantly increased at 40 and 80 mg/kg. The incidences of hemangiosarcoma in all male dosed groups also exceeded the historical range for all routes of administration. Hemangiosarcomas were also present in the spleen of dosed and vehicle control male mice, but the incidences did not increase in a dose-dependent manner and were within or only slightly exceeded the NTP historical control ranges.

Hemangiosarcomas of the liver in male mice have occurred in several previous NTP 2-year studies, and a recent analysis suggests an association between hemangiosarcomas in the liver and secondary iron overload associated with erythrocyte hemolysis (Nyska *et al.*, 2004).

In the studies reviewed by Nyska *et al.* (2004), there is a significant association between Kupffer cell pigmentation associated with red cell hemolysis and the incidence of hemangiosarcoma. The association between hemolysis and pigmentation indicative of hemosiderosis suggests that oxidative stress resulting from iron overload in endothelial cells may contribute to induction of hemangiosarcoma (Nyska *et al.*, 2004; Klaunig and Kamendulis, 2005; Corthals *et al.*, 2006). In the current study however, there was no evidence of pigmentation in the spleen or of Kupffer cell pigmentation in the liver consistent with red cell hemolysis.

Hematology at the end of the 3-month formamide study indicated a slight but significant reduction in hematocrit values and erythrocyte counts and minimal increases in mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration in male mice. In addition, at the end of the 2-year study, extramedullary hematopoiesis was significantly increased in the spleen of male mice that received 80 mg/kg.

In the 2-year study, incidences of minimal to mild mineralization of the testicular arteries and testicular tunic were significantly increased in 80 mg/kg male mice. The strong dose response and significantly increased incidence in the 80 mg/kg group indicate a clear association with the administration of formamide.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of form-amide in male or female F344/N rats administered 20, 40, or 80 mg/kg. There was *clear evidence of carcinogenic activity* of formamide in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver. There was *equivocal evidence of carcinogenic activity* of formamide in female B6C3F1 mice based on increased incidences of hepatocellular adenoma or carcinoma (combined).

An increased incidence of bone marrow hyperplasia occurred in male rats. Mineralization of the testicular arteries and tunic and hematopoietic cell proliferation of the spleen in male mice were also associated with administration of formamide.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	24	15	22	18
Natural deaths		8	2	3
Survivors				
Terminal sacrifice	26	27	26	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(50)	(50)
Intestine large, cecum	(49)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
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)
Carcinoma, metastatic, pancreas		1 (2%)		
Fibrous histiocytoma, metastatic, skin	1 (2%)	1 (2%)		
Hepatocellular adenoma		1 (2%)	1 (2%)	1 (2%)
Hepatocellular carcinoma				2 (4%)
Ito cell tumor benign				1 (2%)
Mesentery	(10)	(12)	(7)	(10)
Carcinoma, metastatic, pancreas		1 (8%)		
Oral mucosa	(22)	(27)	(29)	(20)
Squamous cell carcinoma		1 (4%)		
Pancreas	(50)	(50)	(50)	(50)
Acinus, carcinoma		1 (2%)		
Salivary gland	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(1)	(0)	(0)
Squamous cell papilloma		1 (100%)		
Tooth	(25)	(32)	(30)	(25)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Pericardium, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	7 (14%)	9 (18%)	1 (2%)	6 (12%)
Pheochromocytoma malignant		1 (2%)	1 (2%)	1 (2%)
Bilateral, pheochromocytoma benign	2 (4%)		1 (2%)	

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)		4 (8%)	2 (4%)
Carcinoma	1 (2%)			
Parathyroid gland	(48)	(49)	(49)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	11 (22%)	15 (30%)	21 (42%)	18 (36%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)		
C-cell, adenoma	6 (12%)	5 (10%)	6 (12%)	2 (4%)
C-cell, carcinoma		1 (2%)		
Follicular cell, carcinoma	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Coagulating gland	(0)	(1)	(1)	(0)
Epididymis	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		2 (4%)	2 (4%)
Carcinoma	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Adenoma		3 (6%)	2 (4%)	
Seminal vesicle	(50)	(50)	(50)	(49)
Testis	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	32 (64%)	41 (82%)	44 (88%)	38 (76%)
Interstitial cell, adenoma	12 (24%)	7 (14%)	2 (4%)	7 (14%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(8)	(8)	(5)
Fibrous histiocytoma, metastatic, skin	1 (14%)			
Lymph node, mandibular	(0)	(1)	(0)	(3)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Osteosarcoma, metastatic, skin			1 (2%)	
Thymus	(49)	(45)	(47)	(48)
Thymoma malignant				1 (2%)
Integumentary System				
Mammary gland	(50)	(48)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma		1 (2%)		
Fibroadenoma	2 (4%)	4 (8%)		1 (2%)
Fibroma	1 (2%)			

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)		3 (6%)	
Keratoacanthoma	8 (16%)	3 (6%)	4 (8%)	2 (4%)
Squamous cell papilloma	1 (2%)			
Pinna, neural crest tumor			1 (2%)	
Subcutaneous tissue, fibroma	5 (10%)	4 (8%)	2 (4%)	2 (4%)
Subcutaneous tissue, fibroma, multiple	1 (2%)		1 (2%)	
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)		
Subcutaneous tissue, osteosarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma	1 (2%)	1 (2%)	1 (2%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant		1 (2%)		
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		2 (4%)	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	2 (4%)		
Carcinoma, metastatic, pancreas		1 (2%)		
Chordoma, metastatic, bone	1 (2%)	1 (2%)	1 (2%)	
Fibrous histiocytoma, metastatic, skin	1 (2%)	1 (2%)		
Osteosarcoma, metastatic, skin			1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla			1 (2%)	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Sarcoma			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(1)	(1)
Carcinoma			1 (100%)	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic skin	1 (2%)			
Stromal nephroma			1 (2%)	
Renal tubule, adenoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)		2 (4%)
Leukemia mononuclear	15 (30%)	14 (28%)	17 (34%)	18 (36%)
Mesothelioma mononuclear	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	50	49	50
Total primary neoplasms	120	129	125	113
Total animals with benign neoplasms	45	50	48	49
Total benign neoplasms	94	97	97	84
Total animals with malignant neoplasms	25	27	26	25
Total malignant neoplasms	26	32	27	29
Total animals with metastatic neoplasms	3	3	3	1
Total metastatic neoplasms	13	6	4	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 Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	9/50 (18%)	9/50 (18%)	2/50 (4%)	6/50 (12%)
Adjusted rate[b]	21.4%	21.4%	4.7%	13.9%
Terminal rate[c]	6/26 (23%)	7/27 (26%)	0/26 (0%)	5/29 (17%)
First incidence (days)	654	573	657	643
Poly-3 test[d]	P=0.128N	P=0.602	P=0.023N	P=0.268N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	9/50 (18%)	10/50 (20%)	3/50 (6%)	7/50 (14%)
Adjusted rate	21.4%	23.8%	7.1%	16.1%
Terminal rate	6/26 (23%)	8/27 (30%)	1/26 (4%)	5/29 (17%)
First incidence (days)	654	573	657	590
Poly-3 test	P=0.194N	P=0.497	P=0.055N	P=0.363N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	2.4%	2.4%	6.9%
Terminal rate	0/26 (0%)	1/27 (4%)	0/26 (0%)	2/29 (7%)
First incidence (days)	—[e]	727 (T)	622	577
Poly-3 test	P=0.058	P=0.498	P=0.504	P=0.125
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.4%	9.4%	4.8%	4.6%
Terminal rate	0/26 (0%)	1/27 (4%)	2/26 (8%)	0/29 (0%)
First incidence (days)	589	485	727 (T)	629
Poly-3 test	P=0.584	P=0.182	P=0.500	P=0.510
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)	1/50 (2%)
Adjusted rate	4.8%	9.6%	0.0%	2.3%
Terminal rate	1/26 (4%)	3/27 (11%)	0/26 (0%)	0/29 (0%)
First incidence (days)	622	612	—	723
Poly-3 test	P=0.193N	P=0.332	P=0.236N	P=0.493N
Mammary Gland: Fibroma, Fibroadenoma, or Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	0/50 (0%)	1/50 (2%)
Adjusted rate	9.5%	9.6%	0.0%	2.3%
Terminal rate	3/26 (12%)	3/27 (11%)	0/26 (0%)	0/29 (0%)
First incidence (days)	622	612	—	723
Poly-3 test	P=0.056N	P=0.640	P=0.059N	P=0.172N
Mammary Gland: Fibroma, Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	0/50 (0%)	1/50 (2%)
Adjusted rate	9.5%	12.0%	0.0%	2.3%
Terminal rate	3/26 (12%)	3/27 (11%)	0/26 (0%)	0/29 (0%)
First incidence (days)	622	612	—	723
Poly-3 test	P=0.046N	P=0.497	P=0.059N	P=0.172N
Pancreatic Islets: Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	4.8%	0.0%	9.5%	4.7%
Terminal rate	2/26 (8%)	0/27 (0%)	3/26 (12%)	2/29 (7%)
First incidence (days)	727 (T)	—	693	727 (T)
Poly-3 test	P=0.422	P=0.239N	P=0.343	P=0.684N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	7.2%	0.0%	9.5%	4.7%
Terminal rate	3/26 (12%)	0/27 (12%)	3/26 (12%)	2/29 (7%)
First incidence (days)	727 (T)	—	693	727 (T)
Poly-3 test	P=0.588	P=0.120N	P=0.508	P=0.487N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	11/50 (22%)	15/50 (30%)	21/50 (42%)	18/50 (36%)
Adjusted rate	26.0%	35.1%	47.9%	39.9%
Terminal rate	7/26 (27%)	11/27 (41%)	13/26 (50%)	10/29 (35%)
First incidence (days)	561	485	496	496
Poly-3 test	P=0.104	P=0.249	P=0.025	P=0.121
Preputial Gland: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.4%	7.1%	4.7%	4.7%
Terminal rate	0/26 (0%)	0/27 (0%)	0/26 (0%)	1/29 (3%)
First incidence (days)	721	485	591	693
Poly-3 test	P=0.516	P=0.312	P=0.510	P=0.511
Preputial Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	4.8%	7.1%	9.3%	9.2%
Terminal rate	0/26 (0%)	0/27 (0%)	1/26 (4%)	1/29 (3%)
First incidence (days)	707	485	591	657
Poly-3 test	P=0.285	P=0.508	P=0.351	P=0.356
Prostate Gland: Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	7.3%	4.8%	0.0%
Terminal rate	0/26 (0%)	3/27 (11%)	2/26 (8%)	0/29 (0%)
First incidence (days)	—	727 (T)	727 (T)	—
Poly-3 test	P=0.395N	P=0.116	P=0.239	—[f]
Skin: Keratoacanthoma				
Overall rate	8/50 (16%)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted rate	19.0%	7.2%	9.5%	4.7%
Terminal rate	6/26 (23%)	2/27 (7%)	3/26 (12%)	2/29 (7%)
First incidence (days)	622	588	649	727 (T)
Poly-3 test	P=0.042N	P=0.099N	P=0.172N	P=0.041N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	9/50 (18%)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted rate	21.4%	7.2%	9.5%	4.7%
Terminal rate	7/26 (27%)	2/27 (7%)	3/26 (12%)	2/29 (7%)
First incidence (days)	622	588	649	727 (T)
Poly-3 test	P=0.023N	P=0.059N	P=0.110N	P=0.022N
Skin: Basal Cell Adenoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.4%	0.0%	7.1%	0.0%
Terminal rate	1/26 (4%)	0/27 (0%)	1/26 (4%)	0/29 (0%)
First incidence (days)	727 (T)	—	657	—
Poly-3 test	P=0.485N	P=0.502N	P=0.313	P=0.494N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Basal Cell Adenoma				
Overall rate	10/50 (20%)	3/50 (6%)	7/50 (14%)	2/50 (4%)
Adjusted rate	23.7%	7.2%	16.3%	4.7%
Terminal rate	8/26 (31%)	2/27 (7%)	4/26 (15%)	2/29 (7%)
First incidence (days)	622	588	649	727 (T)
Poly-3 test	P=0.022N	P=0.034N	P=0.280N	P=0.011N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	6/50 (12%)	4/50 (8%)	3/50 (6%)	2/50 (4%)
Adjusted rate	14.3%	9.6%	7.1%	4.7%
Terminal rate	4/26 (15%)	3/27 (11%)	2/26 (8%)	1/29 (3%)
First incidence (days)	582	658	721	665
Poly-3 test	P=0.086N	P=0.378N	P=0.240N	P=0.124N
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	7/50 (14%)	6/50 (12%)	3/50 (6%)	2/50 (4%)
Adjusted rate	16.6%	14.2%	7.1%	4.7%
Terminal rate	4/26 (15%)	4/27 (15%)	2/26 (8%)	1/29 (3%)
First incidence (days)	582	477	721	665
Poly-3 test	P=0.035N	P=0.499N	P=0.155N	P=0.072N
Testis: Adenoma				
Overall rate	44/50 (88%)	48/50 (96%)	46/50 (92%)	45/50 (90%)
Adjusted rate	94.3%	98.4%	96.5%	92.9%
Terminal rate	25/26 (96%)	27/27 (100%)	26/26 (100%)	28/29 (97%)
First incidence (days)	477	477	512	518
Poly-3 test	P=0.298N	P=0.253	P=0.499	P=0.564N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/50 (12%)	6/50 (12%)	6/50 (12%)	2/50 (4%)
Adjusted rate	14.3%	14.6%	14.3%	4.6%
Terminal rate	2/26 (8%)	6/27 (22%)	6/26 (23%)	0/29 (0%)
First incidence (days)	680	727 (T)	727 (T)	593
Poly-3 test	P=0.086N	P=0.608	P=0.622	P=0.122N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	6/50 (12%)	7/50 (14%)	6/50 (12%)	2/50 (4%)
Adjusted rate	14.3%	17.0%	14.3%	4.6%
Terminal rate	2/26 (8%)	7/27 (26%)	6/26 (23%)	0/29 (0%)
First incidence (days)	680	727 (T)	727 (T)	593
Poly-3 test	P=0.072N	P=0.484	P=0.622	P=0.122N
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	14/50 (28%)	17/50 (34%)	18/50 (36%)
Adjusted rate	33.7%	31.1%	37.2%	38.8%
Terminal rate	3/26 (12%)	5/27 (19%)	6/26 (23%)	5/29 (17%)
First incidence (days)	561	561	512	518
Poly-3 test	P=0.279	P=0.489N	P=0.446	P=0.385
All Organs: Benign Neoplasms				
Overall rate	45/50 (90%)	50/50 (100%)	48/50 (96%)	49/50 (98%)
Adjusted rate	96.5%	100.0%	99.0%	98.0%
Terminal rate	26/26 (100%)	27/27 (100%)	26/26 (100%)	28/29 (97%)
First incidence (days)	477	477	496	496
Poly-3 test	P=0.606	P=0.194	P=0.409	P=0.582

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	27/50 (54%)	26/50 (52%)	25/50 (50%)
Adjusted rate	52.7%	55.5%	54.1%	52.1%
Terminal rate	7/26 (27%)	9/27 (33%)	7/26 (27%)	9/29 (31%)
First incidence (days)	394	477	496	518
Poly-3 test	P=0.479N	P=0.473	P=0.527	P=0.558N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	50/50 (100%)	49/50 (98%)	50/50 (100%)
Adjusted rate	99.1%	100.0%	99.8%	100.0%
Terminal rate	26/26 (100%)	27/27 (100%)	26/26 (100%)	29/29 (100%)
First incidence (days)	394	477	496	496
Poly-3 test	P=0.702	P=0.957	P=0.973	P=0.957

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, liver, lung, pancreatic islets, pituitary gland, preputial gland, prostate gland, testis, 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 is the P value corresponding to pairwise comparison 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 Formamide[a]

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	24	15	22	18
Natural deaths		8	2	3
Survivors				
Terminal sacrifice	26	27	26	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(50)	(50)
Inflammation	1 (2%)			1 (2%)
Intestine large, cecum	(49)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	8 (16%)	9 (18%)	11 (22%)	10 (20%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation, chronic active			1 (2%)	
Mineralization			1 (2%)	
Perforation			1 (2%)	
Ulcer			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Basophilic focus	32 (64%)	34 (68%)	33 (66%)	30 (60%)
Clear cell focus	21 (42%)	19 (38%)	24 (48%)	16 (32%)
Degeneration, cystic	7 (14%)	6 (12%)	2 (4%)	5 (10%)
Eosinophilic focus	14 (28%)	16 (32%)	8 (16%)	13 (26%)
Fatty change, focal	9 (18%)	9 (18%)	12 (24%)	5 (10%)
Fatty change, diffuse	14 (28%)	14 (28%)	13 (26%)	9 (18%)
Hematopoietic cell proliferation	4 (8%)	4 (8%)	5 (10%)	5 (10%)
Hepatodiaphragmatic nodule	4 (8%)	7 (14%)	8 (16%)	6 (12%)
Hyperplasia, granulocytic				1 (2%)
Inflammation	38 (76%)	36 (72%)	38 (76%)	37 (74%)
Mixed cell focus	5 (10%)	10 (20%)	22 (44%)	7 (14%)
Necrosis	2 (4%)	4 (8%)	5 (10%)	6 (12%)
Pigmentation		1 (2%)		
Regeneration	1 (2%)		1 (2%)	
Thrombosis	1 (2%)			
Vacuolization cytoplasmic, focal			1 (2%)	
Vacuolization, cytoplasmic, diffuse	1 (2%)			1 (2%)
Bile duct, cyst	1 (2%)			
Bile duct, fibrosis		1 (2%)		
Bile duct, hyperplasia	49 (98%)	48 (96%)	47 (94%)	44 (88%)
Centrilobular, degeneration	10 (20%)	8 (16%)	8 (16%)	6 (12%)
Oval cell, hyperplasia	23 (46%)	15 (30%)	20 (40%)	12 (24%)
Serosa, cyst	1 (2%)			
Serosa, fibrosis	1 (2%)			

[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 Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Alimentary System (continued)				
Mesentery	(10)	(12)	(7)	(10)
Hemorrhage			1 (14%)	
Fat, necrosis	9 (90%)	10 (83%)	6 (86%)	10 (100%)
Oral mucosa	(22)	(27)	(29)	(20)
Gingival, hyperplasia, squamous	21 (95%)	27 (100%)	29 (100%)	20 (100%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Basophilic focus			2 (4%)	
Cyst	1 (2%)			
Infiltration cellular, mononuclear cell	4 (8%)	2 (4%)	7 (14%)	3 (6%)
Inflammation, chronic active	23 (46%)	24 (48%)	25 (50%)	27 (54%)
Vacuolization, cytoplasmic		1 (2%)		
Acinus, atrophy	21 (42%)	21 (42%)	21 (42%)	24 (48%)
Acinus, hyperplasia	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Acinus, hyperplasia, focal	1 (2%)			
Duct, cyst		2 (4%)	2 (4%)	1 (2%)
Salivary gland	(50)	(50)	(50)	(50)
Vacuolization, cytoplasmic		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema				2 (4%)
Erosion				1 (2%)
Hyperplasia, squamous				2 (4%)
Inflammation	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Edema	1 (2%)	1 (2%)		
Erosion		3 (6%)		1 (2%)
Hyperplasia	1 (2%)			
Inflammation				1 (2%)
Mineralization	1 (2%)	1 (2%)		
Ulcer			1 (2%)	
Tongue	(0)	(1)	(0)	(0)
Tooth	(25)	(32)	(30)	(25)
Malformation	1 (4%)			
Peridontal tissue, inflammation	24 (96%)	32 (100%)	30 (100%)	25 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	48 (96%)	50 (100%)	48 (96%)	45 (90%)
Thrombosis	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Atrium, thrombosis	1 (2%)			
Endocardium, hyperplasia				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	9 (18%)	18 (36%)	20 (40%)	11 (22%)
Degeneration, cystic	2 (4%)			1 (2%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia	12 (24%)	6 (12%)	9 (18%)	13 (26%)
Hypertrophy	7 (14%)	8 (16%)	10 (20%)	7 (14%)
Infiltration cellular, mononuclear cell		1 (2%)	9 (18%)	9 (18%)
Vacuolization cytoplasmic	31 (62%)	29 (58%)	33 (66%)	29 (58%)

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)		
Hyperplasia	12 (24%)	13 (26%)	15 (30%)	21 (42%)
Infiltration cellular, mononuclear cell	2 (4%)	2 (4%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	4 (8%)	3 (6%)	2 (4%)
Parathyroid gland	(48)	(49)	(49)	(49)
Hyperplasia			1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	13 (26%)	18 (36%)	20 (40%)	19 (38%)
Cyst	3 (6%)	2 (4%)	3 (6%)	4 (8%)
Pars distalis, hyperplasia	24 (48%)	23 (46%)	19 (38%)	18 (36%)
Pars intermedia, hyperplasia		1 (2%)		
Pars nervosa, Rathke's cleft, hyperplasia, tubular				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
C-cell, hyperplasia	22 (44%)	18 (36%)	17 (34%)	18 (36%)
Follicle, cyst				1 (2%)
Follicular cell, hyperplasia			2 (4%)	1 (2%)
General Body System				
None				
Genital System				
Coagulating gland	(0)	(1)	(1)	(0)
Inflammation		1 (100%)		
Epididymis	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation	1 (2%)			
Preputial gland	(50)	(50)	(50)	(50)
Inflammation	50 (100%)	47 (94%)	45 (90%)	46 (92%)
Duct, ectasia	7 (14%)	14 (28%)	2 (4%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)		
Inflammation	28 (56%)	29 (58%)	35 (70%)	30 (60%)
Epithelium, degeneration				1 (2%)
Epithelium, hyperplasia	6 (12%)	7 (14%)	7 (14%)	5 (10%)
Epithelium, vacuolization cytoplasmic	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(49)
Dilatation	1 (2%)			
Inflammation		1 (2%)		
Testis	(50)	(50)	(50)	(50)
Artery, inflammation, chronic active				1 (2%)
Germinal epithelium, atrophy	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Germinal epithelium, degeneration	1 (2%)		1 (2%)	
Interstitial cell, hyperplasia	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	5 (10%)	3 (6%)	3 (6%)
Hyperplasia	19 (38%)	24 (48%)	22 (44%)	29 (58%)

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Hematopoietic System (continued)				
Lymph node	(7)	(8)	(8)	(5)
Deep cervical, ectasia		1 (13%)		3 (60%)
Deep cervical, hemorrhage		2 (25%)		
Deep cervical, hyperplasia				1 (20%)
Deep cervical, hyperplasia, plasma cell				1 (20%)
Mediastinal, ectasia		2 (25%)	1 (13%)	
Mediastinal, hemorrhage		1 (13%)		
Lymph node, mandibular	(0)	(1)	(0)	(3)
Ectasia				1 (33%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	7 (14%)	4 (8%)	3 (6%)	5 (10%)
Hemorrhage			1 (2%)	
Hyperplasia, histiocytic				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen			1 (2%)	
Fibrosis		1 (2%)		
Hematopoietic cell proliferation	41 (82%)	34 (68%)	36 (72%)	43 (86%)
Hyperplasia, granulocytic				1 (2%)
Hyperplasia, histiocytic		1 (2%)	3 (6%)	
Hyperplasia, lymphoid, focal		1 (2%)		
Hyperplasia, focal		1 (2%)		1 (2%)
Pigmentation	29 (58%)	31 (62%)	32 (64%)	34 (68%)
Capsule, fibrosis				1 (2%)
Capsule, hemorrhage				1 (2%)
Lymphoid follicle, atrophy		1 (2%)		1 (2%)
Lymphoid follicle, depletion cellular		2 (4%)		
Red pulp, atrophy		1 (2%)		
Thymus	(49)	(45)	(47)	(48)
Atrophy	46 (94%)	40 (89%)	41 (87%)	42 (88%)
Integumentary System				
Mammary gland	(50)	(48)	(50)	(50)
Cyst	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Hyperplasia	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Edema	1 (2%)			
Hemorrhage	1 (2%)			
Inflammation	3 (6%)			
Ulcer	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperplasia, granulocytic				1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Edema				1 (2%)
Hemorrhage	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hydrocephalus			1 (2%)	1 (2%)
Mineralization		1 (2%)	1 (2%)	1 (2%)

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage		2 (4%)		
Inflammation	1 (2%)	2 (4%)	4 (8%)	3 (6%)
Metaplasia, squamous		1 (2%)	1 (2%)	
Alveolar, epithelium, hyperplasia	10 (20%)	12 (24%)	10 (20%)	16 (32%)
Alveolus, infiltration cellular, histiocyte	27 (54%)	33 (66%)	31 (62%)	31 (62%)
Artery, thrombosis		1 (2%)		
Vein, hemorrhage			1 (2%)	
Vein, inflammation				1 (2%)
Nose	(50)	(50)	(50)	(50)
Glands, hyperplasia			1 (2%)	
Lateral wall, inflammation	6 (12%)	4 (8%)	7 (14%)	11 (22%)
Nasolacrimal duct, inflammation	2 (4%)	4 (8%)	4 (8%)	5 (10%)
Nasopharyngeal duct, inflammation	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Nasopharyngeal duct, ulcer		1 (2%)		
Olfactory epithelium, metaplasia	1 (2%)		1 (2%)	
Respiratory epithelium, hyperplasia	19 (38%)	20 (40%)	25 (50%)	24 (48%)
Septum, inflammation	15 (30%)	11 (22%)	18 (36%)	17 (34%)
Turbinate, inflammation	11 (22%)	4 (8%)	18 (36%)	16 (32%)
Trachea	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	1 (2%)	4 (8%)	2 (4%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Choroid, hyperplasia		1 (2%)		
Ciliary body, iris, inflammation		1 (2%)	1 (2%)	
Cornea, inflammation		1 (2%)	1 (2%)	
Lens, degeneration	1 (2%)	2 (4%)		5 (10%)
Retina, atrophy	2 (4%)	3 (6%)		3 (6%)
Retina, degeneration	2 (4%)		1 (2%)	4 (8%)
Retina, necrosis	1 (2%)			
Sclera, mineralization	27 (54%)	30 (60%)	26 (52%)	22 (44%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	2 (4%)	
Infiltration cellular, lymphoid	1 (2%)			
Infiltration cellular, mononuclear cell	3 (6%)			
Inflammation	9 (18%)	8 (16%)	4 (8%)	10 (20%)
Zymbal's gland	(0)	(0)	(1)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Cyst	1 (2%)	1 (2%)	1 (2%)	
Mineralization	37 (74%)	43 (86%)	43 (86%)	44 (88%)
Necrosis		1 (2%)		
Nephropathy	49 (98%)	48 (96%)	50 (100%)	47 (94%)
Pigmentation	8 (16%)	7 (14%)	3 (6%)	4 (8%)
Renal tubule, degeneration	1 (2%)			
Transitional epithelium, hyperplasia		1 (2%)	1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)	1 (2%)	
Inflammation		1 (2%)	1 (2%)	
Ulcer		1 (2%)		
Transitional epithelium, hyperplasia			1 (2%)	

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

Table B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Formamide. 80

Table B2 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Formamide. 83

Table B3 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Formamide. 86

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	16	14	14
Natural deaths	2	4	2	4
Survivors				
Died last week of study		1		
Terminal sacrifice	38	29	34	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Lipoma			1 (2%)	
Intestine large, cecum	(50)	(49)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)		1 (2%)	
Hepatocellular adenoma, multiple	1 (2%)			
Mesentery	(18)	(16)	(12)	(9)
Oral mucosa	(15)	(9)	(12)	(7)
Gingival, squamous cell carcinoma	1 (7%)			
Pancreas	(50)	(50)	(50)	(50)
Salivary gland	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(1)	(0)
Tooth	(19)	(11)	(11)	(14)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Parathyroid gland	(46)	(47)	(46)	(47)
Adenoma			1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	22 (44%)	21 (42%)	15 (30%)	15 (30%)
Pars distalis, carcinoma	2 (4%)		1 (2%)	
Pars intermedia, carcinoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)	1 (2%)		
C-cell, adenoma	4 (8%)	7 (14%)	6 (12%)	5 (10%)
C-cell, carcinoma		1 (2%)		1 (2%)
Follicular cell, adenoma		2 (4%)	1 (2%)	3 (6%)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Adenoma	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Carcinoma			1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Granulosa-theca tumor benign		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Carcinoma			2 (4%)	
Polyp stromal	7 (14%)	7 (14%)	5 (10%)	10 (20%)
Sarcoma		1 (2%)	1 (2%)	1 (2%)
Sarcoma stromal			1 (2%)	
Schwannoma malignant	1 (2%)			
Cervix, sarcoma stromal				1 (2%)
Vagina	(0)	(2)	(0)	(0)
Leiomyosarcoma		1 (50%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(6)	(5)	(3)	(2)
Deep cervical, carcinoma, metastatic, thyroid gland				1 (50%)
Lymph node, mandibular	(1)	(0)	(1)	(1)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(47)	(47)	(46)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	1 (2%)	1 (2%)	
Fibroadenoma	19 (38%)	17 (34%)	14 (28%)	20 (40%)
Fibroadenoma, multiple	14 (28%)	7 (14%)	4 (8%)	5 (10%)
Fibroma	1 (2%)	1 (2%)		
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)			1 (2%)
Basal cell carcinoma		1 (2%)		
Fibroma		1 (2%)		
Keratoacanthoma	2 (4%)	1 (2%)		1 (2%)
Squamous cell papilloma		1 (2%)		
Trichoepithelioma	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)			
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Skeletal muscle	(1)	(0)	(1)	(0)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	2 (4%)		1 (2%)	
Granular cell tumor benign	1 (2%)			
Meningioma malignant				1 (2%)
Neuroblastoma			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)			
Mediastinum, alveolar/bronchiolar carcinoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Astrocytoma malignant, metastatic, brain			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia granulocytic			1 (2%)	
Leukemia mononuclear	10 (20%)	11 (22%)	12 (24%)	11 (22%)
Lymphoma malignant		1 (2%)		
Mesothelioma malignant	2 (4%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	46	40	46
Total primary neoplasms	100	87	78	82
Total animals with benign neoplasms	43	39	31	41
Total benign neoplasms	80	70	54	65
Total animals with malignant neoplasms	15	16	20	16
Total malignant neoplasms	20	17	24	17
Total animals with metastatic neoplasms	3		2	1
Total metastatic neoplasms	3		2	1

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

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Clitoral Gland: Adenoma				
Overall rate[a]	2/50 (4%)	1/50 (2%)	4/50 (8%)	3/49 (6%)
Adjusted rate[b]	4.4%	2.4%	9.0%	7.1%
Terminal rate[c]	2/38 (5%)	1/30 (3%)	3/34 (9%)	3/32 (9%)
First incidence (days)	730 (T)	730 (T)	658	730 (T)
Poly-3 test[d]	P=0.272	P=0.539N	P=0.324	P=0.464
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	5/50 (10%)	3/49 (6%)
Adjusted rate	4.4%	2.4%	11.2%	7.1%
Terminal rate	2/38 (5%)	1/30 (3%)	4/34 (12%)	3/32 (9%)
First incidence (days)	730 (T)	730 (T)	658	730 (T)
Poly-3 test	P=0.260	P=0.539N	P=0.205	P=0.464
Mammary Gland: Fibroadenoma				
Overall rate	33/50 (66%)	24/50 (48%)	18/50 (36%)	25/50 (50%)
Adjusted rate	69.2%	55.6%	39.5%	55.8%
Terminal rate	26/38 (68%)	17/30 (57%)	13/34 (38%)	18/32 (56%)
First incidence (days)	478	505	562	612
Poly-3 test	P=0.094N	P=0.123N	P=0.002N	P=0.125N
Mammary Gland: Fibroma or Fibroadenoma				
Overall rate	33/50 (66%)	25/50 (50%)	18/50 (36%)	25/50 (50%)
Adjusted rate	69.2%	57.1%	39.5%	55.8%
Terminal rate	26/38 (68%)	17/30 (57%)	13/34 (38%)	18/32 (56%)
First incidence (days)	478	505	562	612
Poly-3 test	P=0.087N	P=0.157N	P=0.002N	P=0.125N
Mammary Gland: Fibroma, Fibroadenoma, or Carcinoma				
Overall rate	35/50 (70%)	26/50 (52%)	18/50 (36%)	25/50 (50%)
Adjusted rate	73.4%	59.4%	39.5%	55.8%
Terminal rate	28/38 (74%)	18/30 (60%)	13/34 (38%)	18/32 (56%)
First incidence (days)	478	505	562	612
Poly-3 test	P=0.034N	P=0.108N	P<0.001N	P=0.053N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/50 (44%)	21/50 (42%)	15/50 (30%)	15/50 (30%)
Adjusted rate	46.8%	48.6%	33.6%	33.6%
Terminal rate	18/38 (47%)	14/30 (47%)	12/34 (35%)	9/32 (28%)
First incidence (days)	478	545	708	527
Poly-3 test	P=0.065N	P=0.518	P=0.139N	P=0.138N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	24/50 (48%)	21/50 (42%)	16/50 (32%)	15/50 (30%)
Adjusted rate	50.9%	48.6%	35.5%	33.6%
Terminal rate	19/38 (50%)	14/30 (47%)	12/34 (35%)	9/32 (28%)
First incidence (days)	478	545	562	527
Poly-3 test	P=0.033N	P=0.495N	P=0.095N	P=0.067N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	8.7%	7.2%	0.0%	4.6%
Terminal rate	4/38 (11%)	2/30 (7%)	0/34 (0%)	2/32 (6%)
First incidence (days)	730 (T)	395	—[e]	730 (T)
Poly-3 test	P=0.197N	P=0.550N	P=0.065N	P=0.365N

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Thyroid Gland (C-Cell): Adenoma				
Overall rate	5/50 (10%)	8/50 (16%)	6/50 (12%)	5/50 (10%)
Adjusted rate	10.9%	18.7%	13.4%	11.6%
Terminal rate	5/38 (13%)	4/30 (13%)	5/34 (15%)	5/32 (16%)
First incidence (days)	730 (T)	505	604	730 (T)
Poly-3 test	P=0.481N	P=0.230	P=0.483	P=0.592
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	9/50 (18%)	6/50 (12%)	6/50 (12%)
Adjusted rate	10.9%	21.1%	13.4%	13.8%
Terminal rate	5/38 (13%)	5/30 (17%)	5/34 (15%)	5/32 (16%)
First incidence (days)	730 (T)	505	604	630
Poly-3 test	P=0.548	P=0.153	P=0.483	P=0.464
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	4.9%	2.3%	6.9%
Terminal rate	0/38 (0%)	2/30 (7%)	1/34 (3%)	3/32 (9%)
First incidence (days)	—	730 (T)	730 (T)	730 (T)
Poly-3 test	P=0.094	P=0.213	P=0.493	P=0.108
Uterus: Stromal Polyp				
Overall rate	7/50 (14%)	7/50 (14%)	5/50 (10%)	10/50 (20%)
Adjusted rate	15.3%	16.5%	11.1%	22.7%
Terminal rate	7/38 (18%)	4/30 (13%)	3/34 (9%)	6/32 (19%)
First incidence (days)	730 (T)	353	590	533
Poly-3 test	P=0.226	P=0.554	P=0.394N	P=0.266
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	7/50 (14%)	7/50 (14%)	5/50 (10%)	11/50 (22%)
Adjusted rate	15.3%	16.5%	11.1%	24.9%
Terminal rate	7/38 (18%)	4/30 (13%)	3/34 (9%)	6/32 (19%)
First incidence (days)	730 (T)	353	590	533
Poly-3 test	P=0.148	P=0.554	P=0.394N	P=0.189
All Organs: Mononuclear Cell Leukemia				
Overall rate	10/50 (20%)	11/50 (22%)	12/50 (24%)	11/50 (22%)
Adjusted rate	21.2%	25.2%	26.1%	24.4%
Terminal rate	4/38 (11%)	5/30 (17%)	7/34 (21%)	5/32 (16%)
First incidence (days)	654	469	464	497
Poly-3 test	P=0.424	P=0.420	P=0.381	P=0.454
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	39/50 (78%)	31/50 (62%)	41/50 (82%)
Adjusted rate	90.2%	84.5%	66.8%	88.5%
Terminal rate	36/38 (95%)	25/30 (83%)	23/34 (68%)	29/32 (91%)
First incidence (days)	478	353	562	527
Poly-3 test	P=0.398N	P=0.289N	P=0.003N	P=0.533N
All Organs: Malignant Neoplasms				
Overall rate	15/50 (30%)	16/50 (32%)	20/50 (40%)	16/50 (32%)
Adjusted rate	30.6%	35.7%	42.0%	33.9%
Terminal rate	6/38 (16%)	8/30 (27%)	10/34 (29%)	5/32 (16%)
First incidence (days)	402	395	464	255
Poly-3 test	P=0.405	P=0.381	P=0.172	P=0.450

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	46/50 (92%)	40/50 (80%)	46/50 (92%)
Adjusted rate	98.0%	93.4%	82.7%	93.3%
Terminal rate	37/38 (97%)	27/30 (90%)	26/34 (77%)	29/32 (91%)
First incidence (days)	402	353	464	255
Poly-3 test	P=0.203N	P=0.261N	P=0.010N	P=0.247N

(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 is the P value corresponding to pairwise comparison 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 Formamide[a]

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	16	14	14
Natural deaths	2	4	2	4
Survivors				
Died last week of study		1		
Terminal sacrifice	38	29	34	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(49)	(50)	(50)
Erosion		1 (2%)		
Inflammation			1 (2%)	1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	4 (8%)	7 (14%)	7 (14%)	3 (6%)
Intestine small, ileum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Angiectasis	4 (8%)	1 (2%)	4 (8%)	2 (4%)
Basophilic focus	44 (88%)	46 (92%)	47 (94%)	45 (90%)
Clear cell focus	7 (14%)	6 (12%)	5 (10%)	5 (10%)
Degeneration, cystic				1 (2%)
Eosinophilic focus	10 (20%)	8 (16%)	11 (22%)	14 (28%)
Fatty change, focal	12 (24%)	8 (16%)	10 (20%)	8 (16%)
Fatty change, diffuse	7 (14%)	5 (10%)	7 (14%)	4 (8%)
Hematopoietic cell proliferation	4 (8%)	3 (6%)	6 (12%)	2 (4%)
Hepatodiaphragmatic nodule	3 (6%)	7 (14%)	3 (6%)	5 (10%)
Inflammation	39 (78%)	45 (90%)	46 (92%)	41 (82%)
Mixed cell focus	19 (38%)	12 (24%)	15 (30%)	18 (36%)
Necrosis	3 (6%)	1 (2%)		4 (8%)
Regeneration				1 (2%)
Vacuolization, cytoplasmic, focal		3 (6%)	3 (6%)	2 (4%)
Vacuolization, cytoplasmic, diffuse			2 (4%)	1 (2%)
Bile duct, cyst		1 (2%)	2 (4%)	
Bile duct, hyperplasia	27 (54%)	28 (56%)	15 (30%)	16 (32%)
Centrilobular, degeneration	8 (16%)	7 (14%)	6 (12%)	5 (10%)
Oval cell, hyperplasia	13 (26%)	22 (44%)	16 (32%)	17 (34%)
Serosa, inflammation		1 (2%)		
Mesentery	(18)	(16)	(12)	(9)
Fat, necrosis	17 (94%)	16 (100%)	12 (100%)	9 (100%)
Oral mucosa	(15)	(9)	(12)	(7)
Gingival, hyperplasia, squamous	14 (93%)	9 (100%)	12 (100%)	7 (100%)
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)	1 (2%)		
Infiltration cellular, mononuclear cell	9 (18%)	1 (2%)	6 (12%)	6 (12%)
Inflammation, chronic active	8 (16%)	12 (24%)	16 (32%)	14 (28%)
Vacuolization cytoplasmic		1 (2%)		
Acinus, atrophy	7 (14%)	9 (18%)	10 (20%)	14 (28%)
Acinus, hyperplasia	1 (2%)			
Artery, inflammation, chronic active			1 (2%)	
Duct, cyst	3 (6%)	3 (6%)	5 (10%)	1 (2%)

[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 Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Alimentary System (continued)				
Salivary gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Inflammation		1 (2%)	1 (2%)	
Necrosis				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema		2 (4%)		
Erosion			1 (2%)	
Hyperplasia, squamous		1 (2%)	1 (2%)	
Inflammation	1 (2%)	2 (4%)	3 (6%)	
Mineralization				1 (2%)
Ulcer	1 (2%)	1 (2%)	2 (4%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Amyloid deposition				1 (2%)
Mineralization			1 (2%)	1 (2%)
Gland, cyst	2 (4%)	1 (2%)		
Tongue	(0)	(0)	(1)	(0)
Hyperplasia, squamous			1 (100%)	
Tooth	(19)	(11)	(11)	(14)
Peridontal tissue, inflammation	19 (100%)	11 (100%)	11 (100%)	14 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, inflammation, focal		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Cardiomyopathy	44 (88%)	47 (94%)	40 (80%)	37 (74%)
Degeneration				1 (2%)
Inflammation	1 (2%)	1 (2%)	2 (4%)	
Thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	39 (78%)	38 (76%)	38 (76%)	43 (86%)
Degeneration, cystic	5 (10%)	7 (14%)	5 (10%)	10 (20%)
Hyperplasia	20 (40%)	22 (44%)	8 (16%)	15 (30%)
Hypertrophy	11 (22%)	10 (20%)	11 (22%)	13 (26%)
Infiltration cellular, mononuclear cell	2 (4%)			
Necrosis				1 (2%)
Vacuolization cytoplasmic	30 (60%)	16 (32%)	23 (46%)	19 (38%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	2 (4%)	4 (8%)	1 (2%)
Infiltration cellular, mononuclear cell	3 (6%)		1 (2%)	
Necrosis				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(46)	(47)	(46)	(47)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	36 (72%)	25 (50%)	23 (46%)	24 (48%)
Cyst	9 (18%)	10 (20%)	12 (24%)	5 (10%)
Cytoplasmic alteration				1 (2%)
Hemorrhage		1 (2%)		
Pars distalis, cyst	1 (2%)			1 (2%)
Pars distalis, hyperplasia	24 (48%)	21 (42%)	26 (52%)	25 (50%)
Pars nervosa, cyst				1 (2%)

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	24 (48%)	24 (48%)	34 (68%)	27 (54%)
Follicle, cyst	1 (2%)			
Follicular cell, hyperplasia	2 (4%)			
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Cyst	1 (2%)			1 (2%)
Hyperplasia	5 (10%)	7 (14%)	6 (12%)	8 (16%)
Inflammation	36 (72%)	39 (78%)	43 (86%)	37 (76%)
Duct, cyst	11 (22%)	14 (28%)	23 (46%)	16 (33%)
Ovary	(50)	(50)	(50)	(50)
Congestion				1 (2%)
Cyst	9 (18%)		9 (18%)	5 (10%)
Necrosis			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Adenomyosis	1 (2%)		1 (2%)	2 (4%)
Cyst	1 (2%)		1 (2%)	
Decidual reaction			1 (2%)	
Hemorrhage		1 (2%)		
Inflammation		1 (2%)	4 (8%)	1 (2%)
Endometrium, hyperplasia, cystic	8 (16%)	7 (14%)	6 (12%)	5 (10%)
Vagina	(0)	(2)	(0)	(0)
Inflammation		1 (50%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia	14 (28%)	16 (32%)	18 (36%)	13 (26%)
Hyperplasia, histiocytic	1 (2%)			
Inflammation, granulomatous	1 (2%)			
Myelofibrosis	1 (2%)	1 (2%)		1 (2%)
Lymph node	(6)	(5)	(3)	(2)
Ectasia			1 (33%)	
Hyperplasia, lymphoid	1 (17%)			
Deep cervical, ectasia	1 (17%)	2 (40%)		
Deep cervical, hyperplasia, plasma cell		1 (20%)		
Mediastinal, ectasia	1 (17%)			
Mediastinal, hyperplasia, lymphoid		1 (20%)		
Mediastinal, inflammation			1 (33%)	
Lymph node, mandibular	(1)	(0)	(1)	(1)
Ectasia	1 (100%)			1 (100%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	2 (4%)	1 (2%)	4 (8%)	
Hyperplasia, histiocytic			1 (2%)	
Hyperplasia, lymphoid	1 (2%)	1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	40 (80%)	32 (64%)	40 (80%)	40 (80%)
Hyperplasia, lymphoid, focal			1 (2%)	
Pigmentation	39 (78%)	39 (78%)	41 (82%)	38 (7%)
Capsule, thrombosis				1 (2%)

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Hematopoietic System (continued)				
Thymus	(48)	(47)	(47)	(46)
Atrophy	45 (94%)	44 (94%)	43 (91%)	45 (98%)
Pigmentation		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	24 (48%)	19 (38%)	20 (40%)	6 (12%)
Hyperplasia	15 (30%)	7 (14%)	7 (14%)	7 (14%)
Hyperplasia, atypical		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Hyperplasia, squamous		1 (2%)		
Inflammation	1 (2%)	1 (2%)	1 (2%)	
Ulcer		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis	1 (2%)	1 (2%)		
Skeletal muscle	(1)	(0)	(1)	(0)
Cyst			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis			2 (4%)	
Hemorrhage		2 (4%)	1 (2%)	1 (2%)
Mineralization	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation	5 (10%)	2 (4%)	5 (10%)	8 (16%)
Metaplasia, squamous	1 (2%)		1 (2%)	
Alveolar epithelium, hyperplasia	12 (24%)	6 (12%)	15 (30%)	6 (12%)
Alveolus, infiltration cellular, histiocyte	45 (90%)	43 (86%)	46 (92%)	43 (86%)
Serosa, fibrosis			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Gland, cyst	1 (2%)			
Lateral wall, inflammation	4 (8%)	5 (10%)	3 (6%)	2 (4%)
Nasolacrimal duct, inflammation	6 (12%)	6 (12%)	5 (10%)	2 (4%)
Nasopharyngeal duct, inflammation		3 (6%)		
Nerve, degeneration			1 (2%)	
Olfactory epithelium, metaplasia			1 (2%)	2 (4%)
Respiratory epithelium, hyperplasia	37 (74%)	31 (62%)	25 (50%)	22 (44%)
Septum, inflammation	10 (20%)	9 (18%)	12 (24%)	9 (18%)
Turbinate, inflammation	20 (40%)	11 (22%)	12 (24%)	9 (18%)
Trachea	(50)	(50)	(50)	(50)
Inflammation	7 (14%)	6 (12%)	3 (6%)	7 (14%)

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Inflammation		1 (2%)		
Anterior chamber, ciliary body iris, inflammation				1 (2%)
Cornea, inflammation			2 (4%)	1 (2%)
Iris, inflammation			1 (2%)	
Lens, degeneration		2 (4%)	1 (2%)	3 (6%)
Retina, atrophy		1 (2%)	1 (2%)	2 (4%)
Retina, degeneration		1 (2%)	1 (2%)	
Sclera, mineralization	5 (10%)	2 (4%)		
Harderian gland	(50)	(50)	(50)	(50)
Degeneration		1 (2%)		
Infiltration cellular, mononuclear cell		1 (2%)		1 (2%)
Inflammation	13 (26%)	11 (22%)	17 (34%)	17 (34%)
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	1 (2%)			
Cyst			1 (2%)	
Infarct	3 (6%)			2 (4%)
Infiltration cellular, mononuclear cell		1 (2%)		
Inflammation	2 (4%)			
Mineralization	43 (86%)	42 (84%)	36 (72%)	43 (86%)
Nephropathy	44 (88%)	45 (90%)	46 (92%)	45 (90%)
Pigmentation	3 (6%)	6 (12%)	2 (4%)	2 (4%)
Pelvis, inflammation, suppurative			1 (2%)	
Transitional epithelium, hyperplasia	4 (8%)		1 (2%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell			1 (2%)	1 (2%)
Inflammation	1 (2%)	1 (2%)		

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

Table C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Formamide. 92

Table C2 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Formamide. 96

Table C3 Historical Incidence of Hemangiosarcoma in Control Male B6C3F1 Mice. 99

Table C4 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide. 100

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	8	6	14
Natural deaths	7		8	3
Survivors				
Terminal sacrifice	39	42	36	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Periesophageal tissue, hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Gallbladder	(45)	(48)	(45)	(46)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas				1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Epithelium, carcinoma		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma		3 (6%)		1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas				1 (2%)
Cholangiocarcinoma			1 (2%)	
Hemangioma				1 (2%)
Hemangiosarcoma	1 (2%)	5 (10%)	6 (12%)	3 (6%)
Hemangiosarcoma, multiple			1 (2%)	5 (10%)
Hepatoblastoma	1 (2%)	1 (2%)	3 (6%)	
Hepatoblastoma, multiple				1 (2%)
Hepatocellular adenoma	17 (34%)	12 (24%)	20 (40%)	18 (36%)
Hepatocellular adenoma, multiple	7 (14%)	12 (24%)	5 (10%)	10 (20%)
Hepatocellular carcinoma	12 (24%)	11 (22%)	9 (18%)	18 (36%)
Hepatocellular carcinoma, multiple	3 (6%)	4 (8%)	3 (6%)	1 (2%)
Hepatocholangiocarcinoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Mesentery	(3)	(1)	(1)	(1)
Carcinoma, metastatic, pancreas				1 (100%)
Oral mucosa	(7)	(5)	(5)	(7)
Pancreas	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas				1 (2%)
Tooth	(15)	(14)	(9)	(2)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Hemangioma	1 (2%)			
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Bilateral, carcinoma, metastatic, pancreas				1 (2%)
Subcapsular, adenoma	2 (4%)	1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		
Parathyroid gland	(43)	(39)	(46)	(47)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	1 (2%)			
Pars distalis, carcinoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, adenoma		3 (6%)	1 (2%)	1 (2%)
Follicle, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Testis	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Bilateral, interstitial cell, adenoma		1 (2%)		
Interstitial cell, adenoma		1 (2%)		1 (2%)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		1 (2%)
Lymph node	(4)	(2)	(1)	(1)
Mediastinal, carcinoma, metastatic, pancreas				1 (100%)
Pancreatic, carcinoma, metastatic, pancreas				1 (100%)
Renal, carcinoma, metastatic, pancreas				1 (100%)
Lymph node, mandibular	(49)	(48)	(49)	(48)
Lymph node, mesenteric	(50)	(47)	(50)	(49)
Carcinoma, metastatic, pancreas				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Hemangiosarcoma, multiple			1 (2%)	
Thymus	(47)	(46)	(50)	(46)
Carcinoma, metastatic, pancreas				1 (2%)
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, hemangioma				1 (2%)
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, sarcoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(0)	(2)
Carcinoma, metastatic, pancreas				1 (50%)
Hepatoblastoma, metastatic, liver				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	1 (2%)			
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	8 (16%)	3 (6%)	6 (12%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma	6 (12%)	4 (8%)	4 (8%)	11 (22%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)	2 (4%)	
Carcinoma, metastatic, Harderian gland	1 (2%)	2 (4%)	1 (2%)	
Carcinoma, metastatic, liver	1 (2%)			
Carcinoma, metastatic, pancreas				1 (2%)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Hepatoblastoma, metastatic, liver			1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	4 (8%)	3 (6%)	6 (12%)
Hepatocellular carcinoma, metastatic, uncertain primary site				2 (4%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		1 (2%)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Mediastinum, cholangiocarcinoma, metastatic, liver			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Pleura	(1)	(0)	(0)	(0)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	8 (16%)	2 (4%)	4 (8%)
Carcinoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Bilateral, adenoma				1 (2%)
Bilateral, carcinoma		1 (2%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas				1 (2%)
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Renal tubule, carcinoma				1 (2%)
Renal tubule, cholangiocarcinoma, metastatic, liver			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Lymphoma malignant	2 (4%)		2 (4%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	39	43	46
Total primary neoplasms	78	85	76	90
Total animals with benign neoplasms	31	29	32	33
Total benign neoplasms	45	45	37	39
Total animals with malignant neoplasms	28	27	29	36
Total malignant neoplasms	33	40	39	51
Total animals with metastatic neoplasms	8	7	7	11
Total metastatic neoplasms	11	11	14	30
Total animals with malignant neoplasms uncertain primary site				2

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

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	8/50 (16%)	2/50 (4%)	5/50 (10%)
Adjusted rate[b]	12.9%	16.8%	4.5%	11.1%
Terminal rate[c]	4/39 (10%)	6/42 (14%)	2/36 (6%)	2/33 (6%)
First incidence (days)	456	478	728 (T)	658
Poly-3 test[d]	P=0.301N	P=0.402	P=0.145N	P=0.523N
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.4%	6.4%	6.7%	2.2%
Terminal rate	2/39 (5%)	3/42 (7%)	3/36 (8%)	0/33 (0%)
First incidence (days)	728 (T)	728 (T)	728 (T)	708
Poly-3 test	P=0.355N	P=0.514	P=0.496	P=0.506N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	11/50 (22%)	5/50 (10%)	5/50 (10%)
Adjusted rate	15.0%	23.1%	11.1%	11.1%
Terminal rate	5/39 (13%)	9/42 (21%)	5/36 (14%)	2/33 (6%)
First incidence (days)	456	478	728 (T)	658
Poly-3 test	P=0.188N	P=0.230	P=0.406N	P=0.402N
Small Intestine (Jejunum): Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	6.3%	0.0%	2.2%
Terminal rate	0/39 (0%)	2/42 (5%)	0/36 (0%)	0/33 (0%)
First incidence (days)	—[e]	478	—[f]	650
Poly-3 test	P=0.633N	P=0.127	—	P=0.498
Liver: Hemangiosarcoma				
Overall rate	1/50 (2%)	5/50 (10%)	7/50 (14%)	8/50 (16%)
Adjusted rate	2.2%	10.6%	15.2%	17.5%
Terminal rate	1/39 (3%)	4/42 (10%)	4/36 (11%)	3/33 (9%)
First incidence (days)	728 (T)	655	449	635
Poly-3 test	P=0.018	P=0.110	P=0.032	P=0.016
Liver: Hepatocellular Adenoma				
Overall rate	24/50 (48%)	24/50 (48%)	25/50 (50%)	28/50 (56%)
Adjusted rate	50.8%	51.3%	53.4%	61.5%
Terminal rate	21/39 (54%)	23/42 (55%)	19/36 (53%)	22/33 (67%)
First incidence (days)	456	705	343	618
Poly-3 test	P=0.153	P=0.565	P=0.484	P=0.200
Liver: Hepatocellular Carcinoma				
Overall rate	15/50 (30%)	15/50 (30%)	12/50 (24%)	19/50 (38%)
Adjusted rate	30.9%	30.5%	25.8%	39.8%
Terminal rate	7/39 (18%)	10/42 (24%)	8/36 (22%)	10/33 (30%)
First incidence (days)	455	478	343	449
Poly-3 test	P=0.199	P=0.570N	P=0.371N	P=0.244
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	33/50 (66%)	34/50 (68%)	32/50 (64%)	36/50 (72%)
Adjusted rate	67.0%	69.1%	67.2%	74.7%
Terminal rate	24/39 (62%)	28/42 (67%)	23/36 (64%)	24/33 (73%)
First incidence (days)	455	478	343	449
Poly-3 test	P=0.238	P=0.498	P=0.578	P=0.266

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	2.1%	6.7%	2.2%
Terminal rate	1/39 (3%)	1/42 (2%)	3/36 (8%)	0/33 (0%)
First incidence (days)	728 (T)	728 (T)	728 (T)	485
Poly-3 test	P=0.549	P=0.754N	P=0.302	P=0.760
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	16/50 (32%)	16/50 (32%)	14/50 (28%)	20/50 (40%)
Adjusted rate	33.0%	32.6%	30.1%	41.3%
Terminal rate	8/39 (21%)	11/42 (26%)	10/36 (28%)	10/33 (30%)
First incidence (days)	455	478	343	449
Poly-3 test	P=0.210	P=0.567N	P=0.466N	P=0.264
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	34/50 (68%)	34/50 (68%)	34/50 (68%)	37/50 (74%)
Adjusted rate	69.0%	69.1%	71.4%	75.7%
Terminal rate	25/39 (64%)	28/42 (67%)	25/36 (69%)	24/33 (73%)
First incidence (days)	455	478	343	449
Poly-3 test	P=0.237	P=0.584	P=0.487	P=0.302
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	10/50 (20%)	4/50 (8%)	7/50 (14%)	1/50 (2%)
Adjusted rate	21.4%	8.6%	15.5%	2.3%
Terminal rate	7/39 (18%)	4/42 (10%)	6/36 (17%)	1/33 (3%)
First incidence (days)	456	728 (T)	664	728 (T)
Poly-3 test	P=0.010N	P=0.072N	P=0.325N	P=0.005N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	5/50 (10%)	6/50 (12%)	11/50 (22%)
Adjusted rate	13.2%	10.7%	13.2%	24.3%
Terminal rate	5/39 (13%)	5/42 (12%)	3/36 (8%)	8/33 (24%)
First incidence (days)	705	728 (T)	633	635
Poly-3 test	P=0.059	P=0.480N	P=0.621	P=0.137
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	16/50 (32%)	9/50 (18%)	13/50 (26%)	12/50 (24%)
Adjusted rate	34.1%	19.3%	28.5%	26.6%
Terminal rate	12/39 (31%)	9/42 (21%)	9/36 (25%)	9/33 (27%)
First incidence (days)	456	728 (T)	633	635
Poly-3 test	P=0.389N	P=0.080N	P=0.359N	P=0.286N
Spleen: Hemangiosarcoma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.4%	4.3%	4.4%	6.7%
Terminal rate	2/39 (5%)	1/42 (2%)	0/36 (0%)	0/33 (0%)
First incidence (days)	728 (T)	655	633	646
Poly-3 test	P=0.379	P=0.681N	P=0.693	P=0.497
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	0.0%	6.4%	2.2%	2.2%
Terminal rate	0/39 (0%)	3/42 (7%)	1/36 (3%)	0/33 (0%)
First incidence (days)	—	728 (T)	728 (T)	659
Poly-3 test	P=0.586	P=0.124	P=0.498	P=0.498

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.2%	6.4%	2.2%	2.2%
Terminal rate	1/39 (3%)	3/42 (7%)	1/36 (3%)	0/33 (0%)
First incidence (days)	728 (T)	728 (T)	728 (T)	659
Poly-3 test	P=0.449N	P=0.316	P=0.759	P=0.758
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	7/50 (14%)	7/50 (14%)	11/50 (22%)
Adjusted rate	6.6%	14.9%	15.2%	23.8%
Terminal rate	3/39 (8%)	6/42 (14%)	4/36 (11%)	3/33 (9%)
First incidence (days)	728 (T)	655	449	635
Poly-3 test	P=0.019	P=0.173	P=0.164	P=0.021
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/50 (10%)	7/50 (14%)	7/50 (14%)	12/50 (24%)
Adjusted rate	11.0%	14.9%	15.2%	26.0%
Terminal rate	5/39 (13%)	6/42 (14%)	4/36 (11%)	4/33 (12%)
First incidence (days)	728 (T)	655	449	635
Poly-3 test	P=0.035	P=0.405	P=0.390	P=0.057
All Organs: Benign Neoplasms				
Overall rate	31/50 (62%)	29/50 (58%)	32/50 (64%)	33/50 (66%)
Adjusted rate	64.5%	61.0%	67.9%	71.3%
Terminal rate	26/39 (67%)	27/42 (64%)	24/36 (67%)	24/33 (73%)
First incidence (days)	456	478	343	618
Poly-3 test	P=0.207	P=0.444N	P=0.447	P=0.311
All Organs: Malignant Neoplasms				
Overall rate	28/50 (56%)	27/50 (54%)	29/50 (58%)	36/50 (72%)
Adjusted rate	56.2%	54.8%	59.1%	72.0%
Terminal rate	18/39 (46%)	21/42 (50%)	18/36 (50%)	19/33 (58%)
First incidence (days)	455	478	203	449
Poly-3 test	P=0.041	P=0.525N	P=0.464	P=0.074
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	39/50 (78%)	43/50 (86%)	46/50 (92%)
Adjusted rate	82.2%	79.0%	86.0%	92.0%
Terminal rate	31/39 (80%)	32/42 (76%)	29/36 (81%)	29/33 (88%)
First incidence (days)	455	478	203	449
Poly-3 test	P=0.059	P=0.437N	P=0.405	P=0.122

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, 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 is the P value corresponding to pairwise comparison 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
Historical Incidence of Hemangiosarcoma in Control Male B6C3F1 Mice[a]

Study	Incidence in Controls		
	Liver	Spleen	All Organs
Historical Incidence: Water Gavage Studies			
Acrylonitrile	2/50	1/50	3/50
Elmiron® (sodium pentosanpolysulfate)	2/50	0/49	6/50
Formamide	1/50	2/50	3/50
5-Hydroxymethyl-2-furfural	1/50	1/50	2/50
Overall Historical Incidence: Water Gavage Studies			
Total (%)	6/200 (3.0%)	4/199 (2.0%)	14/200 (7.0%)
Mean ± standard deviation	3.0% ± 1.2%	2.0% ± 1.6%	7.0% ± 3.5%
Range	2%-4%	0%-4%	4%-12%
Overall Historical Incidence: All Routes			
Total (%)	33/1,496 (2.2%)	24/1,483 (1.6%)	76/1,499 (5.1%)
Mean ± standard deviation	2.3% ± 1.6%	1.7% ± 1.2%	5.2% ± 3.2%
Range	0%-8%	0%-4%	0%-12%

[a] Data as of March 2, 2007

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide[a]

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	8	6	14
Natural deaths	7		8	3
Survivors				
Terminal sacrifice	39	42	36	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Artery, inflammation, chronic			1 (2%)	
Muscularis, inflammation, chronic	1 (2%)			
Gallbladder	(45)	(48)	(45)	(46)
Cyst		1 (2%)		
Inflammation, chronic		1 (2%)	1 (2%)	3 (7%)
Ulcer				1 (2%)
Epithelium, cytoplasmic alteration		1 (2%)		2 (4%)
Epithelium, hyperplasia		1 (2%)	2 (4%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Hemorrhage			1 (2%)	
Artery, inflammation, chronic			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Artery, inflammation, chronic	1 (2%)			
Intestine large, rectum	(50)	(50)	(50)	(50)
Artery, inflammation, chronic		1 (2%)		
Intestine small, duodenum	(50)	(50)	(50)	(50)
Artery, inflammation, chronic	1 (2%)			
Epithelium, hyperplasia, focal				1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation, granulomatous		1 (2%)		
Inflammation, chronic				1 (2%)
Peyer's patch, hyperplasia, lymphoid	2 (4%)			1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	2 (4%)	4 (8%)	3 (6%)	4 (8%)
Clear cell focus	29 (58%)	27 (54%)	21 (42%)	8 (16%)
Eosinophilic focus	16 (32%)	21 (42%)	14 (28%)	9 (18%)
Fatty change	31 (62%)	25 (50%)	15 (30%)	1 (2%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule				1 (2%)
Inflammation, acute	1 (2%)			1 (2%)
Inflammation, chronic	16 (32%)	16 (32%)	11 (22%)	15 (30%)
Mineralization	1 (2%)	1 (2%)		
Mixed cell focus	10 (20%)	12 (24%)	13 (26%)	11 (22%)
Necrosis	5 (10%)	9 (18%)	7 (14%)	11 (22%)
Tension lipidosis	4 (8%)	5 (10%)	1 (2%)	6 (12%)
Bile duct, dilatation		1 (2%)		
Bile duct, hyperplasia		1 (2%)		
Centrilobular, degeneration	1 (2%)			

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

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Centrilobular, necrosis	1 (2%)			1 (2%)
Serosa, hyperplasia		1 (2%)		
Vein, thrombosis				1 (2%)
Mesentery	(3)	(1)	(1)	(1)
Inflammation, chronic	1 (33%)			
Artery, inflammation, chronic	1 (33%)		1 (100%)	
Fat, necrosis	1 (33%)	1 (100%)		
Oral mucosa	(7)	(5)	(5)	(7)
Inflammation, chronic				1 (14%)
Gingival, inflammation, chronic	7 (100%)	5 (100%)	5 (100%)	6 (86%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	1 (2%)	3 (6%)		2 (4%)
Acinus, cytoplasmic alteration		1 (2%)	1 (2%)	1 (2%)
Duct, cyst			1 (2%)	1 (2%)
Duct, inflammation, chronic		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Inflammation, granulomatous	1 (2%)			
Artery, parotid gland, inflammation, chronic			1 (2%)	
Artery, parotid gland, mineralization			1 (2%)	1 (2%)
Parotid gland, atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation, chronic	1 (2%)			
Ulcer	1 (2%)		1 (2%)	1 (2%)
Artery, inflammation, chronic	1 (2%)			
Epithelium, hyperplasia, focal	1 (2%)		1 (2%)	
Epithelium, vacuolization cytoplasmic		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Hemorrhage, acute		1 (2%)		
Inflammation, chronic			4 (8%)	1 (2%)
Mineralization			1 (2%)	
Ulcer			1 (2%)	3 (6%)
Epithelium, glands, ectasia		1 (2%)		
Epithelium, glands, hyperplasia, focal			1 (2%)	
Epithelium, hyperplasia, focal		1 (2%)		
Glands, ectasia		1 (2%)	2 (4%)	2 (4%)
Tooth	(15)	(14)	(9)	(2)
Dysplasia	2 (13%)	1 (7%)	2 (22%)	
Malformation	14 (93%)	13 (93%)	7 (78%)	2 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, inflammation, chronic	1 (2%)			
Heart	(50)	(50)	(50)	(50)
Thrombosis				1 (2%)
Artery, inflammation, chronic	1 (2%)	1 (2%)	2 (4%)	
Atrium, thrombosis	1 (2%)	1 (2%)		3 (6%)
Epicardium, hyperplasia	1 (2%)			
Epicardium, infiltration cellular, mononuclear cell	1 (2%)			
Myocardium, infiltration cellular, mononuclear cell	9 (18%)	10 (20%)	2 (4%)	12 (24%)
Myocardium, mineralization			2 (4%)	2 (4%)
Myocardium, necrosis	3 (6%)	1 (2%)	1 (2%)	4 (8%)
Myocardium, necrosis, focal		1 (2%)		
Nerve, inflammation, chronic				1 (2%)
Ventricle, atrophy			1 (2%)	

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Angiectasis		1 (2%)	1 (2%)	1 (2%)
Hyperplasia	2 (4%)			2 (4%)
Hypertrophy	15 (30%)	17 (34%)	17 (35%)	13 (26%)
Pigmentation	1 (2%)			2 (4%)
Vacuolization cytoplasmic				1 (2%)
Vacuolization cytoplasmic, focal	17 (34%)	14 (28%)	11 (22%)	16 (32%)
Subcapsular, hyperplasia	50 (100%)	49 (98%)	42 (86%)	45 (90%)
Subcapsular, hypertrophy, focal	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	35 (70%)	24 (48%)	24 (48%)	9 (18%)
Parathyroid gland	(43)	(39)	(46)	(47)
Right, atrophy				1 (2%)
Pituitary gland	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Cyst	5 (10%)	2 (4%)	2 (4%)	2 (4%)
Pars distalis, hyperplasia, focal	3 (6%)	4 (8%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)	3 (6%)	2 (4%)	5 (10%)
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic		1 (2%)		1 (2%)
C-cell, hyperplasia				1 (2%)
Follicle, hyperplasia	1 (2%)			
Follicle, hyperplasia, focal	12 (24%)	15 (30%)	8 (16%)	8 (16%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	1 (2%)		3 (6%)
Inflammation, chronic	1 (2%)			1 (2%)
Artery, inflammation, chronic		1 (2%)		
Preputial gland	(50)	(50)	(50)	(50)
Cyst	19 (38%)	11 (22%)	11 (22%)	10 (20%)
Infiltration cellular, lymphoid			1 (2%)	
Infiltration cellular, chronic				1 (2%)
Inflammation, suppurative				1 (2%)
Inflammation, granulomatous	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)	4 (8%)		1 (2%)
Duct, cyst		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid			1 (2%)	
Inflammation, suppurative	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy				2 (4%)
Fibrosis	1 (2%)		1 (2%)	
Inflammation, suppurative	1 (2%)			
Inflammation, chronic		1 (2%)		

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Genital System (continued)				
Testis	(50)	(50)	(50)	(50)
Granuloma sperm				1 (2%)
Artery, inflammation, chronic	1 (2%)		1 (2%)	
Artery, mineralization		2 (4%)	5 (10%)	35 (70%)
Artery, thrombosis			1 (2%)	
Germinal epithelium, degeneration	1 (2%)	4 (8%)	1 (2%)	4 (8%)
Germinal epithelium, inflammation, chronic				1 (2%)
Germinal epithelium, mineralization		1 (2%)	1 (2%)	
Tunic, inflammation, chronic	1 (2%)			1 (2%)
Tunic, mineralization	1 (2%)		5 (10%)	27 (54%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	34 (68%)	39 (78%)	35 (70%)	34 (68%)
Lymph node	(4)	(2)	(1)	(1)
Bronchial, hyperplasia, lymphoid	1 (25%)			
Lumbar, hyperplasia, lymphoid	1 (25%)			
Mediastinal, hyperplasia, lymphoid	2 (50%)			
Pancreatic, hyperplasia		1 (50%)		
Renal, hyperplasia, lymphoid	2 (50%)			
Lymph node, mandibular	(49)	(48)	(49)	(48)
Atrophy	11 (22%)	5 (10%)	6 (12%)	13 (27%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, lymphoid	2 (4%)	5 (10%)	5 (10%)	2 (4%)
Necrosis, lymphoid	2 (4%)			1 (2%)
Lymph node, mesenteric	(50)	(47)	(50)	(49)
Atrophy	10 (20%)	3 (6%)	6 (12%)	14 (29%)
Hyperplasia, lymphoid	4 (8%)	1 (2%)	4 (8%)	
Necrosis, lymphoid	1 (2%)		1 (2%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Atrophy	2 (4%)	6 (12%)	3 (6%)	7 (14%)
Hematopoietic cell proliferation	14 (28%)	14 (28%)	20 (40%)	28 (56%)
Hyperplasia, plasma cell	1 (2%)			
Lymphoid follicle, hyperplasia	4 (8%)	1 (2%)	4 (8%)	
Lymphoid follicle, necrosis	1 (2%)			
Thymus	(47)	(46)	(50)	(46)
Atrophy	41 (87%)	39 (85%)	37 (74%)	44 (96%)
Cyst	19 (40%)	9 (20%)	15 (30%)	9 (20%)
Ectopic parathyroid gland			1 (2%)	
Hyperplasia, lymphoid	1 (2%)		2 (4%)	
Necrosis	1 (2%)			
Epithelial cell, hyperplasia			1 (2%)	
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Ulcer				3 (6%)
Subcutaneous tissue, edema				1 (2%)
Subcutaneous tissue, inflammation, chronic	1 (2%)			
Subcutaneous tissue, necrosis			1 (2%)	

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Cranium, hyperplasia		1 (2%)		
Skeletal muscle	(1)	(1)	(0)	(2)
Inflammation, granulomatous	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	1 (2%)			
Cyst epithelial inclusion	1 (2%)			
Hemorrhage, acute		1 (2%)		
Hydrocephalus	1 (2%)			
Necrosis		1 (2%)		
Artery, inflammation, chronic		1 (2%)	1 (2%)	
Meninges, ventricle, inflammation, granulomatous	1 (2%)			
Meninges, infiltration cellular, lymphocyte		1 (2%)		
Vein, infiltration cellular, mononuclear cell		1 (2%)		
Peripheral nerve	(0)	(0)	(1)	(0)
Axon, degeneration			1 (100%)	
Spinal cord	(0)	(0)	(1)	(0)
Hemorrhage			1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Inflammation, chronic			3 (6%)	1 (2%)
Metaplasia, osseous			2 (4%)	
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	1 (2%)		1 (2%)	8 (16%)
Arteriole, thrombosis	1 (2%)			
Artery, mineralization	1 (2%)			
Bronchus, hypertrophy	1 (2%)			
Serosa, inflammation, chronic				1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	4 (8%)	4 (8%)	1 (2%)	1 (2%)
Inflammation, chronic		2 (4%)	1 (2%)	1 (2%)
Polyp, inflammatory	1 (2%)	1 (2%)	1 (2%)	
Glands, hyperplasia				1 (2%)
Glands, hyperplasia, focal	1 (2%)			
Glands, inflammation, suppurative	4 (8%)	1 (2%)	1 (2%)	
Nasolacrimal duct, infiltration cellular, polymorphonuclear	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Nasopharyngeal duct, inflammation, chronic				1 (2%)
Olfactory epithelium, amyloid deposition	1 (2%)			
Olfactory epithelium, atrophy, focal	8 (16%)	5 (10%)	1 (2%)	2 (4%)
Olfactory epithelium, metaplasia	1 (2%)			
Olfactory epithelium, metaplasia, focal		1 (2%)		
Respiratory epithelium, atrophy, focal		2 (4%)		
Respiratory epithelium, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	
Respiratory epithelium, metaplasia, squamous		1 (2%)		
Vomeronasal organ, infiltration cellular, polymorphonuclear	2 (4%)	10 (20%)	3 (6%)	4 (8%)
Pleura	(1)	(0)	(0)	(0)
Hyperplasia	1 (100%)			

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Degeneration				1 (2%)
Anterior chamber, inflammation, suppurative				1 (2%)
Cornea, inflammation, chronic	3 (6%)		2 (4%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	4 (8%)	8 (16%)	5 (10%)	10 (20%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Casts protein	1 (2%)			
Fibrosis, focal			1 (2%)	
Infarct	1 (2%)	1 (2%)	3 (6%)	
Inflammation, suppurative				1 (2%)
Inflammation, chronic		2 (4%)		1 (2%)
Metaplasia, osseous	1 (2%)	1 (2%)	3 (6%)	4 (8%)
Mineralization	16 (32%)	15 (30%)	2 (4%)	5 (10%)
Necrosis				1 (2%)
Nephropathy	43 (86%)	46 (92%)	41 (82%)	45 (90%)
Artery, inflammation, chronic	2 (4%)	1 (2%)	1 (2%)	
Glomerulus, inflammation, chronic	1 (2%)			
Glomerulus, necrosis, fibrinoid	1 (2%)			
Papilla, necrosis	1 (2%)			
Pelvis, inflammation, suppurative	1 (2%)			2 (4%)
Renal tubule, accumulation, hyaline droplet	1 (2%)			
Renal tubule, cyst	8 (16%)	5 (10%)	4 (8%)	5 (10%)
Renal tubule, hyperplasia	1 (2%)	2 (4%)	5 (10%)	1 (2%)
Renal tubule, mineralization			1 (2%)	
Vein, infiltration cellular, lymphocyte		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	1 (2%)		1 (2%)	
Inflammation, suppurative	1 (2%)			
Inflammation, chronic		2 (4%)		1 (2%)
Artery, inflammation, chronic	1 (2%)		1 (2%)	
Artery, mineralization	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)			

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

Table D1 Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Formamide 108

Table D2 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Formamide 112

Table D3 Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F1 Mice 115

Table D4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Formamide 116

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	7	8	8	8
Natural deaths	5	3	10	3
Survivors				
Died last week of study		1		
Terminal sacrifice	38	38	31	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(47)	(45)	(46)	(49)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Intestine large, cecum	(49)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Leiomyosarcoma			1 (2%)	
Intestine, large, colon	(50)	(50)	(50)	(50)
Intestine, large, rectum	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Leiomyosarcoma				1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	1 (2%)
Hepatocellular adenoma	4 (8%)	9 (18%)	11 (22%)	10 (20%)
Hepatocellular adenoma, multiple	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Hepatocellular carcinoma	4 (8%)	3 (6%)		5 (10%)
Hepatocellular, carcinoma, multiple		1 (2%)		1 (2%)
Mesentery	(8)	(3)	(8)	(2)
Fibrosarcoma	1 (13%)			
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(49)	(49)	(50)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(0)	(0)	(0)	(1)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		2 (4%)		
Pheochromocytoma malignant				2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(42)	(32)	(35)	(42)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Pars distalis, adenoma, multiple			1 (2%)	
Pars intermedia, adenoma	1 (2%)	1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
Follicle, adenoma	2 (4%)	2 (4%)	2 (4%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Ovary	(50)	(50)	(50)	(49)
Cystadenoma	1 (2%)	3 (6%)	4 (8%)	1 (2%)
Granulosa cell tumor benign		1 (2%)		
Granulosa cell tumor malignant		1 (2%)		
Hemangioma				1 (2%)
Luteoma				1 (2%)
Teratoma benign		1 (2%)		
Teratoma malignant			1 (2%)	
Bilateral, luteoma		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Polyp stromal				2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(8)	(4)	(6)	(5)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung			1 (17%)	
Mediastinal, hepatocellular carcinoma, metastatic, liver				1 (20%)
Renal, teratoma malignant, metastatic, ovary			1 (17%)	
Lymph node, mandibular	(49)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Spleen	(50)	(49)	(49)	(50)
Hemangiosarcoma	1 (2%)			
Thymus	(49)	(50)	(49)	(50)
Thymoma benign		1 (2%)		

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	2 (4%)	3 (6%)	
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)	6 (12%)	4 (8%)	1 (2%)
Subcutaneous tissue, sarcoma, multiple			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Skeletal muscle	(2)	(5)	(5)	(0)
Fibrosarcoma, metastatic, mesentery	1 (50%)			
Hemangiosarcoma	1 (50%)		1 (20%)	
Lipoma		1 (20%)		
Teratoma malignant, metastatic, ovary			1 (20%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Spinal cord	(1)	(0)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	2 (4%)	1 (2%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)		1 (2%)	
Alveolar/bronchiolar carcinoma	2 (4%)		3 (6%)	3 (6%)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)
Carcinoma, metastatic, Harderian gland	2 (4%)			
Hepatocellular carcinoma, metastatic, liver	2 (4%)	2 (4%)		3 (6%)
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma metastatic, skin		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Pleura	(0)	(0)	(1)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)	
Special Senses System				
Eye	(49)	(50)	(49)	(50)
Harderian gland	(49)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	2 (4%)	7 (14%)
Carcinoma	3 (6%)			1 (2%)
Bilateral, adenoma			1 (2%)	
Bilateral, carcinoma	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Osteosarcoma, metastatic, bone				1 (2%)
Perirenal tissue, hemangioma				1 (2%)
Perirenal tissue, hemangiosarcoma				1 2%)
Urinary bladder	(50)	(50)	(50)	(50)

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	2 (4%)	2 (4%)	
Lymphoma malignant	10 (20%)	6 (12%)	6 (12%)	5 (10%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	36	36	33	35
Total primary neoplasms	50	54	51	55
Total animals with benign neoplasms	17	23	23	24
Total benign neoplasms	22	31	27	30
Total animals with malignant neoplasms	26	20	22	19
Total malignant neoplasms	28	23	24	25
Total animals with metastatic neoplasms	5	3	3	4
Total metastatic neoplasms	14	3	4	7

[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 Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	4/50 (8%)	3/50 (6%)	3/50 (6%)	7/50 (14%)
Adjusted rate[b]	8.9%	6.5%	6.9%	14.8%
Terminal rate[c]	3/38 (8%)	1/39 (3%)	0/31 (0%)	5/39 (13%)
First incidence (days)	665	575	401	623
Poly-3 test[d]	P=0.153	P=0.483N	P=0.518N	P=0.293
Harderian Gland: Carcinoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	8.6%	0.0%	0.0%	2.1%
Terminal rate	1/38 (3%)	0/39 (0%)	0/31 (0%)	0/39 (0%)
First incidence (days)	374	—[e]	—	590
Poly-3 test	P=0.123N	P=0.063N	P=0.074N	P=0.173N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	3/50 (6%)	3/50 (6%)	8/50 (16%)
Adjusted rate	17.1%	6.5%	6.9%	16.7%
Terminal rate	4/38 (11%)	1/39 (3%)	0/31 (0%)	5/39 (13%)
First incidence (days)	374	575	401	590
Poly-3 test	P=0.417	P=0.102N	P=0.123N	P=0.589N
Liver: Hepatocellular Adenoma				
Overall rate	6/50 (12%)	12/50 (24%)	13/50 (26%)	12/50 (24%)
Adjusted rate	13.2%	26.2%	29.4%	25.4%
Terminal rate	3/38 (8%)	11/39 (28%)	9/31 (29%)	11/39 (28%)
First incidence (days)	653	674	407	694
Poly-3 test	P=0.149	P=0.096	P=0.051	P=0.109
Liver: Hepatocellular Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	0/50 (0%)	6/50 (12%)
Adjusted rate	8.9%	8.7%	0.0%	12.6%
Terminal rate	3/38 (8%)	3/39 (8%)	0/31 (0%)	3/39 (8%)
First incidence (days)	653	665	—	657
Poly-3 test	P=0.329	P=0.633N	P=0.068N	P=0.403
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	9/50 (18%)	15/50 (30%)	13/50 (26%)	18/50 (36%)
Adjusted rate	19.8%	32.6%	29.4%	37.8%
Terminal rate	6/38 (16%)	13/39 (33%)	9/31 (29%)	14/39 (36%)
First incidence (days)	653	665	407	657
Poly-3 test	P=0.056	P=0.124	P=0.209	P=0.044
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rate	8.9%	4.4%	4.7%	8.5%
Terminal rate	4/38 (11%)	2/39 (5%)	1/31 (3%)	3/39 (8%)
First incidence (days)	727 (T)	727 (T)	675	694
Poly-3 test	P=0.517	P=0.328N	P=0.360N	P=0.614N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate	4.5%	0.0%	7.0%	8.5%
Terminal rate	2/38 (5%)	0/39 (0%)	1/31 (3%)	3/39 (8%)
First incidence (days)	727 (T)	—	675	693
Poly-3 test	P=0.122	P=0.233N	P=0.478	P=0.362

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	4/50 (8%)	8/50 (16%)
Adjusted rate	11.2%	4.4%	9.4%	16.9%
Terminal rate	5/38 (13%)	2/39 (5%)	2/31 (7%)	6/39 (15%)
First incidence (days)	727 (T)	727 (T)	675	693
Poly-3 test	P=0.113	P=0.208N	P=0.530N	P=0.313
Mammary Gland: Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.5%	4.4%	7.0%	0.0%
Terminal rate	2/38 (5%)	1/39 (3%)	1/31 (3%)	0/39 (0%)
First incidence (days)	727 (T)	665	618	—
Poly-3 test	P=0.184N	P=0.685N	P=0.482	P=0.226N
Ovary: Cystadenoma				
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	1/49 (2%)
Adjusted rate	2.2%	6.6%	9.3%	2.2%
Terminal rate	0/38 (0%)	3/39 (8%)	2/31 (7%)	0/39 (0%)
First incidence (days)	493	727 (T)	587	657
Poly-3 test	P=0.507N	P=0.307	P=0.162	P=0.756N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/49 (6%)	1/50 (2%)
Adjusted rate	6.7%	2.2%	7.2%	2.1%
Terminal rate	2/38 (5%)	1/39 (3%)	3/31 (10%)	1/39 (3%)
First incidence (days)	681	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.300N	P=0.300N	P=0.628	P=0.289N
Skin: Sarcoma				
Overall rate	1/50 (2%)	6/50 (12%)	5/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	12.7%	11.6%	2.1%
Terminal rate	0/38 (0%)	2/39 (5%)	2/31 (7%)	0/39 (0%)
First incidence (days)	665	575	587	623
Poly-3 test	P=0.329N	P=0.064	P=0.092	P=0.749N
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.5%	0.0%	4.7%	6.4%
Terminal rate	2/38 (5%)	0/39 (0%)	2/31 (7%)	2/39 (5%)
First incidence (days)	727 (T)	—	727 (T)	723
Poly-3 test	P=0.249	P=0.233N	P=0.674	P=0.523
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	0/50 (0%)	2/50 (4%)	5/50 (10%)
Adjusted rate	4.5%	0.0%	4.7%	10.6%
Terminal rate	2/38 (5%)	0/39 (0%)	2/31 (7%)	4/39 (10%)
First incidence (days)	727 (T)	—	727 (T)	723
Poly-3 test	P=0.049	P=0.233N	P=0.674	P=0.237
All Organs: Malignant Lymphoma				
Overall rate	10/50 (20%)	6/50 (12%)	6/50 (12%)	5/50 (10%)
Adjusted rate	21.9%	12.9%	13.8%	10.4%
Terminal rate	8/38 (21%)	3/39 (8%)	3/31 (10%)	2/39 (5%)
First incidence (days)	594	513	401	563
Poly-3 test	P=0.109N	P=0.191N	P=0.232N	P=0.108N

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
All Organs: Benign Neoplasms				
Overall rate	17/50 (34%)	23/50 (46%)	23/50 (46%)	24/50 (48%)
Adjusted rate	36.9%	49.1%	50.0%	50.2%
Terminal rate	13/38 (34%)	19/39 (49%)	14/31 (45%)	20/39 (51%)
First incidence (days)	493	575	401	623
Poly-3 test	P=0.156	P=0.161	P=0.140	P=0.135
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	20/50 (40%)	22/50 (44%)	19/50 (38%)
Adjusted rate	52.7%	40.9%	47.0%	38.2%
Terminal rate	16/38 (42%)	11/39 (28%)	8/31 (26%)	9/39 (23%)
First incidence (days)	367	513	401	563
Poly-3 test	P=0.131N	P=0.166N	P=0.363N	P=0.106N
All Organs: Benign or Malignant Neoplasms				
Overall rate	36/50 (72%)	36/50 (72%)	33/50 (66%)	35/50 (70%)
Adjusted rate	72.7%	73.2%	69.3%	70.4%
Terminal rate	25/38 (66%)	26/39 (67%)	18/31 (58%)	25/39 (64%)
First incidence (days)	367	513	401	563
Poly-3 test	P=0.409N	P=0.566	P=0.443N	P=0.487N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[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 is the P value corresponding to pairwise comparison 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 D3
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F1 Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Water Gavage Studies			
Acrylonitrile	14/50	7/50	20/50
Elmiron® (sodium pentosanpolysulfate)	7/50	3/50	10/50
Formamide	6/50	4/50	9/50
5-Hydroxymethyl-2-furfural	14/50	2/50	14/50
Overall Historical Incidence: Water Gavage Studies			
Total (%)	41/200 (20.5%)	16/200 (8.0%)	53/200 (26.5%)
Mean ± standard deviation	20.5% ± 8.7%	8.0% ± 4.3%	26.5% ± 10.0%
Range	12%-28%	4%-14%	18%-40%
Overall Historical Incidence: All Routes			
Total (%)	402/1,593 (25.2%)	159/1,593 (10.0%)	505/1,593 (31.7%)
Mean ± standard deviation	25.8% ± 15.8%	10.2% ± 6.6%	32.4% ± 17.5%
Range	2%-62%	0%-28%	8%-64%

[a] Data as of March 2, 2007

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Formamide[a]

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	7	8	8	8
Natural deaths	5	3	10	3
Survivors				
Died last week of study		1		
Terminal sacrifice	38	38	31	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Perforation			1 (2%)	
Gallbladder	(47)	(45)	(46)	(49)
Cyst	1 (2%)			
Inflammation, chronic	1 (2%)			
Epithelium, cytoplasmic alteration				1 (2%)
Intestine large, cecum	(49)	(50)	(50)	(50)
Edema	1 (2%)			
Lymphoid tissue, hyperplasia, lymphoid			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Serosa, inflammation, chronic			1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Epithelium, hyperplasia, focal	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Epithelium, cyst	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Artery, Peyer's patch, mineralization			1 (2%)	
Epithelium, hyperplasia, focal				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			2 (4%)	
Basophilic focus			1 (2%)	2 (4%)
Clear cell focus	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Eosinophilic focus	12 (24%)	15 (30%)	8 (16%)	7 (14%)
Fatty change	6 (12%)	7 (14%)	4 (8%)	2 (4%)
Hematopoietic cell proliferation	3 (6%)	4 (8%)	1 (2%)	1 (2%)
Infarct			1 (2%)	
Inflammation, chronic	29 (58%)	27 (54%)	21 (42%)	21 (42%)
Mineralization				1 (2%)
Mixed cell focus	4 (8%)	5 (10%)	4 (8%)	6 (12%)
Necrosis	1 (2%)			2 (4%)
Tension lipidosis	3 (6%)	9 (18%)	5 (10%)	6 (12%)
Centrilobular, necrosis			1 (2%)	
Centrilobular, vacuolization cytoplasmic	1 (2%)			
Mesentery	(8)	(3)	(8)	(2)
Fat, necrosis	5 (63%)	3 (100%)	8 (100%)	2 (100%)
Oral mucosa	(1)	(0)	(0)	(0)
Gingival, inflammation, chronic	1 (100%)			

[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 Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Alimentary System (continued)				
Pancreas	(49)	(49)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)	
Inflammation, chronic			2 (4%)	
Acinus, atrophy		1 (2%)	3 (6%)	2 (4%)
Acinus, cytoplasmic alteration	1 (2%)			1 (2%)
Artery, thrombosis			1 (2%)	
Duct, cyst			1 (2%)	3 (6%)
Duct, cytoplasmic alteration				1 (2%)
Duct, inflammation, chronic	1 (2%)		1 (2%)	
Salivary glands	(49)	(50)	(50)	(50)
Atrophy, diffuse				1 (2%)
Parotid gland, atrophy		1 (2%)	1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Epithelium, hyperplasia	1 (2%)			1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	1 (2%)		1 (2%)
Ulcer		1 (2%)		
Epithelium, glands, cytoplasmic alteration	1 (2%)			1 (2%)
Epithelium, glands, hyperplasia				1 (2%)
Glands, ectasia	1 (2%)			
Glands, hyperplasia	1 (2%)			
Tooth	(0)	(0)	(0)	(1)
Malformation				1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Artery, inflammation, chronic	2 (4%)			
Atrium, thrombosis			1 (2%)	1 (2%)
Coronary artery, inflammation, chronic	1 (2%)			
Myocardium, infiltration cellular, mononuclear cell	2 (4%)	7 (14%)	4 (8%)	3 (6%)
Myocardium, mineralization		3 (6%)	1 (2%)	
Myocardium, necrosis			1 (2%)	
Ventricle, thrombosis				1 (2%)
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Atrophy				1 (2%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)		
Hyperplasia		2 (4%)		1 (2%)
Hypertrophy	1 (2%)	1 (2%)	4 (8%)	
Pigmentation		3 (6%)		
Vacuolization cytoplasmic, focal	3 (6%)		2 (4%)	
Subcapsular hyperplasia	49 (100%)	49 (98%)	48 (98%)	49 (98%)
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia, focal	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	4 (8%)	
Parathyroid gland	(42)	(32)	(35)	(42)
Cyst	1 (2%)			2 (5%)

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis	2 (4%)	1 (2%)		
Cyst	3 (6%)	4 (8%)		1 (2%)
Fibrosis		1 (2%)		
Hemorrhage, chronic		1 (2%)		
Pars distalis, hyperplasia, focal	11 (22%)	11 (22%)	10 (20%)	10 (20%)
Pars intermedia, hyperplasia, focal	1 (2%)		1 (2%)	1 (2%)
Thyroid gland	(49)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	2 (4%)	
Infiltration cellular, lymphoid		1 (2%)		
Inflammation, suppurative		2 (4%)		
Inflammation, granulomatous				1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Follicle, hyperplasia, focal	14 (29%)	6 (12%)	10 (20%)	5 (10%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Duct, cyst				1 (2%)
Ovary	(50)	(50)	(50)	(49)
Angiectasis	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Cyst	24 (48%)	19 (38%)	11 (22%)	23 (47%)
Mineralization		1 (2%)		
Thrombosis	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Bilateral, thrombosis	1 (2%)			
Follicle, atrophy	33 (66%)	29 (58%)	35 (70%)	37 (76%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)	2 (4%)	1 (2%)
Dilatation				2 (4%)
Inflammation, suppurative	1 (2%)	1 (2%)		
Inflammation, chronic active				1 (2%)
Thrombosis			1 (2%)	
Endometrium, hyperplasia, cystic	45 (90%)	48 (96%)	45 (90%)	42 (84%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Hyperplasia	8 (16%)	10 (20%)	13 (26%)	5 (10%)
Infiltration cellular, histiocyte	1 (2%)			
Lymph node	(8)	(4)	(6)	(5)
Bronchial, hyperplasia, lymphoid		1 (25%)		
Iliac, necrosis		1 (25%)		
Lumbar, hematopoietic, cell proliferation	1 (13%)			
Lumbar, hyperplasia, lymphoid	1 (13%)			
Mediastinal, hematopoietic cell proliferation	1 (13%)			
Mediastinal, hyperplasia, lymphoid	2 (25%)			
Mediastinal, inflammation, chronic			1 (17%)	
Renal, hyperplasia, lymphoid	1 (13%)			

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(49)	(50)	(50)	(50)
Atrophy	3 (6%)	2 (4%)	6 (12%)	4 (8%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	8 (16%)		5 (10%)	6 (12%)
Necrosis, lymphoid	1 (2%)		1 (2%)	
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Atrophy	7 (14%)	9 (18%)	8 (16%)	7 (14%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, lymphoid	4 (8%)	1 (2%)	4 (8%)	3 (6%)
Infiltration cellular, polymorphonuclear			1 (2%)	
Inflammation, suppurative				1 (2%)
Necrosis, lymphoid	2 (4%)	1 (2%)	1 (2%)	
Spleen	(50)	(49)	(49)	(50)
Atrophy	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	17 (34%)	17 (35%)	15 (31%)	8 (16%)
Hyperplasia, lymphoid				3 (6%)
Hyperplasia, plasma cell	1 (2%)		1 (2%)	
Lymphoid follicle, hyperplasia	3 (6%)	4 (8%)		2 (4%)
Lymphoid follicle, necrosis	3 (6%)			1 (2%)
Thymus	(49)	(50)	(49)	(50)
Angiectasis				1 (2%)
Atrophy	16 (33%)	19 (38%)	12 (24%)	17 (34%)
Cyst	7 (14%)	9 (18%)	7 (14%)	
Hyperplasia, lymphoid	9 (18%)	11 (22%)	7 (14%)	8 (16%)
Necrosis, lymphoid	1 (2%)	1 (2%)	1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia, cystic	1 (2%)	1 (2%)		
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Inflammation, granulomatous			1 (2%)	
Ulcer			2 (4%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Hyperostosis	3 (6%)	6 (12%)	3 (6%)	2 (4%)
Skeletal muscle	(2)	(5)	(5)	(0)
Fibrosis		3 (60%)		
Inflammation, chronic			1 (20%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Arteriole, inflammation, chronic	1 (2%)			
Artery, meninges, inflammation, chronic				1 (2%)
Hypothalamus, compression	1 (2%)	2 (4%)	1 (2%)	
Meninges, infiltration cellular, lymphocyte	2 (4%)		1 (2%)	
Vein, infiltration cellular, lymphocyte	1 (2%)			
Spinal cord	(1)	(0)	(0)	(0)
Hemorrhage	1 (100%)			

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Formamide

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation		1 (2%)		
Inflammation, granulomatous				1 (2%)
Inflammation, focal, chronic	1 (2%)			
Inflammation, chronic	2 (4%)			
Metaplasia, osseous	1 (2%)		1 (2%)	1 (2%)
Necrosis			1 (2%)	
Alveolar epithelium, hyperplasia	2 (4%)	5 (10%)	2 (4%)	
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Vein, thrombosis			2 (4%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic	1 (2%)		2 (4%)	
Glands, hyperplasia	1 (2%)			
Glands inflammation, suppurative			2 (4%)	
Nasolacrimal duct, infiltration cellular, polymorphonuclear	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Olfactory epithelium, atrophy, focal		2 (4%)	1 (2%)	1 (2%)
Respiratory epithelium, hyperplasia, focal	1 (2%)			1 (2%)
Respiratory epithelium, necrosis			1 (2%)	
Vomeronasal organ, infiltration cellular, polymorphonuclear	9 (18%)	11 (22%)	2 (4%)	5 (10%)
Pleura	(0)	(0)	(1)	(0)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Degeneration	1 (2%)	1 (2%)		
Cornea, inflammation, chronic	1 (2%)			1 (2%)
Retrobulbar, inflammation, chronic	1 (2%)			
Harderian gland	(49)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hyperplasia, focal	7 (14%)	4 (8%)	3 (6%)	5 (10%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	1 (2%)			
Infarct	2 (4%)	1 (2%)	1 (2%)	
Infiltration cellular, lymphoid		1 (2%)		
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mineralization	6 (12%)	5 (10%)	1 (2%)	3 (6%)
Nephropathy	35 (70%)	23 (46%)	28 (56%)	38 (76%)
Arteriole, inflammation, chronic		1 (2%)		
Artery, inflammation, chronic	1 (2%)			
Glomerulus, amyloid deposition				1 (2%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	2 (4%)		
Renal tubule, cyst				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Artery, inflammation, chronic				1 (2%)

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Two assays were performed as reported by Mortelmans *et al.* (1986). Formamide was sent to the laboratories as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 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. A third assay used *Escherichia coli* strain WP *uvrA* pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100 and activation in buffer or rat liver S9 only for 20 minutes at 37° C. In all three assays, top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of formamide. In the absence of toxicity, 10,000 µg/plate was selected as the high dose. All trials were repeated.

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.

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assay for induction of sex-linked recessive lethal (SLRL) mutations was performed with adult flies as described by Foureman *et al.* (1994). Formamide was supplied as a coded aliquot by Radian Corporation. Formamide was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, formamide was retested by injection into adult males.

To administer formamide by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector that automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of formamide at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were allowed to feed for 72 hours on a solution of 5% sucrose containing formamide (dissolved in distilled water). In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of formamide dissolved in saline and allowed to recover for 24 hours. A concurrent saline control group was also included. In the adult exposures, treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings was treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a

Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls (Mason *et al.,* 1992) using a normal approximation to the binomial test (Margolin *et al.,* 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

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 B6C3F1 mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronucleated cells in 2,000 normochromatic erythrocytes (NCEs) in each of 10 animals 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 with 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). 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 three independent Ames assays, formamide (concentrations up to the maximum of 10,000 µg/plate) did not induce mutagenic activity in any of several strains of *S. typhimurium* tested with and without rat or hamster liver S9 activation enzymes (Table E1; Mortelmans *et al.*, 1986) or in *E. coli* strain WP *uvrA* pKM101 with and without 10% rat liver S9 (Table E1). Negative results were obtained with formamide in a test for induction of sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* treated with formamide either in feed (2,500 or 5,000 ppm) or by abdominal injection (21,570 ppm) (Table E2). No increases in micronucleated normochromatic erythrocytes were observed in male or female B6C3F1 mice treated with formamide (up to 160 mg/kg) by gavage for 3 months; no significant effects on the percentage of polychromatic (immature) erythrocytes was seen in either male or female mice, indicating the absence of formamide-induced bone marrow toxicity (Table E3).

Table E1
Mutagenicity of Formamide in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute[c]							
TA100	0	123 ± 12	137 ± 6	111 ± 7	107 ± 6	79 ± 9	125 ± 3
	100	124 ± 4	137 ± 6	127 ± 4	94 ± 4	88 ± 6	117 ± 7
	333	113 ± 15	145 ± 12	124 ± 4	99 ± 6	95 ± 4	114 ± 9
	1,000	128 ± 7	136 ± 6	121 ± 8	133 ± 7	85 ± 8	116 ± 5
	3,333	135 ± 10	131 ± 5	105 ± 15	107 ± 9	85 ± 7	118 ± 7
	10,000	125 ± 4	143 ± 8	123 ± 4	107 ± 15	99 ± 4	114 ± 7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		1,468 ± 33	1,265 ± 37	507 ± 37	865 ± 15	811 ± 10	1,402 ± 15
TA1535	0	40 ± 3	31 ± 2	14 ± 1	17 ± 3	14 ± 1	11 ± 1
	100	34 ± 3	35 ± 3	12 ± 5	11 ± 2	13 ± 1	16 ± 1
	333	44 ± 4	40 ± 3	13 ± 2	13 ± 1	11 ± 1	12 ± 2
	1,000	40 ± 4	36 ± 2	14 ± 1	14 ± 2	13 ± 3	12 ± 1
	3,333	41 ± 1	35 ± 4	11 ± 2	10 ± 2	14 ± 2	12 ± 2
	10,000	47 ± 7[e]	35 ± 5	11 ± 1	11 ± 3	16 ± 2	13 ± 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		779 ± 22	958 ± 12	118 ± 5	126 ± 11	118 ± 8	136 ± 7
TA1537	0	10 ± 3	10 ± 1	12 ± 2	12 ± 2	8 ± 3	13 ± 1
	100	10 ± 1	7 ± 2	14 ± 2	15 ± 1	12 ± 2	7 ± 2
	333	8 ± 2	9 ± 0	12 ± 1	11 ± 2	10 ± 2	9 ± 2
	1,000	7 ± 2	8 ± 1	13 ± 1	10 ± 0	13 ± 1	9 ± 4
	3,333	10 ± 1	9 ± 1	10 ± 1	11 ± 3	11 ± 1	10 ± 1
	10,000	8 ± 1	7 ± 1	8 ± 2	8 ± 1	11 ± 1	8 ± 1
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		167 ± 25	198 ± 11	131 ± 19	92 ± 3	169 ± 10	173 ± 8
TA98	0	16 ± 2	18 ± 3	24 ± 2	36 ± 3	23 ± 2	40 ± 3
	100	20 ± 1	20 ± 3	27 ± 4	36 ± 6	26 ± 1	37 ± 2
	333	17 ± 4	20 ± 2	25 ± 3	34 ± 1	26 ± 1	30 ± 3
	1,000	15 ± 1	14 ± 1	21 ± 4	36 ± 4	22 ± 3	33 ± 2
	3,333	11 ± 1	21 ± 3	27 ± 4	35 ± 3	22 ± 7	32 ± 3
	10,000	16 ± 3	16 ± 3	27 ± 2	36 ± 2	26 ± 2	33 ± 4
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		969 ± 44	1,801 ± 98	265 ± 11	961 ± 33	441 ± 17	1,498 ± 54

Table E1
Mutagenicity of Formamide in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at Case Western Reserve University[c]							
TA100	0	112 ± 14	85 ± 2	170 ± 22	107 ± 11	174 ± 6	110 ± 24
	33	101 ± 6	76 ± 3				
	100	118 ± 3	73 ± 1	199 ± 5	115 ± 14	163 ± 6	110 ± 15
	333	124 ± 8	75 ± 2	180 ± 9	108 ± 11	170 ± 5	140 ± 11
	1,000	132 ± 8	77 ± 4	181 ± 7	106 ± 10	188 ± 11	131 ± 8
	3,333	110 ± 2	72 ± 1	190 ± 2	117 ± 8	185 ± 8	144 ± 11
	10,000			211 ± 5	107 ± 4	192 ± 12	120 ± 9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		284 ± 16	1,499 ± 90	1,894 ± 15	2,677 ± 145	1,407 ± 262	2,356 ± 381
TA1535	0	7 ± 1	7 ± 2	7 ± 1	7 ± 1	6 ± 1	8 ± 1
	33	7 ± 1	3 ± 1				
	100	6 ± 1	5 ± 2	8 ± 1	5 ± 1	5 ± 0	5 ± 0
	333	7 ± 1	3 ± 0	8 ± 5	5 ± 1	6 ± 2	6 ± 2
	1,000	6 ± 2	4 ± 1	11 ± 1	5 ± 1	6 ± 0	3 ± 2
	3,333	6 ± 1	2 ± 1	9 ± 2	7 ± 1	4 ± 1	4 ± 1
	10,000			11 ± 4	11 ± 3	8 ± 1	5 ± 0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		817 ± 17	601 ± 19	190 ± 15	87 ± 15	142 ± 27	77 ± 39
TA1537	0	4 ± 1	5 ± 1	8 ± 1	8 ± 2	6 ± 1	10 ± 3
	33	6 ± 3	6 ± 0				
	100	5 ± 1	4 ± 1	7 ± 2	4 ± 1	10 ± 3	4 ± 1
	333	4 ± 1	5 ± 1	7 ± 1	7 ± 1	9 ± 2	6 ± 1
	1,000	5 ± 1	4 ± 1	7 ± 0	3 ± 0	6 ± 1	3 ± 1
	3,333	6 ± 2	5 ± 2	7 ± 0	7 ± 2	6 ± 2	7 ± 2
	10,000			5 ± 2	5 ± 1	6 ± 1	3 ± 0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		690 ± 57	777 ± 53	213 ± 8	95 ± 32	263 ± 5	145 ± 12
TA98	0	17 ± 3	14 ± 1	19 ± 2	17 ± 3	16 ± 2	15 ± 1
	33	15 ± 1	13 ± 1				
	100	14 ± 1	14 ± 2	20 ± 3	16 ± 2	16 ± 2	21 ± 3
	333	12 ± 2	16 ± 1	20 ± 3	13 ± 1	23 ± 2	23 ± 2
	1,000	15 ± 1	17 ± 2	15 ± 1	17 ± 2	14 ± 2	12 ± 1
	3,333	14 ± 2	16 ± 2	17 ± 1	22 ± 1	22 ± 3	15 ± 1
	10,000			17 ± 2	17 ± 2	11 ± 2	21 ± 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		116 ± 1	692 ± 56	1,359 ± 81	2,121 ± 299	786 ± 115	898 ± 47

Table E1
Mutagenicity of Formamide in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate				
		–S9		+10% rat S9		
		Trial 1	Trial 2	Trial 1	Trial 2	
Study performed at SITEK Research Laboratories						
TA100	0	60 ± 6	85 ± 3	92 ± 9	83 ± 6	
	1,500	69 ± 6	84 ± 3	84 ± 2	78 ± 5	
	3,500	67 ± 6	88 ± 2	109 ± 9	83 ± 4	
	5,000	83 ± 4	82 ± 9	89 ± 2	84 ± 6	
	7,500	70 ± 3	76 ± 7	85 ± 6	78 ± 8	
	10,000	83 ± 4	81 ± 5	85 ± 4	76 ± 8	
Trial summary		Negative	Negative	Negative	Negative	
Positive control		557 ± 8	659 ± 29	1,213 ± 40	1,101 ± 49	
		–S9			+10% rat S9	
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2
TA98	0	13 ± 1	22 ± 3	21 ± 2	32 ± 2	32 ± 4
	1,500	17 ± 2	24 ± 1	20 ± 2	37 ± 4	26 ± 1
	3,500	22 ± 0	24 ± 2	24 ± 4	37 ± 1	32 ± 2
	5,000	25 ± 1	20 ± 1	20 ± 1	32 ± 2	35 ± 4
	7,500	19 ± 4	17 ± 1	21 ± 1	35 ± 1	30 ± 3
	10,000	21 ± 3	18 ± 2	22 ± 1	38 ± 1	32 ± 3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		514 ± 13	654 ± 17	804 ± 26	1,416 ± 42	1,484 ± 86

Table E1
Mutagenicity of Formamide in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	Trial 2	Trial 3
Study performed at SITEK Research Laboratories (continued)				
***Escherichia coli* WP *uvrA* pKM101 (Analogous to TA102)**				
	0	242 ± 5	203 ± 12	232 ± 21
	750			255 ± 11
	1,000			277 ± 2
	1,500	255 ± 16	239 ± 8	230 ± 4
	3,500	281 ± 9	239 ± 23	234 ± 5
	5,000	232 ± 10	220 ± 14	241 ± 3
	7,500	246 ± 21	227 ± 2	
	10,000	245 ± 14	208 ± 19	
Trial summary		Negative	Negative	Negative
Positive control		2,071 ± 45	1,955 ± 18	1,913 ± 40

		+10% rat S9 Trial 1	Trial 2	Trial 3	Trial 4
E. coli (continued)	0	244 ± 15	245 ± 12	260 ± 20	254 ± 16
	750				313 ± 12
	1,000				313 ± 20
	1,500	251 ± 17	275 ± 7	264 ± 19	265 ± 13
	3,500	268 ± 18	280 ± 7	286 ± 9	273 ± 5
	5,000	282 ± 19	246 ± 26	268 ± 9	259 ± 24
	7,500	304 ± 11	274 ± 3	287 ± 3	
	10,000	372 ± 8	219 ± 7	283 ± 12	
Trial summary		Equivocal	Negative	Negative	Negative
Positive control		1,262 ± 35	1,164 ± 25	823 ± 43	1,046 ± 74

[a] 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The detailed protocol and these data are presented by Mortelmans *et al*. (1986).
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and 1535), 9-aminoacridine (TA1537), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (pKM101). The positive control for metabolic activation was 2-aminoanthracene.
[e] Slight toxicity

TABLE E2
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Formamide[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[b]
Feeding	2,500	0	1	3/2,531	0/751	4/1,294	7/4,576 (0.15%)
	0			5/3,134	1/2,695	3/2,106	9/7,935 (0.11%)
	5,000	0	0	0/463	0/13	0/215	0/691 (0.00%)
	0			0/321	1/299	0/227	1/847 (0.12%)
							P=0.377[c]
Injection	21,570	14	18	0/2,718	0/1,321	2/1,127	2/5,166 (0.04%)
	0			2/3,080	2/2,204	4/2,065	8/7,349 (0.11%)
							P=0.914

[a] Study was performed at the University of Wisconsin-Madison. The detailed protocol is presented by Foureman *et al.* (1994). The mean mutant frequency from 518 negative control experiments is 0.074% (Mason *et al.*, 1992).
[b] Total number of lethal mutations/number of X chromosomes tested for three mating trials
[c] Significance of total number of lethal mutations/total number of X chromosomes tested by a normal approximation to the binomial test (Margolin *et al*., 1983).

Table E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Formamide 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[b]
Male					
Water[d]	0	10	1.15 ± 0.21		2.18 ± 0.08
Formamide	10	10	1.20 ± 0.17	0.4420	2.38 ± 0.14
	20	10	1.30 ± 0.23	0.3340	2.21 ± 0.11
	40	10	1.00 ± 0.17	0.6764	1.94 ± 0.06
	80	10	0.95 ± 0.09	0.7316	1.84 ± 0.07
	160	10	0.95 ± 0.14	0.7316	2.15 ± 0.23
			P=0.849[e]		
Female					
Water	0	10	0.85 ± 0.13		2.09 ± 0.08
Formamide	10	10	0.80 ± 0.11	0.5691	2.11 ± 0.08
	20	10	0.80 ± 0.13	0.5691	1.87 ± 0.14
	40	10	0.95 ± 0.12	0.3694	1.84 ± 0.12
	80	10	1.05 ± 0.12	0.2581	1.70 ± 0.08
160	10	0.80 ± 0.15	0.5691	1.78 ± 0.11	
			P=0.461		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al*. (1990); NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle controls, significant at P≤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≤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 Formamide 132
TABLE F2 Hematology Data for Mice in the 3-Month Gavage Study of Formamide. 137

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Male						
Hematology						
n						
Day 4	9	9	10	10	10	10
Day 23	8	10	10	10	10	9
Week 14	10	10	9	10	10	10
Automated hematocrit (%)						
Day 4	40.6 ± 0.3	41.7 ± 0.6	41.2 ± 0.6	40.2 ± 0.6	40.5 ± 0.8	38.9 ± 0.4
Day 23	44.2 ± 0.9	44.1 ± 0.7	45.7 ± 0.6	45.4 ± 0.5	45.4 ± 0.4	48.2 ± 0.4**
Week 14	45.0 ± 0.4	44.7 ± 0.2	44.9 ± 0.5	46.5 ± 0.3*	47.4 ± 0.3**	50.1 ± 0.4**
Hemoglobin (g/dL)						
Day 4	13.6 ± 0.1	13.9 ± 0.2	13.8 ± 0.3	13.5 ± 0.2	13.6 ± 0.3	13.3 ± 0.2
Day 23	14.9 ± 0.2	14.9 ± 0.2	15.2 ± 0.2	15.4 ± 0.2	15.3 ± 0.2	16.5 ± 0.2**
Week 14	15.0 ± 0.1	15.0 ± 0.1	15.0 ± 0.2	15.5 ± 0.1**	15.9 ± 0.1**	17.1 ± 0.1**
Erythrocytes (10^6/μL)						
Day 4	6.94 ± 0.05	7.20 ± 0.13	7.09 ± 0.13	6.88 ± 0.12	7.00 ± 0.16	6.80 ± 0.08
Day 23	7.67 ± 0.15	7.62 ± 0.15	7.87 ± 0.13	7.84 ± 0.09	7.77 ± 0.09	8.48 ± 0.07**
Week 14	8.50 ± 0.06	8.40 ± 0.05	8.52 ± 0.09	8.77 ± 0.06*	8.81 ± 0.08*	8.91 ± 0.06**
Reticulocytes (10^6/μL)						
Day 4	0.22 ± 0.02	0.25 ± 0.04	0.26 ± 0.04	0.25 ± 0.03	0.27 ± 0.03	0.23 ± 0.02
Day 23	0.13 ± 0.02	0.17 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	0.16 ± 0.01
Week 14	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 4	0.05 ± 0.02	0.09 ± 0.03	0.08 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.17 ± 0.05
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0.03 ± 0.02
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.01**	0.03 ± 0.01*
Mean cell volume (fL)						
Day 4	58.5 ± 0.1	57.9 ± 0.5	58.1 ± 0.2	58.5 ± 0.3	57.9 ± 0.2*	57.2 ± 0.3**
Day 23	57.7 ± 0.2	57.9 ± 0.4	58.0 ± 0.3	57.9 ± 0.3	58.5 ± 0.4	56.9 ± 0.4
Week 14	52.9 ± 0.3	53.2 ± 0.1	52.7 ± 0.2	53.1 ± 0.2	53.8 ± 0.2**	56.2 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.1	19.4 ± 0.1	19.5 ± 0.1	19.7 ± 0.2	19.4 ± 0.2	19.5 ± 0.1
Day 23	19.4 ± 0.1	19.6 ± 0.1	19.4 ± 0.1	19.6 ± 0.1	19.7 ± 0.2	19.5 ± 0.2
Week 14	17.6 ± 0.1	17.7 ± 0.1	17.5 ± 0.1	17.7 ± 0.1	18.1 ± 0.1**	19.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.4 ± 0.1	33.5 ± 0.2	33.6 ± 0.2	33.7 ± 0.2	33.5 ± 0.2	34.1 ± 0.2
Day 23	33.6 ± 0.2	33.8 ± 0.1	33.4 ± 0.1	33.8 ± 0.1	33.7 ± 0.2	34.2 ± 0.1
Week 14	33.3 ± 0.2	33.3 ± 0.2	33.3 ± 0.3	33.3 ± 0.2	33.6 ± 0.1	34.2 ± 0.2**
Platelets (10^3/μL)						
Day 4	899.7 ± 17.9	874.3 ± 27.1	904.3 ± 19.9	879.4 ± 15.2	873.1 ± 31.7	825.0 ± 24.8*
Day 23	832.8 ± 27.5	781.2 ± 8.6	837.8 ± 17.2	768.5 ± 16.0	785.2 ± 17.0	774.0 ± 14.1
Week 14	679.1 ± 9.3	675.4 ± 13.8	660.7 ± 10.7	622.7 ± 9.4**	640.3 ± 12.0**	606.6 ± 9.9**
Leukocytes (10^3/μL)						
Day 4	9.11 ± 0.41	9.20 ± 0.46	9.76 ± 0.38	9.48 ± 0.25	9.46 ± 0.50	10.12 ± 0.43
Day 23	10.15 ± 0.40	10.60 ± 0.35	8.70 ± 0.32	9.90 ± 0.54	9.50 ± 0.47	8.84 ± 0.46
Week 14	9.27 ± 0.41	10.03 ± 0.29	9.43 ± 0.24	8.67 ± 0.38	8.32 ± 0.36	8.85 ± 0.35
Segmented neutrophils (10^3/μL)						
Day 4	0.97 ± 0.07	0.92 ± 0.04	1.23 ± 0.12	1.06 ± 0.10	1.18 ± 0.11	1.33 ± 0.11*
Day 23	1.11 ± 0.09	1.03 ± 0.05	0.89 ± 0.10	1.10 ± 0.09	1.03 ± 0.08	1.27 ± 0.06
Week 14	1.28 ± 0.08	1.54 ± 0.10	1.51 ± 0.09	1.50 ± 0.14	1.67 ± 0.16*	1.88 ± 0.19**

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	9	9	10	10	10	10
Day 23	8	10	10	10	10	9
Week 14	10	10	9	10	10	10
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	7.78 ± 0.37	8.06 ± 0.45	8.28 ± 0.44	8.26 ± 0.20	8.09 ± 0.45	8.53 ± 0.41
Day 23	8.80 ± 0.43	9.39 ± 0.34	7.67 ± 0.33	8.55 ± 0.57	8.25 ± 0.45	7.46 ± 0.45
Week 14	7.84 ± 0.41	8.28 ± 0.29	7.65 ± 0.17	6.99 ± 0.33	6.46 ± 0.33**	6.79 ± 0.25*
Monocytes (10^3/μL)						
Day 4	0.32 ± 0.05	0.22 ± 0.04	0.23 ± 0.05	0.14 ± 0.03*	0.18 ± 0.03	0.23 ± 0.03
Day 23	0.23 ± 0.06	0.16 ± 0.03	0.12 ± 0.01	0.22 ± 0.04	0.21 ± 0.04	0.09 ± 0.02
Week 14	0.07 ± 0.03	0.13 ± 0.02	0.16 ± 0.02	0.14 ± 0.02	0.08 ± 0.02	0.09 ± 0.02
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	0.04 ± 0.02	0.00 ± 0.00	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
Day 23	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.01
Week 14	0.09 ± 0.03	0.08 ± 0.03	0.11 ± 0.03	0.08 ± 0.03	0.12 ± 0.01	0.10 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.6 ± 0.4	10.8 ± 0.3	11.0 ± 0.4	10.2 ± 0.3	11.5 ± 0.3	12.8 ± 0.3**
Day 23	13.0 ± 0.4	12.2 ± 0.3	12.6 ± 0.4	12.7 ± 0.6	12.9 ± 0.5	13.3 ± 0.4
Week 14	12.9 ± 0.5	14.0 ± 0.3	15.2 ± 0.3**	14.8 ± 0.7	13.9 ± 0.4	14.2 ± 0.5
Creatinine (mg/dL)						
Day 4	0.41 ± 0.01	0.40 ± 0.00	0.40 ± 0.00	0.40 ± 0.00	0.41 ± 0.01	0.40 ± 0.00
Day 23	0.50 ± 0.00	0.50 ± 0.02	0.50 ± 0.00	0.50 ± 0.00	0.49 ± 0.01	0.51 ± 0.03
Week 14	0.54 ± 0.02	0.56 ± 0.02	0.53 ± 0.02	0.53 ± 0.02	0.58 ± 0.03	0.53 ± 0.02
Total protein (g/dL)						
Day 4	5.4 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.6 ± 0.1	5.4 ± 0.1
Day 23	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
Week 14	6.4 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.1 ± 0.1*
Albumin (g/dL)						
Day 4	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.0	4.3 ± 0.1	4.2 ± 0.1
Day 23	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.0	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.0
Week 14	4.8 ± 0.0	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.0	4.6 ± 0.1	4.5 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 4	76 ± 3	72 ± 3	69 ± 3	66 ± 2*	67 ± 2*	49 ± 2**
Day 23	62 ± 2	57 ± 1	60 ± 2	59 ± 1	55 ± 1**	52 ± 1**
Week 14	97 ± 8	113 ± 11	139 ± 6	116 ± 13	74 ± 3*	57 ± 3**

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Alkaline phosphatase (IU/L)						
Day 4	762 ± 16	735 ± 19	730 ± 23	711 ± 11	721 ± 11	689 ± 8**
Day 23	556 ± 13	532 ± 11	532 ± 19	534 ± 18	536 ± 13	522 ± 8
Week 14	229 ± 4	236 ± 4	235 ± 5	235 ± 4	240 ± 7	265 ± 7**
Creatine kinase (IU/L)						
Day 4	353 ± 46[b]	350 ± 43	347 ± 36	293 ± 29	304 ± 26	310 ± 43
Day 23	229 ± 54	195 ± 22	181 ± 33	185 ± 26	148 ± 11	157 ± 18
Week 14	265 ± 52	228 ± 31	207 ± 15	181 ± 18	184 ± 25	363 ± 60
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 0	16 ± 0	16 ± 1	15 ± 0	16 ± 1	15 ± 1
Day 23	18 ± 1	17 ± 1	18 ± 1	18 ± 0	19 ± 1	18 ± 1
Week 14	44 ± 4	54 ± 8	61 ± 5	51 ± 7	34 ± 3	29 ± 2*
Bile acids (µmol/L)						
Day 4	21.1 ± 1.5	20.1 ± 1.7	20.9 ± 2.8	25.3 ± 2.4	19.3 ± 1.5	26.0 ± 2.4
Day 23	19.6 ± 1.1	15.9 ± 1.6	15.3 ± 0.8*	17.1 ± 1.2	17.7 ± 0.7	15.9 ± 0.6
Week 14	19.1 ± 2.1	17.2 ± 1.3	16.5 ± 1.0	15.9 ± 1.7	16.9 ± 1.7	14.9 ± 1.0
Female						
Hematology						
n						
Day 4	8	10	10	10	10	10
Day 23	10	8	9	10	10	8
Week 14	10	10	10	10	10	10
Automated hematocrit (%)						
Day 4	42.1 ± 0.8	41.4 ± 0.5	40.7 ± 0.8	40.4 ± 0.5	40.9 ± 0.4	40.4 ± 0.3
Day 23	44.1 ± 0.5	44.2 ± 0.7	44.8 ± 0.9	45.7 ± 0.5	46.5 ± 0.5**	48.7 ± 0.4**
Week 14	43.2 ± 0.4	43.8 ± 0.3	43.6 ± 0.4	45.1 ± 0.3**	46.8 ± 0.6**	48.8 ± 0.4**
Hemoglobin (g/dL)						
Day 4	14.1 ± 0.3	14.0 ± 0.2	13.7 ± 0.3	13.7 ± 0.2	13.9 ± 0.1	13.9 ± 0.1
Day 23	15.1 ± 0.1	15.1 ± 0.2	15.2 ± 0.3	15.5 ± 0.1	16.0 ± 0.2**	16.8 ± 0.1**
Week 14	14.4 ± 0.1	14.6 ± 0.1	14.6 ± 0.2	15.1 ± 0.1**	15.5 ± 0.2**	16.6 ± 0.2**
Erythrocytes (10^6/µL)						
Day 4	7.23 ± 0.13	7.09 ± 0.10	6.93 ± 0.15	6.86 ± 0.09	7.05 ± 0.06	7.09 ± 0.07
Day 23	7.58 ± 0.10	7.68 ± 0.14	7.65 ± 0.18	7.88 ± 0.09	8.05 ± 0.11**	8.62 ± 0.08**
Week 14	7.64 ± 0.07	7.73 ± 0.06	7.71 ± 0.06	7.85 ± 0.05*	8.07 ± 0.09**	8.27 ± 0.10**
Reticulocytes (10^6/µL)						
Day 4	0.25 ± 0.02	0.26 ± 0.01	0.29 ± 0.02	0.27 ± 0.01	0.28 ± 0.02	0.22 ± 0.02
Day 23	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.12 ± 0.02	0.11 ± 0.02
Week 14	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Nucleated erythrocytes (10^3/µL)						
Day 4	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.07 ± 0.02	0.02 ± 0.01	0.15 ± 0.03*
Day 23	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.11 ± 0.02**	0.09 ± 0.04**
Week 14	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	8	10	10	10	10	10
Day 23	10	8	9	10	10	8
Week 14	10	10	10	10	10	10
Mean cell volume (fL)						
Day 4	58.3 ± 0.4	58.4 ± 0.2	58.7 ± 0.2	58.9 ± 0.3	58.1 ± 0.5	57.1 ± 0.3
Day 23	58.3 ± 0.3	57.6 ± 0.5	58.6 ± 0.2	57.9 ± 0.2	57.8 ± 0.3	56.5 ± 0.4**
Week 14	56.5 ± 0.1	56.7 ± 0.1	56.6 ± 0.1	57.4 ± 0.1**	58.0 ± 0.1**	59.0 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.2	19.8 ± 0.2	19.8 ± 0.1	19.9 ± 0.1	19.8 ± 0.2	19.6 ± 0.1
Day 23	20.0 ± 0.1	19.8 ± 0.1	19.9 ± 0.1	19.7 ± 0.1	19.9 ± 0.1	19.5 ± 0.1
Week 14	18.9 ± 0.1	18.8 ± 0.1	18.9 ± 0.1	19.3 ± 0.1**	19.3 ± 0.1*	20.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.5 ± 0.08	33.8 ± 0.2	33.7 ± 0.2	33.8 ± 0.1	34.1 ± 0.1**	34.4 ± 0.2**
Day 23	34.2 ± 0.1	34.3 ± 0.1	34.0 ± 0.1	34.1 ± 0.2	34.4 ± 0.2	34.6 ± 0.2
Week 14	33.4 ± 0.1	33.2 ± 0.2	33.4 ± 0.2	33.5 ± 0.2	33.2 ± 0.2	34.0 ± 0.2
Platelets (10^3/µL)						
Day 4	885.0 ± 27.8	867.5 ± 25.9	905.2 ± 27.4	822.8 ± 24.6	917.4 ± 27.2	874.8 ± 15.9
Day 23	785.1 ± 17.9	795.8 ± 19.8	819.8 ± 10.8	730.7 ± 18.3	725.8 ± 11.9*	700.4 ± 20.4**
Week 14	657.3 ± 15.8	667.9 ± 9.7	672.5 ± 8.7	623.1 ± 13.5	651.8 ± 10.6	599.7 ± 10.7**
Leukocytes (10^3/µL)						
Day 4	8.58 ± 0.38	8.60 ± 0.51	9.05 ± 0.28	9.20 ± 0.34	9.71 ± 0.50	10.53 ± 0.38**
Day 23	10.07 ± 0.62	8.88 ± 0.31	9.27 ± 0.41	9.64 ± 0.56	10.15 ± 0.45	9.41 ± 0.52
Week 14	8.45 ± 0.40	8.31 ± 0.33	8.20 ± 0.33	8.52 ± 0.26	8.98 ± 0.37	8.19 ± 0.34
Segmented neutrophils (10^3/µL)						
Day 4	0.89 ± 0.07	0.85 ± 0.06	0.97 ± 0.06	1.01 ± 0.08	1.02 ± 0.10	1.16 ± 0.15
Day 23	1.03 ± 0.13	1.10 ± 0.17	1.07 ± 0.11	1.22 ± 0.12	1.38 ± 0.12	1.54 ± 0.12**
Week 14	1.36 ± 0.10	1.32 ± 0.12	1.67 ± 0.14	1.93 ± 0.23	1.76 ± 0.20	2.03 ± 0.22**
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	7.59 ± 0.39	7.63 ± 0.50	7.96 ± 0.30	8.11 ± 0.28	8.58 ± 0.45	9.23 ± 0.45*
Day 23	8.93 ± 0.57	7.60 ± 0.24	7.98 ± 0.37	8.35 ± 0.55	8.62 ± 0.41	7.81 ± 0.55
Week 14	6.80 ± 0.42	6.68 ± 0.36	6.32 ± 0.27	6.32 ± 0.20	7.00 ± 0.30	5.92 ± 0.38
Monocytes (10^3/µL)						
Day 4	0.06 ± 0.01	0.08 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.08 ± 0.04	0.05 ± 0.02
Day 23	0.10 ± 0.03	0.15 ± 0.04	0.14 ± 0.04	0.07 ± 0.02	0.10 ± 0.03	0.02 ± 0.01
Week 14	0.25 ± 0.04	0.23 ± 0.03	0.17 ± 0.03	0.17 ± 0.03	0.15 ± 0.03*	0.15 ± 0.04*
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	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.08 ± 0.03
Day 23	0.01 ± 0.01	0.03 ± 0.02	0.07 ± 0.03	0.01 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
Week 14	0.04 ± 0.01	0.08 ± 0.02	0.04 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.09 ± 0.02

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Formamide

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Female (continued)						
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	9.7 ± 0.6	10.1 ± 0.3	9.3 ± 0.2	10.7 ± 0.3	10.2 ± 0.5	12.8 ± 0.7**
Day 23	13.5 ± 0.5	12.6 ± 0.4	12.3 ± 0.4	13.2 ± 0.5	13.7 ± 0.4	12.3 ± 0.8
Week 14	12.7 ± 0.2	12.9 ± 0.4	13.2 ± 0.3	12.5 ± 0.5	13.2 ± 0.3	14.5 ± 0.7
Creatinine (mg/dL)						
Day 4	0.40 ± 0.00	0.40 ± 0.00	0.39 ± 0.01	0.40 ± 0.00	0.39 ± 0.01	0.40 ± 0.00
Day 23	0.51 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.53 ± 0.02	0.50 ± 0.00	0.48 ± 0.01
Week 14	0.51 ± 0.01	0.52 ± 0.01	0.51 ± 0.01	0.50 ± 0.00	0.51 ± 0.01	0.48 ± 0.01
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1
Day 23	5.9 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	5.8 ± 0.1	6.0 ± 0.1	5.6 ± 0.1*
Week 14	6.2 ± 0.1	6.4 ± 0.2	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	5.8 ± 0.1**
Albumin (g/dL)						
Day 4	4.6 ± 0.1	4.6 ± 0.0	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.4 ± 0.1
Day 23	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.5 ± 0.0	4.6 ± 0.0	4.3 ± 0.1**
Week 14	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.5 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	65 ± 2	61 ± 2	59 ± 1	57 ± 2*	52 ± 3**	41 ± 3**
Day 23	51 ± 2	49 ± 2	49 ± 1	52 ± 2	51 ± 2	47 ± 1
Week 14	60 ± 5	67 ± 5	62 ± 5	56 ± 4	62 ± 3	60 ± 5
Alkaline phosphatase (IU/L)						
Day 4	576 ± 5	581 ± 8	576 ± 10	581 ± 9	584 ± 14	526 ± 12*
Day 23	405 ± 8	416 ± 10	409 ± 8	415 ± 10	417 ± 10	370 ± 7
Week 14	190 ± 7	221 ± 12	210 ± 6	203 ± 8	200 ± 9	253 ± 6**
Creatine kinase (IU/L)						
Day 4	318 ± 46	332 ± 60	309 ± 33	308 ± 32	313 ± 32	334 ± 41
Day 23	166 ± 25	299 ± 55	185 ± 37	154 ± 18	180 ± 30	227 ± 28
Week 14	188 ± 37	176 ± 29	132 ± 20	158 ± 29	177 ± 32	225 ± 57
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	17 ± 1	16 ± 1	16 ± 0	17 ± 1	15 ± 1
Day 23	19 ± 1	20 ± 1	18 ± 1	19 ± 1	19 ± 1	21 ± 1
Week 14	21 ± 1	22 ± 1	23 ± 1	21 ± 2	25 ± 1	25 ± 2
Bile acids (μmol/L)						
Day 4	19.4 ± 0.9	14.2 ± 0.6**	15.4 ± 0.8	15.6 ± 0.6	16.6 ± 1.5	18.3 ± 1.6
Day 23	12.6 ± 0.7	11.2 ± 0.7	15.0 ± 1.6	12.4 ± 1.4	13.1 ± 0.9	16.3 ± 2.1
Week 14	25.0 ± 3.2	16.6 ± 1.1	17.9 ± 1.7	18.8 ± 2.8	23.7 ± 3.1	23.7 ± 2.5

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
**$P \leq 0.01$
[a] 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 Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Male						
n	10	10	9	10	10	10
Automated hematocrit (%)	49.9 ± 0.8	48.6 ± 0.7	49.8 ± 0.4	50.2 ± 1.4	49.1 ± 0.6	45.9 ± 1.2**
Hemoglobin (g/dL)	16.9 ± 0.2	16.7 ± 0.2	17.0 ± 0.2	17.1 ± 0.4	16.9 ± 0.2	16.1 ± 0.4
Erythrocytes (10^6/µL)	10.90 ± 0.16	10.57 ± 0.15	10.83 ± 0.10	10.88 ± 0.29	10.63 ± 0.14	9.75 ± 0.30**
Reticulocytes (10^6/µL)	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 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)	45.8 ± 0.2	46.1 ± 0.2	46.0 ± 0.1	46.2 ± 0.2	46.2 ± 0.2	47.2 ± 0.4**
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.9 ± 0.1	15.7 ± 0.1	15.7 ± 0.1	15.9 ± 0.1*	16.5 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	34.4 ± 0.2	34.1 ± 0.2	34.0 ± 0.2	34.4 ± 0.2*	35.0 ± 0.1**
Platelets (10^3/µL)	673.1 ± 18.6	692.7 ± 19.1	662.7 ± 31.6	706.1 ± 17.9	793.5 ± 29.8**	935.6 ± 27.4**
Leukocytes (10^3/µL)	5.22 ± 0.33	4.82 ± 0.27	4.66 ± 0.39	5.37 ± 0.59	5.89 ± 0.40	5.73 ± 0.47
Segmented neutrophils (10^3/µL)	0.59 ± 0.07	0.67 ± 0.04	0.54 ± 0.05	0.63 ± 0.10	0.77 ± 0.08	0.93 ± 0.08**
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)	4.52 ± 0.27	4.02 ± 0.27	3.97 ± 0.36	4.58 ± 0.49	4.88 ± 0.35	4.45 ± 0.37
Monocytes (10^3/µL)	0.06 ± 0.01	0.08 ± 0.02	0.10 ± 0.01	0.08 ± 0.02	0.12 ± 0.01**	0.13 ± 0.03*
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)	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.08 ± 0.03	0.12 ± 0.02*	0.23 ± 0.04**
Female						
n	8	6	9	10	10	10
Automated hematocrit (%)	47.9 ± 1.1	45.4 ± 1.1	47.1 ± 0.7	46.8 ± 0.8	46.5 ± 0.5	45.5 ± 0.5
Hemoglobin (g/dL)	16.4 ± 0.4	15.9 ± 0.3	16.2 ± 0.2	16.1 ± 0.2	16.3 ± 0.2	16.0 ± 0.2
Erythrocytes (10^6/µL)	10.22 ± 0.25	9.70 ± 0.24	10.02 ± 0.14	9.90 ± 0.16	9.87 ± 0.11	9.64 ± 0.11
Reticulocytes (10^6/µL)	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01*	0.07 ± 0.01	0.07 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	46.9 ± 0.2	46.7 ± 0.2	47.0 ± 0.2	47.3 ± 0.1	47.1 ± 0.2	47.2 ± 0.2
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.4 ± 0.3	16.2 ± 0.1	16.3 ± 0.1	16.5 ± 0.1*	16.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.4 ± 0.2	35.1 ± 0.5	34.5 ± 0.1	34.5 ± 0.2	35.0 ± 0.2*	35.2 ± 0.2**
Platelets (10^3/µL)	593.0 ± 25.2	711.5 ± 65.6	606.3 ± 33.1	712.9 ± 27.2*	700.1 ± 24.3*	878.0 ± 28.7**
Leukocytes (10^3/µL)	4.41 ± 0.40	3.55 ± 0.23	4.51 ± 0.38	4.56 ± 0.24	5.76 ± 0.32*	6.02 ± 0.22**
Segmented neutrophils (10^3/µL)	0.53 ± 0.07	0.34 ± 0.05	0.39 ± 0.04	0.51 ± 0.07	0.90 ± 0.12	0.94 ± 0.13
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)	3.72 ± 0.37	3.06 ± 0.20	3.95 ± 0.32	3.88 ± 0.22	4.58 ± 0.31	4.70 ± 0.18*
Monocytes (10^3/µL)	0.10 ± 0.03	0.12 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	0.09 ± 0.03	0.16 ± 0.05
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	0.03 ± 0.01	0.10 ± 0.03	0.11 ± 0.02	0.20 ± 0.03**	0.21 ± 0.03**

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

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

Table G1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of Formamide 140

Table G2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Formamide 141

Table G3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Formamide 142

Table G4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Formamide 143

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

	Vehicle Control	160 mg/kg	312 mg/kg	625 mg/kg	1,250 mg/kg	2,500 mg/kg
n	5	5	0	0	0	0
Male						
Necropsy body wt	176 ± 5	150 ± 5**				
Heart						
Absolute	0.666 ± 0.032	0.644 ± 0.013				
Relative	3.781 ± 0.095	4.312 ± 0.080**				
R. Kidney						
Absolute	0.756 ± 0.030	0.713 ± 0.030				
Relative	4.295 ± 0.069	4.768 ± 0.115**				
Liver						
Absolute	9.370 ± 0.558	7.995 ± 0.319				
Relative	53.157 ± 2.021	53.426 ± 0.680				
Lung						
Absolute	1.294 ± 0.087	1.240 ± 0.065				
Relative	7.346 ± 0.371	8.292 ± 0.357				
R. Testis						
Absolute	1.121 ± 0.029	1.032 ± 0.022*				
Relative	6.388 ± 0.201	6.910 ± 0.108				
Thymus						
Absolute	0.417 ± 0.017	0.358 ± 0.031				
Relative	2.371 ± 0.079	2.386 ± 0.157				
Female						
Necropsy body wt	118 ± 2	97 ± 3**				
Heart						
Absolute	0.507 ± 0.013	0.503 ± 0.016				
Relative	4.284 ± 0.077	5.198 ± 0.117**				
R. Kidney						
Absolute	0.554 ± 0.007	0.480 ± 0.016**				
Relative	4.689 ± 0.064	4.965 ± 0.105				
Liver						
Absolute	6.111 ± 0.092	4.702 ± 0.329**				
Relative	51.693 ± 0.876	48.325 ± 1.900				
Lung						
Absolute	0.986 ± 0.049	0.873 ± 0.042				
Relative	8.341 ± 0.433	9.008 ± 0.279				
Thymus						
Absolute	0.373 ± 0.017	0.306 ± 0.016*				
Relative	3.155 ± 0.161	3.157 ± 0.095				

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test

**$P \leq 0.01$

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

Table G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	343 ± 5	348 ± 6	343 ± 6	337 ± 8	317 ± 7**	259 ± 5**
Heart						
Absolute	1.016 ± 0.023	1.012 ± 0.029	1.054 ± 0.024	1.034 ± 0.028	1.019 ± 0.029	0.911 ± 0.015*
Relative	2.962 ± 0.052	2.909 ± 0.058	3.073 ± 0.064	3.067 ± 0.058	3.210 ± 0.037**	3.516 ± 0.063**
R. Kidney						
Absolute	1.046 ± 0.020	1.078 ± 0.024	1.081 ± 0.022	1.073 ± 0.024	1.017 ± 0.021	0.914 ± 0.024**
Relative	3.049 ± 0.035	3.099 ± 0.040	3.150 ± 0.039	3.182 ± 0.046*	3.211 ± 0.054*	3.522 ± 0.041**
Liver						
Absolute	13.04 ± 0.35	12.83 ± 0.29	13.15 ± 0.31	13.08 ± 0.25	12.07 ± 0.29*	10.30 ± 0.31**
Relative	38.006 ± 0.868	36.893 ± 0.355	38.317 ± 0.782	38.832 ± 0.436	38.034 ± 0.192	39.679 ± 0.848
Lung						
Absolute	1.788 ± 0.079	1.843 ± 0.077	1.873 ± 0.078	1.739 ± 0.059	1.731 ± 0.052	1.530 ± 0.054*
Relative	5.210 ± 0.216	5.300 ± 0.201	5.449 ± 0.191	5.172 ± 0.195	5.485 ± 0.243	5.894 ± 0.171
R. Testis						
Absolute	1.498 ± 0.032	1.534 ± 0.028	1.553 ± 0.024	1.547 ± 0.045	1.494 ± 0.033	1.188 ± 0.043**
Relative	4.368 ± 0.073	4.415 ± 0.047	4.529 ± 0.077	4.597 ± 0.135	4.712 ± 0.062*	4.576 ± 0.128*
Thymus						
Absolute	0.316 ± 0.009	0.291 ± 0.015	0.304 ± 0.012	0.291 ± 0.011	0.287 ± 0.011	0.277 ± 0.018
Relative	0.922 ± 0.021	0.841 ± 0.050	0.890 ± 0.046	0.862 ± 0.022	0.904 ± 0.033	1.068 ± 0.069
Female						
Necropsy body wt	188 ± 3	191 ± 4	184 ± 3	177 ± 3*	175 ± 4*	150 ± 5**
Heart						
Absolute	0.634 ± 0.009	0.643 ± 0.015	0.644 ± 0.011	0.644 ± 0.013	0.669 ± 0.014	0.654 ± 0.014
Relative	3.373 ± 0.030	3.376 ± 0.063*	3.501 ± 0.065	3.638 ± 0.041**	3.840 ± 0.083**	4.396 ± 0.094**
R. Kidney						
Absolute	0.661 ± 0.010	0.654 ± 0.015	0.649 ± 0.012	0.628 ± 0.011	0.634 ± 0.015	0.591 ± 0.013**
Relative	3.521 ± 0.054	3.429 ± 0.045	3.522 ± 0.042	3.550 ± 0.045	3.635 ± 0.075	3.976 ± 0.109**
Liver						
Absolute	6.717 ± 0.176	6.743 ± 0.189	6.816 ± 0.136	6.600 ± 0.204	6.574 ± 0.151	5.898 ± 0.202**
Relative	35.703 ± 0.626	35.365 ± 0.632	37.049 ± 0.777	37.286 ± 0.883	37.681 ± 0.710	39.446 ± 0.582**
Lung						
Absolute	1.209 ± 0.051	1.230 ± 0.069	1.251 ± 0.046	1.158 ± 0.038	1.185 ± 0.063	1.029 ± 0.051
Relative	6.446 ± 0.302	6.443 ± 0.312	6.788 ± 0.202	6.575 ± 0.295	6.764 ± 0.266	6.876 ± 0.243
Thymus						
Absolute	0.264 ± 0.009	0.247 ± 0.009	0.249 ± 0.008	0.231 ± 0.007	0.248 ± 0.009	0.228 ± 0.006**
Relative	1.399 ± 0.035	1.295 ± 0.033	1.350 ± 0.038	1.305 ± 0.029	1.420 ± 0.041	1.529 ± 0.037

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test

**$P \leq 0.01$

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

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

	Vehicle Control	160 mg/kg	312 mg/kg	625 mg/kg	1,250 mg/kg	2,500 mg/kg
n	5	5	0	0	0	0
Male						
Necropsy body wt	26.0 ± 0.2	24.5 ± 0.5*				
Heart						
Absolute	0.145 ± 0.005	0.177 ± 0.005**				
Relative	5.600 ± 0.165	7.240 ± 0.226**				
R. Kidney						
Absolute	0.262 ± 0.003	0.221 ± 0.004**				
Relative	10.100 ± 0.086	9.025 ± 0.158**				
Liver						
Absolute	1.470 ± 0.032	1.393 ± 0.056				
Relative	56.619 ± 0.949	56.831 ± 1.544				
Lung						
Absolute	0.204 ± 0.006	0.182 ± 0.009				
Relative	7.876 ± 0.239	7.441 ± 0.439				
R. Testis						
Absolute	0.100 ± 0.003	0.090 ± 0.003*				
Relative	3.865 ± 0.098	3.686 ± 0.109				
Thymus						
Absolute	0.053 ± 0.002	0.024 ± 0.002**				
Relative	2.034 ± 0.081	1.001 ± 0.100**				
Female						
n						
Necropsy body wt	19.5 ± 0.5	18.0 ± 0.8				
Heart						
Absolute	0.110 ± 0.005	0.136 ± 0.004**				
Relative	5.609 ± 0.139	7.645 ± 0.442**				
R. Kidney						
Absolute	0.151 ± 0.007	0.128 ± 0.004*				
Relative	7.694 ± 0.221	7.129 ± 0.104				
Liver						
Absolute	1.082 ± 0.040	0.958 ± 0.060				
Relative	55.344 ± 1.107	53.190 ± 1.161				
Lung						
Absolute	0.178 ± 0.008	0.162 ± 0.009				
Relative	9.107 ± 0.295	9.119 ± 0.692				
Thymus						
Absolute	0.072 ± 0.004	0.032 ± 0.002**				
Relative	3.699 ± 0.179	1.782 ± 0.159**				

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test

**$P \leq 0.01$

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

Table G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Formamide[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.4 ± 0.9	38.3 ± 0.9	36.8 ± 1.5	35.8 ± 1.4	34.4 ± 0.9*	32.6 ± 0.8**
Heart						
Absolute	0.168 ± 0.006	0.217 ± 0.012**	0.163 ± 0.004	0.170 ± 0.006	0.165 ± 0.005	0.196 ± 0.009
Relative	4.381 ± 0.175	5.656 ± 0.259	4.447 ± 0.097	4.771 ± 0.131	4.802 ± 0.109	6.043 ± 0.368**
R. Kidney						
Absolute	0.306 ± 0.010	0.304 ± 0.006	0.304 ± 0.010	0.311 ± 0.009	0.296 ± 0.011	0.282 ± 0.010
Relative	7.967 ± 0.236	7.957 ± 0.184	8.288 ± 0.170	8.781 ± 0.279*	8.591 ± 0.152*	8.647 ± 0.197*
Liver						
Absolute	1.689 ± 0.045	1.842 ± 0.033	1.636 ± 0.079	1.663 ± 0.075	1.705 ± 0.049	1.767 ± 0.033
Relative	43.966 ± 0.728	48.246 ± 0.766	44.428 ± 0.899	46.493 ± 1.076	49.521 ± 0.576**	54.298 ± 1.088**
Lung						
Absolute	0.336 ± 0.008	0.334 ± 0.007	0.334 ± 0.015	0.316 ± 0.015	0.289 ± 0.014**	0.228 ± 0.008**
Relative	8.825 ± 0.402	8.738 ± 0.176	9.147 ± 0.433	8.870 ± 0.323	8.403 ± 0.342	7.005 ± 0.226**
R. Testis						
Absolute	0.115 ± 0.002	0.119 ± 0.004	0.118 ± 0.004	0.111 ± 0.006	0.121 ± 0.003	0.108 ± 0.004
Relative	3.006 ± 0.065	3.124 ± 0.112	3.221 ± 0.070	3.119 ± 0.145	3.514 ± 0.057**	3.290 ± 0.102**
Thymus						
Absolute	0.046 ± 0.003	0.044 ± 0.002	0.046 ± 0.003	0.042 ± 0.003	0.039 ± 0.002	0.035 ± 0.002**
Relative	1.200 ± 0.073	1.156 ± 0.065	1.242 ± 0.058	1.168 ± 0.081	1.135 ± 0.051	1.073 ± 0.047
Female						
Necropsy body wt	29.1 ± 0.7	30.4 ± 1.4	29.0 ± 1.2	28.3 ± 1.4	28.3 ± 0.8	26.5 ± 0.4
Heart						
Absolute	0.156 ± 0.011	0.154 ± 0.010	0.164 ± 0.008	0.155 ± 0.007	0.189 ± 0.022	0.150 ± 0.007
Relative	5.382 ± 0.406	5.161 ± 0.414	5.687 ± 0.261	5.580 ± 0.401	6.660 ± 0.745	5.687 ± 0.276
R. Kidney						
Absolute	0.177 ± 0.007	0.170 ± 0.005	0.164 ± 0.004	0.163 ± 0.005	0.169 ± 0.004	0.158 ± 0.003*
Relative	6.074 ± 0.185	5.651 ± 0.169	5.713 ± 0.220	5.805 ± 0.121	5.990 ± 0.089	5.974 ± 0.058
Liver						
Absolute	1.408 ± 0.041	1.391 ± 0.050	1.339 ± 0.031	1.295 ± 0.053	1.404 ± 0.050	1.409 ± 0.024
Relative	48.396 ± 0.861	45.943 ± 0.800	46.507 ± 1.114	45.905 ± 0.918	49.540 ± 0.645	53.202 ± 0.899**
Lung						
Absolute	0.338 ± 0.015	0.307 ± 0.017	0.314 ± 0.012	0.289 ± 0.021*	0.289 ± 0.017*	0.209 ± 0.010**
Relative	11.620 ± 0.375	10.254 ± 0.664	10.925 ± 0.441	10.341 ± 0.716	10.158 ± 0.411	7.903 ± 0.427**
Thymus						
Absolute	0.041 ± 0.002	0.053 ± 0.003*	0.052 ± 0.003*	0.047 ± 0.003	0.050 ± 0.002	0.043 ± 0.001
Relative	1.409 ± 0.078	1.766 ± 0.104*	1.779 ± 0.103**	1.642 ± 0.067	1.756 ± 0.062*	1.614 ± 0.043

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test

**$P \leq 0.01$

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

Table H1 Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of Formamide 146

Table H2 Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of Formamide 146

Table H3 Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of Formamide 147

Table H4 Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Formamide 147

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	343 ± 5	343 ± 6	337 ± 8	317 ± 7*
L. Cauda epididymis	0.166 ± 0.006	0.180 ± 0.007	0.173 ± 0.006	0.174 ± 0.005
L. Epididymis	0.454 ± 0.007	0.485 ± 0.012	0.475 ± 0.007	0.470 ± 0.008
L. Testis	1.558 ± 0.026	1.606 ± 0.027	1.601 ± 0.034	1.523 ± 0.049
Spermatid measurements				
Spermatid heads (10^7/g testis)	148.1 ± 5.6	153.1 ± 8.5	144.7 ± 5.8	149.6 ± 6.3
Spermatid heads (10^7/testis)	213.6 ± 9.2	223.8 ± 13.8	211.1 ± 9.0	207.8 ± 10.3
Spermatid heads (10^7/g cauda)	446.6 ± 28.5	363.5 ± 22.2*	440.2 ± 17.1	433.4 ± 23.9
Spermatid heads (10^7/cauda)	73.26 ± 4.21	65.40 ± 4.54	75.65 ± 3.21	75.15 ± 4.19
Epididymal spermatozoal measurements				
Motility (%)	81.14 ± 1.24	79.58 ± 0.81	77.92 ± 0.53	79.01 ± 1.11

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

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
n	10	10	10	10
Necropsy body wt (g)	188 ± 3	184 ± 3	177 ± 3*	175 ± 4**
Estrous cycle length (days)	4.80 ± 0.25	4.80 ± 0.11	5.25 ± 0.34	5.00 ± 0.00
Estrous stages[b] (% of cycle)				
Diestrus	65.0	59.2	50.8	60.0
Proestrus	10.0	9.2	10.8	15.0
Estrus	20.0	22.5	30.0	20.0
Metestrus	5.0	9.2	8.3	4.2
Uncertain diagnoses	0.0	0.0	0.0	0.8

* Significantly different ($P \leq 0.05$) from the control group by William's test
**$P \leq 0.01$
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control for estrous cycle length are not significant by Dunn's test.
[b] Evidence shows that females administered 40 mg/kg differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages; 40 mg/kg females spent more time in estrus and less time in diestrus than vehicle control females.

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.4 ± 0.9	36.8 ± 1.5	35.8 ± 1.4	34.4 ± 0.9
L. cauda epididymis	0.0156 ± 0.0012	0.0165 ± 0.0012	0.0150 ± 0.0008	0.0155 ± 0.0006
L. epididymis	0.0456 ± 0.0021	0.0480 ± 0.0012	0.0448 ± 0.0016	0.0472 ± 0.0008
L. testis	0.1106 ± 0.0041	0.1150 ± 0.0029	0.1071 ± 0.0062	0.1152 ± 0.0027
Spermatid measurements				
Spermatid heads (10^7/g testis)	251.3 ± 12.2	238.7 ± 14.2	223.1 ± 13.3	205.6 ± 11.1[b]
Spermatid heads (10^7/testis)	23.63 ± 1.43	22.07 ± 0.64	20.44 ± 2.07	21.34 ± 1.48
Spermatid heads (10^7/cauda)	9.720 ± 1.692	14.510 ± 2.804	11.100 ± 1.502	11.450 ± 1.312
Spermatic heads (10^7/g cauda)	633.0 ± 109.6	759.6 ± 85.0	727.6 ± 92.9	754.6 ± 104.2
Epididymal spermatozoal measurements				
Motility (%)	76.76 ± 0.80	77.74 ± 0.64	75.90 ± 0.82	76.74 ± 0.71

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

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

	Vehicle Control	20 mg/kg	40 mg/kg	80 mg/kg
n	10	10	10	10
Necropsy body wt (g)	29.1 ± 0.7	29.0 ± 1.2	28.3 ± 1.4	28.3 ± 0.8
Estrous cycle length (days)	4.45 ± 0.30	4.44 ± 0.13[b]	4.31 ± 0.19[c]	4.07 ± 0.11
Estrous stages[d] (% of cycle)				
Diestrus	49.2	40.0	42.5	36.7
Proestrus	7.5	3.3	4.2	9.2
Estrus	22.5	32.5	28.3	35.0
Metestrus	18.3	17.5	17.5	19.2
Uncertain diagnoses	2.5	6.7	7.5	0.0

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control were not significant by Dunnett's test (body weights) or Dunn's test (estrous cycle length).
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[d] Evidence shows that dosed females differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages; dosed females spent more time in estrus and less time in diestrus and proestrus than vehicle control females.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Formamide

Formamide was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) (lot 08003HQ), and Acros Organics/Fisher Scientific (Fair Lawn, NJ) (lot A012538501). Lot 08003HQ was used in the 2-week and 3-month studies, and lot A012538501 was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory, Battelle Columbus Operations (Columbus, OH); the analytical chemistry laboratory also conducted stability analyses of lot A012538501. Reports on analyses performed in support of the formamide studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a colorless liquid, were identified as formamide by infrared and proton nuclear magnetic resonance (NMR) spectroscopy, and lot A012538501 was also identified as formamide by the analytical chemistry laboratory using ultraviolet (UV)/visible spectroscopy and boiling point and relative density determinations. All spectra were consistent with the structure, literature spectra (*Aldrich*, 1983, 1985, 1997), and spectra of analytical standards and frozen reference samples of formamide. Representative infrared and NMR spectra are presented in Figures I1 and I2. The boiling point (210.0° C at 756.9 mm Hg) and relative density (1.133 at 25° C) were consistent with literature values (*Merck*, 1996).

The moisture content of lot A012538501 was determined by the analytical chemistry laboratory using Karl Fischer titration. The purity of lot 08003HQ was determined by the analytical chemistry laboratory using gas chromatography (GC) by a system that included a Varian gas chromatograph with a flame ionization detector (Varian Inc., Palo Alto, CA), helium carrier gas at a flow rate of 10 mL/minute, a DB-WAX column (30 m × 0.53 mm, 1-µm film) (Agilent J&W, Folsom, CA), and a temperature program of 100° C for 4 minutes, then increased to 220° C at 15° C/minute with a 3-minute hold at 220° C. The study laboratory determined the purity of this lot relative to a frozen reference standard from the same lot using high-performance liquid chromatography (HPLC) by system A (Table I1). The purity of lot A012538501 was determined by the analytical chemistry laboratory using HPLC (system B) and thin-layer chromatography (TLC). One- and two-dimensional TLC was performed on precoated silica gel $60F_{254}$ plates (20 cm × 20 cm, 250 µm thickness) (EM Science; Gibbstown, NJ), that were developed in a chamber saturated with acetone:toluene (75:25). Spots are visualized with a sequence of spray reagents (0.5% v/v sodium hypochlorite, absolute ethanol, 1% soluble starch, and 1% potassium iodide) and shortwave UV light. The study laboratory determined the purity of lot A012538501 relative to a frozen reference standard from this same lot using HPLC by a system similar to system A.

For lot 08003HQ, GC indicated one major peak with no impurities greater than 0.05% relative to the major peak area; HPLC indicated a relative purity of 100%. The overall purity of lot 08003HQ was determined to be approximately 100%. For lot A012538501, HPLC by system B indicated one major peak with no impurities greater than 0.05% relative to the major peak area, and HPLC by a system similar to system A indicated an average relative purity of 101%. Karl Fischer titration indicated 0.06% water. TLC indicated one major spot and three minor spots. The overall purity of lot A012538501 was determined to be approximately 100%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory. HPLC was performed using system C (Table I1). These studies indicated that formamide was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at 25° (lot 08003HQ) or 5° C (lot A012538501) in the original shipping containers (sealed amber glass bottles). Periodic reanalyses of the bulk chemical were performed by the study laboratory using HPLC by system A, and no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing formamide with deionized water to give the required concentration (Table I2). The dose formulations were stored at approximately 5° C in amber glass bottles sealed with Teflon®-lined lids for up to 35 (2-week and 3-month studies) or 50 (2-year studies) days.

Because the dose formulations were solutions, no homogeneity studies were performed. Stability studies of a 0.6 mg/mL dose formulation of a lot not used in the animal studies were performed by the analytical chemistry laboratory using HPLC by a system similar to system A (Table I1). Stability was confirmed for at least 50 days for dose formulations stored in the dark in glass containers under ambient and refrigerated conditions, and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of formamide were conducted by the study laboratory using HPLC by system A. During the 2-week studies, the dose formulations were analyzed once; all five dose formulations for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all five 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 of these dose formulations were also analyzed (Table I4). All dose formulations analyzed for rats (15) and mice (15) were within 10% of the target concentrations; all 15 animal room samples for rats and mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months (Table I5). All dose formulations analyzed for rats (27) and mice (30) were within 10% of the target concentrations; all animal room samples for rats (12) and mice (12) were within 10% of the target concentrations.

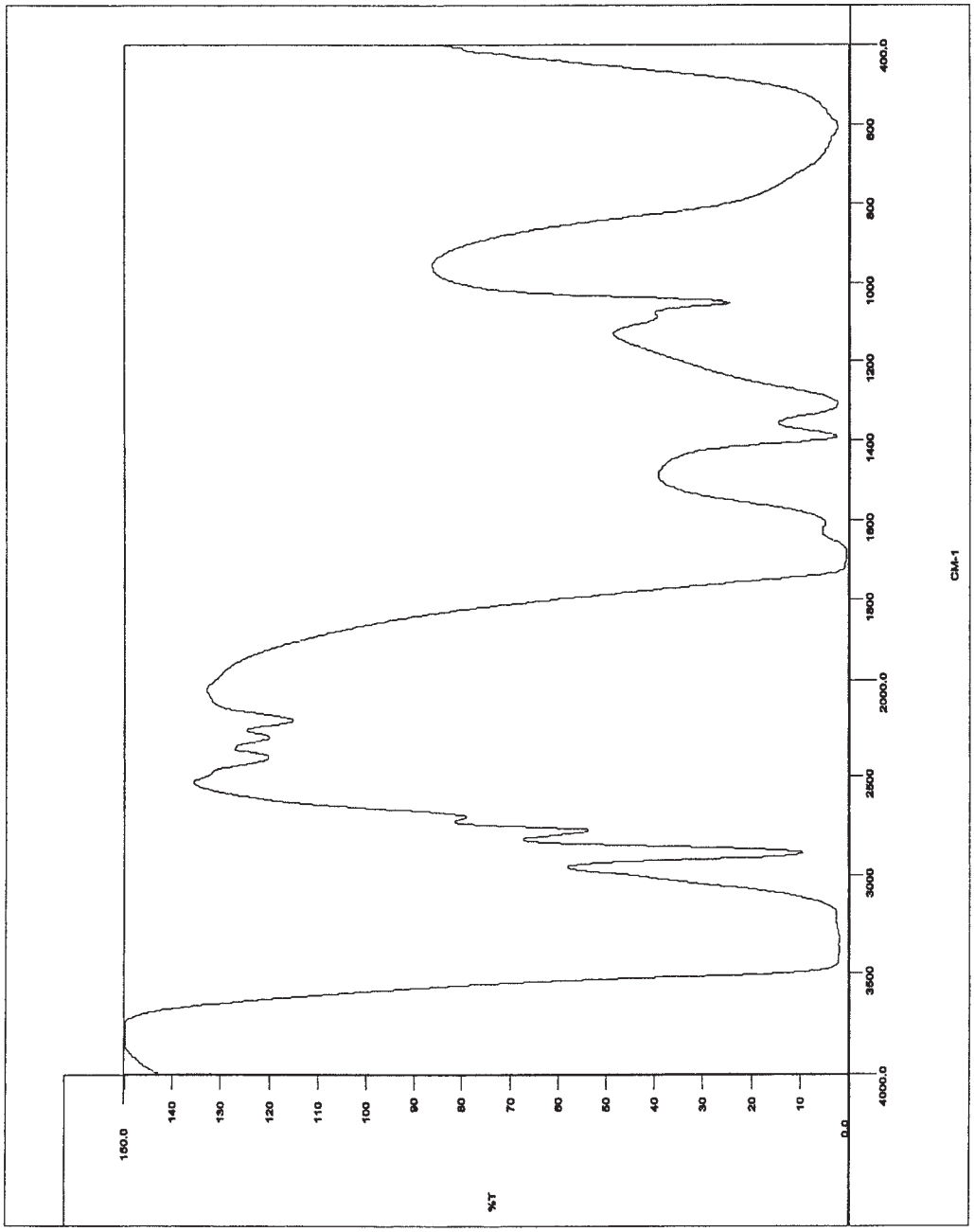

Figure I1
Infrared Absorption Spectrum of Formamide

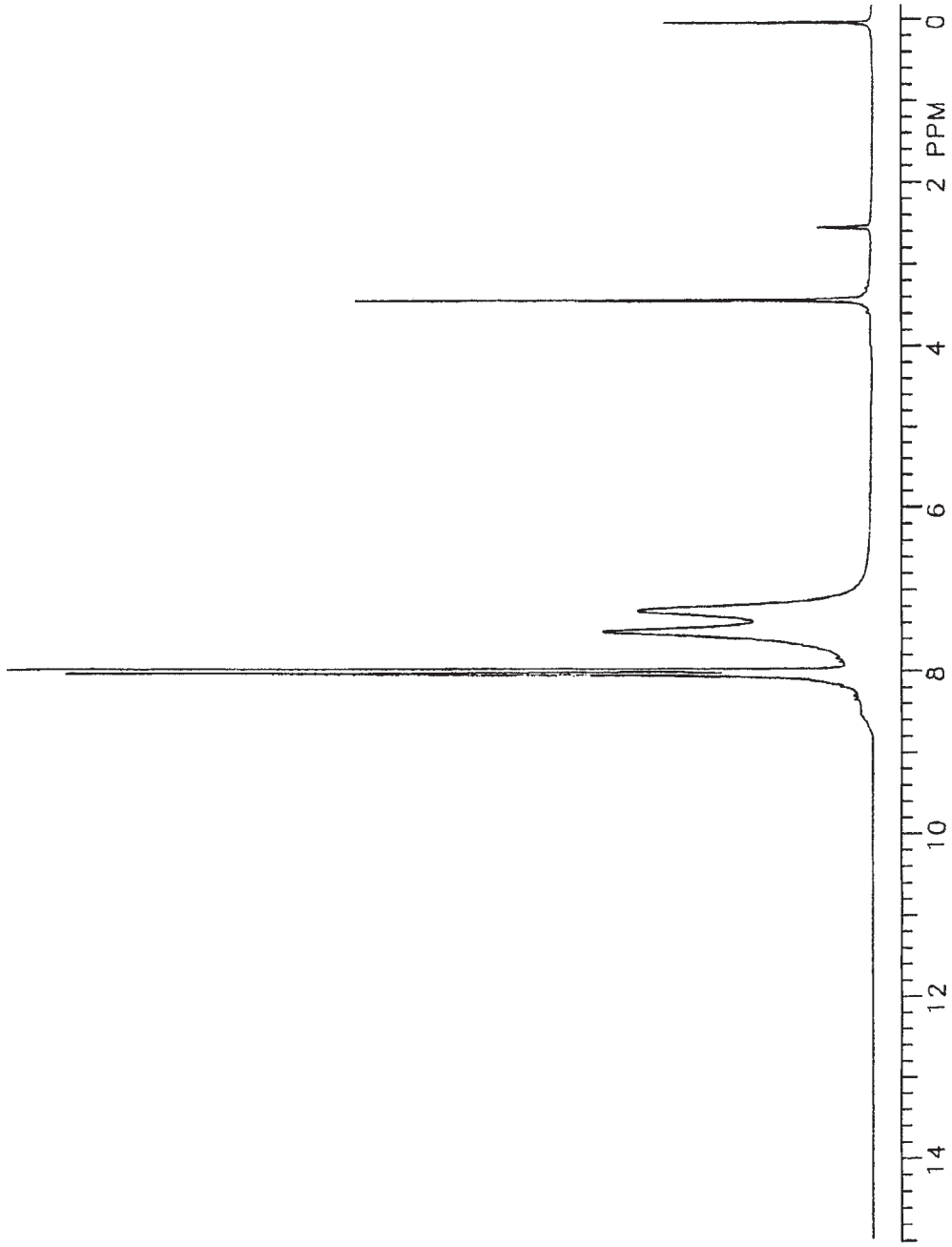

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Formamide

Table I1
High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Formamide[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (210 nm) light	Phenomenex Prodigy™ ODS(3), 250 mm × 4.6 mm, 5 µm (Phenomenex, Torrance, CA)	Methanol:Milli-Q® water (10:90), isocratic; flow rate 0.8 mL/minute; *N,N*-dimethylformamide as internal standard
System B		
Ultraviolet (210 nm) light	Alltech Alltima™ C18, 250 mm × 3.2 mm, 5 µm (Alltech Associates, Inc., Deerfield, IL)	A) Methanol:water (50:50) and B) methanol: water (10:90); linear gradient from 100% B to 100% A in 25 minutes, held for 1 minute, then linear to 100% B over 35 minutes; flow rate 1 mL/minute
System C		
Ultraviolet (210 nm) light	Alltech Alltima™ C18, 250 mm × 3.2 mm, 5 µm (Alltech Associates, Inc.)	Methanol:water (10:90) isocratic; flow rate 1.0 mL/minute; *N,N*-dimethylformamide as internal standard

[a] High-performance liquid chromatographs were manufactured by Waters Corp. (Milford, MA)

Table I2
Preparation and Storage of Dose Formulations in the Gavage Studies of Formamide

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
An accurately weighed amount of formamide was placed in a graduated glass cylinder, diluted to volume with deionized water, capped, and shaken vigorously until mixed.	An accurately weighted amount of formamide was added to a glass beaker partially filled with deionized water, diluted to volume with deionized water, and stirred with a magnetic stirrer for approximately 15 minutes.	The appropriate volume of formamide was measured with a volumetric pipet or graduated cylinder and transferred to a calibrated glass mixing container partially filled with deionized water, diluted to volume with deionized water, capped, and shaken vigorously until mixed.
Chemical Lot Number		
08003HQ	08003HQ	A012538501
Maximum Storage Time		
35 days	35 days	50 days
Storage Conditions		
Stored in amber glass bottles sealed with Teflon®-lined lids at approximately 5° C.	Same as 2-week studies	Same as 2-week studies
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
June 2, 1997	June 4-5, 1997	32	32.08	0
		62.5	62.86	+1
		125	124.4	0
		250	243.5	–3
		500	499.6	0
	June 27, 30, 1997[b]	32	33.33	+4
		62.5	62.65	0
		125	128.5	+3
		250	257.9	+3
		500	516.3	+3
Mice				
June 2, 1997	June 4-5, 1997	16	16.19	+1
		31.2	32.16	+3
		62.5	62.86	+1
		125	124.4	0
		250	243.5	–3
	June 27, 30, 1997[b]	16	16.48	+3
		31.2	32.27	+3
		62.5	65.12	+4
		125	131.9	+6
		250	260.5	+4

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 32 mg/mL=160 mg/kg, 62.5 mg/mL=312 mg/kg, 125 mg/mL=625 mg/kg, 250 mg/mL=1,250 mg/kg, 500 mg/mL=2,500 mg/kg. For mice, dosing volume=10 mL/kg; 16 mg/mL=160 mg/kg, 31.2 mg/mL=312 mg/kg, 62.5 mg/mL=625 mg/kg, 125 mg/mL=1,250 mg/kg, 250 mg/mL=2,500 mg/kg.
[b] Animal room samples

Table I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Formamide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
October 6, 1997	October 6, 1997	2	2.023	+1
		4	4.126	+3
		8	8.266	+3
		16	16.70	+4
		32	32.86	+3
	November 10, 1997[b]	2	2.042	+2
		4	4.152	+4
		8	8.196	+2
		16	16.45	+3
		32	33.66	+5
December 1, 1997	December 2, 1997	2	1.985	–1
		4	4.031	+1
		8	8.157	+2
		16	16.54	+3
		32	34.25	+7
	January 5, 1998[b]	2	2.065	+3
		4	4.221	+6
		8	8.051	+1
		16	15.87	–1
		32	31.30	–2
December 29, 1997	December 30, 1997	2	2.075	+4
		4	4.105	+3
		8	8.098	+1
		16	16.75	+5
		32	33.02	+3
	January 16, 1998[b]	2	2.060	+3
		4	4.001	0
		8	8.456	+6
		16	16.98	+6
		32	32.51	+2

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Formamide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
October 6, 1997	October 6, 1997	1	1.013	+1
		2	2.023	+1
		4	4.126	+3
		8	8.266	+3
		16	16.70	+4
	November 10, 1997[b]	1	0.9828	–2
		2	2.005	0
		4	4.109	+3
		8	8.136	+2
		16	16.41	+3
December 1, 1997	December 2, 1997	1	0.9718	–3
		2	1.985	–1
		4	4.031	+1
		8	8.157	+2
		16	16.54	+3
	January 5, 1998[b]	1	0.9901	–1
		2	2.059	+3
		4	4.109	+3
		8	8.086	+1
		16	15.45	–3
December 29, 1997	December 30, 1997	1	1.022	+2
		2	2.075	+4
		4	4.105	+3
		8	8.098	+1
		16	16.75	+5
	January 16, 1998[b]	1	1.042	+4
		2	2.109	+5
		4	4.110	+3
		8	7.777	–3
		16	16.79	+5

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 2 mg/mL=10 mg/kg, 4 mg/mL=20 mg/kg, 8 mg/mL=40 mg/kg, 16 mg/mL=80 mg/kg, 32 mg/mL=160 mg/kg. For mice, dosing volume=10 mL/kg; 1 mg/mL=10 mg/kg, 2 mg/mL=20 mg/kg, 4 mg/mL=40 mg/kg, 8 mg/mL=80 mg/kg, 16 mg/mL=160 mg/kg.
[b] Animal room samples

Table I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Formamide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
March 7, 2001	March 8-9, 2001	4	3.763	–6
		8	7.768	–3
		16	15.36	–4
	May 2, 2001[b]	4	4.177	+4
		8	8.370	+5
		16	16.63	+4
May 30, 2001	May 30, 2001	4	4.010	0
		8	7.937	–1
		16	16.17	+1
August 22, 2001	August 23, 2001	4	4.024	+1
		8	8.221	+3
		16	15.93	0
	October 12, 2001[b]	4	3.996	0
		8	8.146	+2
		16	16.24	+1
November 14, 2001	November 16-19, 2001	4	3.993	0
		8	8.214	+3
		16	16.23	+1
February 6, 2002	February 7, 2002	4	4.080	+2
		8	8.130	+2
		16	16.12	+1
May 1, 2002	May 3, 2002	4	4.090	+2
		8	8.461	+6
		16	17.08	+7
	June 26, 2002[b]	4	3.831	–4
		8	7.704	–4
		16	15.45	–3
July 24, 2002	July 24, 2002	4	4.019	0
		8	7.990	0
		16	15.96	0
October 16, 2002	October 17, 2002	4	4.088	+2
		8	8.113	+1
		16	16.01	0
January 8, 2003	January 13, 2003	4	3.991	0
		8	8.030	0
		16	16.04	0
	February 28, 2003[b]	4	4.048	+1
		8	8.160	+2
		16	16.35	+2

Table I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Formamide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
August 22, 2001	August 23, 2001	2	2.069	+3
		4	4.024	+1
		8	8.221	+3
	October 12, 2001[b]	2	2.041	+2
		4	3.981	0
		8	8.014	0
November 14, 2001	November 16-19, 2001	2	2.067	+3
		4	3.993	0
		8	8.214	+3
February 6, 2002	February 7, 2002	2	2.099	+5
		4	4.080	+2
		8	8.130	+2
May 1, 2002	May 3, 2002	2	2.113	+6
		4	4.090	+2
		8	8.461	+6
	June 26, 2002[b]	2	1.958	–2
		4	3.876	–3
		8	7.827	–2
July 24, 2002	July 24, 2002	2	2.084	+4
		4	4.019	0
		8	7.990	0
October 16, 2002	October 17, 2002	2	2.059	+3
		4	4.088	+2
		8	8.113	+1
January 8, 2003	January 13, 2003	2	2.024	+1
		4	3.991	0
		8	8.030	0
	February 28, 2003[b]	2	2.095	+5
		4	4.058	+1
		8	8.147	+2
April 2, 2003	April 8, 2003	2	1.980	–1
April 9, 2003	April 9, 2003	4	4.101	+3
		8	8.433	+5
June 25, 2003	June 26, 2003	2	2.071	+4
		4	4.195	+5
		8	8.432	+5

Table I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Formamide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
September 17, 2003	September 17, 2003	2	2.037	+2
		4	4.112	+3
		8	8.252	+3
	October 9, 2003[b]	2	1.991	0
		4	4.045	+1
		8	8.171	+2

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 4 mg/mL=20 mg/kg, 8 mg/mL=40 mg/kg, 16 mg/mL=80 mg/kg. For mice, dosing volume=10 mL/kg; 2 mg/mL=20 mg/kg, 4 mg/mL=40 mg/kg, 8 mg/mL=80 mg/kg.
[b] Animal room samples

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

TABLE J1 Ingredients of NTP-2000 Rat and Mouse Ration 164
TABLE J2 Vitamins and Minerals in NTP-2000 Rat and Mouse Ration 164
TABLE J3 Nutrient Composition of NTP-2000 Rat and Mouse Ration 165
TABLE J4 Contaminant Levels in NTP-2000 Rat and Mouse Ration 166

Table J1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

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

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

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.67	13.3 – 15.7	31
Crude fat (% by weight)	8.1 ± 0.25	7.6 – 8.6	31
Crude fiber (% by weight)	9.0 ± 0.42	8.0 – 9.9	31
Ash (% by weight)	5.2 ± 0.26	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)	4,999 ± 1065	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]	7.6 ± 1.25	5.9 – 11.4	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 (%)	1.011 ± 0.056	0.873 – 1.140	31
Phosphorus (%)	0.607 ± 0.033	0.555 – 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 J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.33 ± 0.152	0.17 – 0.50	31
Cadmium (ppm)	0.05 ± 0.016	0.04 – 0.09	31
Lead (ppm)	0.07 ± 0.025	0.05 – 0.17	31
Mercury (ppm)	<0.02		31
Selenium (ppm)	0.22 ± 0.053	0.14 – 0.36	31
Aflatoxins (ppb)	<5.00		31
Nitrate nitrogen (ppm)[c]	13.8 ± 3.92	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)	25.0 ± 64.0	10.0 – 360.0	31
Coliform (MPN/g)	3.1 ± 0.2	3.0 – 3.6	31
Escherichia coli (MPN/g)	<10		31
Salmonella (MPN/g)	Negative		31
Total nitrosoamines (ppb)[e]	4.3 ± 1.46	2.3 – 8.4	31
N-Nitrosodimethylamine (ppb)[e]	2.6 ± 1.29	1.2 – 6.9	31
N-Nitrosopyrrolidine (ppb)[e]	1.7 ± 0.77	0.9 – 3.2	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	0.123 ± 0.072	0.020 – 0.259	31
Methyl parathion	<0.02		31
Ethyl parathion	<0.02		31
Malathion	0.307 ± 0.430	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 K
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from two male and two female extra rats and mice at the start of the 2-week studies and from five male and five female extra rats and mice at study start and from up to five male and five female sentinel rats and mice at 4 weeks and at termination of the 3-month studies. During the 2-year studies, samples were collected from five male and five female sentinel rats and mice at 1, 6, 12, and 18 months and from up to five male and five female 80 mg/kg rats and mice at the end of the studies. Fecal samples were taken from sentinel mice at 18 months. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and analyzed at the study laboratory or sent to MA Bioservices, Inc. (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

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

Method and Test	Time of Analysis
RATS (continued)	
2-Year Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
RCV/SDA	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, study termination
PVM	Study termination
RCV/SDA	Study termination
MICE	
2-Week Study	
ELISA	
Ectromelia virus	Study start
EDIM (epizootic diarrhea of infant mice)	Study start
GDVII (mouse encephalomyelitis virus)	Study start
LCM (lymphocytic choriomeningitis virus)	Study start
Mouse adenovirus	Study start
MHV (mouse hepatitis virus)	Study start
PVM	Study start
Reovirus 3	Study start
Sendai	Study start
Hemagglutination Inhibition	
K (papovavirus)	Study start
MVM (minute virus of mice)	Study start
Polyoma virus	Study start
3-Month Study	
ELISA	
Ectromelia virus	Study start, 1 month, study termination
EDIM	Study start, 1 month, study termination
GDVII	Study start, 1 month, study termination
LCM	Study start, 1 month, study termination
Mouse adenoma virus	Study start, 1 month, study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV	Study start, 1 month, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study start, 1 month, study termination
Reovirus 3	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination

Method and Test	Time of Analysis
MICE (continued)	
3-Month Study (continued)	
Immunofluorescence Assay	
Mouse adenoma virus	Study start
MCMV	Study termination
Parvovirus	Study start, 1 month, study termination
Reovirus 3	Study start
2-Year Study	
ELISA	
Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM	1, 6, 12, and 18 months, study termination
GDVII	1, 6, 12, and 18 months, study termination
LCM	1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	1, 6, 12, and 18 months, study termination
MCMV	Study termination
MHV	1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus 3	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Ectromelia virus	Study termination
EDIM	18 months
GDVII	1 month
LCM	18 months and study termination
MCMV	Study termination
Parvovirus	1, 6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter spp. (fecal)	18 months

RESULTS

All test results were negative.