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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Tetralin (CAS No. 119-64-2) in F344/N Rats and B6C3F1 Mice and a Toxicology Study of Tetralin in Male NBR Rats (Inhalation Studies)

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRALIN

(CAS NO. 119-64-2)

IN F344/N RATS AND B6C3F1 MICE

AND A TOXICOLOGY STUDY OF TETRALIN IN MALE NBR RATS

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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FOREWORD

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

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

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

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SUMMARY

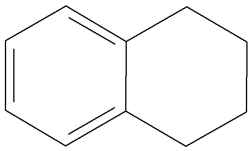
Background
Tetralin is widely used as a solvent for naphthalene, waxes, resins, oils, fats, polishes, and cleaning products. We studied tetralin to determine if it caused cancer in rats or mice.

Methods
We exposed groups of 50 male and female rats and mice to air containing 30, 60, or 120 parts per million (ppm) tetralin six hours per day for two years. Similar groups of 50 animals were exposed to clean air in the same inhalation chambers six hours per day as the untreated control groups. Tissues from more than 40 sites were examined for every animal.

Results
Survival and body weights of rats and mice were not adversely affected by exposure to tetralin. Male rats exposed to tetralin had higher rates of tumors of the kidney, and female rats exposed to decalin had increased rates of rare tumors in the liver and polyps in the uterus. There were also slightly increased rates of testicular tumors in male rats and hemangiosarcomas of the spleen in female mice. In all groups of male and female rats and mice exposed to tetralin by inhalation there was extensive hyperplasia and metaplasia of the epithelium of the nose. All male and female mice exposed to tetralin had granules in the urinary bladder.

Conclusions
We conclude that tetralin caused cancer of the kidney in male rats and of the liver and uterus in female rats. Increases in testicular tumors in male rats and hemangiosarcomas of the spleen in female mice may have been related to exposure to tetralin. There was no evidence that tetralin increased tumor rates in male mice. Noncancerous lesions in the nose in all groups of exposed animals and of the urinary bladder in all exposed mice were attributed to exposure to tetralin.

ABSTRACT



TETRALIN

CAS No. 119-64-2

Chemical Formula: $C_{10}H_{12}$ Molecular Weight: 132.21

Synonyms: Benzocyclohexane; $\Delta^{5,7,9}$-naphthalene; naphthalene 1,2,3,4-tetrahydride; tetrahydronaphthalene; 1,2,3,4-tetrahydronaphthalene; tetraline

Trade name: Tetranap

Tetralin is used as an industrial solvent primarily for naphthalene, fats, resins, oils, and waxes; as a solvent and stabilizer for shoe polishes and floor waxes; as a solvent for pesticides, rubber, asphalt, and aromatic hydrocarbons (e.g., anthracene); as a dye solvent carrier in the textile industry; as a substitute for turpentine in lacquers, paints, and varnishes; in paint thinners and as a paint remover; in alkali-resistant lacquers for cleaning printing ink from rollers and type; as a constituent of motor fuels and lubricants; for the removal of naphthalene in gas distribution systems; and as an insecticide for clothes moths. Tetralin was nominated by the National Cancer Institute for carcinogenicity and disposition studies because of its structure, high production volume, and high potential for worker and consumer exposure. Male and female F344/N rats and B6C3F1 mice were exposed to tetralin (at least 97% pure) by inhalation for 2 weeks, 3 months, or 2 years; male NCI Black Reiter (NBR) rats were exposed to tetralin by inhalation for 2 weeks. Male NBR rats do not produce α2u-globulin; the NBR rats were included to study the relationship of α2u-globulin and renal lesion induction. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

2-Week Studies in Rats

Groups of five male (F344/N and NBR) and five female (F344/N) rats were exposed to tetralin at air concentrations of 0, 7.5, 15, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 12 exposures. All rats survived to the end of the studies. The final mean body weight of female rats exposed to 120 ppm and mean body weight gains of female rats exposed to 30 ppm or greater were significantly less than those of the chamber controls. Final mean body weights of exposed groups of male NBR rats and mean body weight gains of all exposed groups of male rats were significantly less than those of the chamber controls. Dark-

stained urine was observed in all 120 ppm rats. Squinting, weeping, or matted fur around the eyes were noted in the majority of F344/N rats exposed to 120 ppm. The α2u-globulin concentrations in the kidney of male F344/N rats were significantly greater in all exposed groups than in the chamber control group. The absolute kidney weight of 60 ppm females and the relative kidney weights of male F344/N rats exposed to 30 ppm or greater and female rats exposed to 15 ppm or greater were significantly increased. The absolute liver weight of 120 ppm NBR male rats and the relative liver weights of male and female rats exposed to 60 or 120 ppm were significantly increased. In the nose, the incidences of mononuclear cell cellular infiltration were generally significantly increased in all exposed groups of rats, and incidences of olfactory epithelium degeneration and glandular hypertrophy occurred in all male F344/N rats exposed to 120 ppm.

2-Week Study in Mice

Groups of five male and five female mice were exposed to tetralin at air concentrations of 0, 7.5, 15, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 13 exposures. All mice survived to the end of the study. Mean body weights of male and female mice were similar to those of the chamber controls. Dark-stained urine was observed in most of the exposed mice. The absolute and relative liver weights of 60 and 120 ppm males and 30 and 120 ppm females and the relative liver weights of 60 ppm females were significantly greater than those of the chamber controls. In the nose, the incidences of olfactory epithelium atrophy were significantly increased in 60 and 120 ppm males and females. Glandular dilatation occurred in all 120 ppm females, and glandular hyperplasia occurred in all 120 ppm males and females.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed to tetralin at air concentrations of 0, 7.5, 15, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. The same exposure concentrations were given to additional groups of 10 male and 10 female clinical pathology study rats for up to 6 weeks and five male renal toxicity study rats for 2 weeks. All rats survived to the end of the study. During the first 4 weeks of exposure, dark-stained urine was observed in the catch pans of rats exposed to 30, 60, or 120 ppm. Tetralin induced a minimal decrease in the erythron in both sexes that resulted in a hematopoietic response. Tetralin increased urine aspartate aminotransferase and urine lactate dehydrogenase activities (males and females) and glucose/creatinine ratio (males), suggestive of renal injury. The absolute kidney weights of 60 and 120 ppm females and the relative kidney weights of males and females exposed to 15 ppm or greater were significantly greater than those of the chamber controls. Concentrations of α2u-globulin in the kidney of exposed male rats were generally greater than those of the chamber controls at all time points and greater at 6 and 14 weeks than at 2 weeks. There were significantly increased incidences of olfactory epithelium necrosis in rats exposed to 30 ppm or greater and of olfactory epithelium regeneration in 60 and 120 ppm rats.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed to tetralin at air concentrations of 0, 7.5, 15, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. All mice survived to the end of the study. Mean body weights of 120 ppm males were significantly less than those of the chamber controls. Dark-stained urine was observed in the catch pans of mice exposed to 30, 60, or 120 ppm during the first month of the study. Tetralin induced a minimal decrease in the erythron in both sexes that resulted in a hematopoietic response. The relative liver weights of 120 ppm males and 30 ppm or greater females were significantly greater than those of the chamber controls. Incidences of olfactory epithelium metaplasia in 60 and 120 ppm males and females, respiratory epithelium hyaline droplet accumulation in 120 ppm males and 60 and 120 ppm females, cytoplasmic eosinophilic granules within the transitional epithelium lining the urinary bladder in all exposed groups of males and females, and ovarian atrophy and uterine atrophy in 60 and 120 ppm females were significantly increased.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to tetralin at air concentrations of 0, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week

for 105 weeks. Additional groups of five male and five female rats were exposed to the same concentrations for 12 months. Survival of all exposed groups of rats was similar to that of the chamber controls. Mean body weights of 120 ppm females were 6% less than those of the chamber controls after week 29. Dark-stained urine was observed in all exposed groups of rats. Creatinine-adjusted levels of all urinary metabolites increased with increasing exposure concentration in males and females.

In the standard evaluation of the kidney, there were slightly increased incidences of cortical renal tubule adenoma in male rats. In the combined analysis of single and step sections, the incidence of cortical renal tubule adenoma was significantly increased in the 120 ppm group. In the combined analysis, there was also a significantly increased incidence of renal tubule hyperplasia in the 120 ppm group. In 120 ppm males in the standard evaluation, the severity of chronic nephropathy was increased and the incidence of transitional epithelial hyperplasia in the renal pelvis was significantly increased.

Three hepatocellular adenomas occurred in 120 ppm females, and one hepatocellular carcinoma each was observed in the 60 and 120 ppm groups.

The incidences of uterine stromal polyp and endometrium hyperplasia were significantly increased in 120 ppm females. Incidences of interstitial cell adenoma and germinal epithelium atrophy of the testis in 30 and 120 ppm males were significantly greater than those in the chamber controls.

The incidences of olfactory epithelium degeneration, metaplasia, basal cell hyperplasia, suppurative inflammation, and mineralization (except 30 ppm females) in the nose were significantly increased in all exposed groups of rats. The incidences of glandular dilatation were significantly increased in 120 ppm males and all exposed groups of females. The incidences of respiratory epithelium chronic inflammation were significantly increased in males exposed to 60 or 120 ppm and all exposed groups of females.

The incidences of lens cataract in 120 ppm females were significantly increased.

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to tetralin at air concentrations of 0, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Additional groups of five male and five female mice were exposed to the same concentrations for 12 months. Survival of 60 and 120 ppm female mice was significantly greater than that of the chamber controls. The mean body weights of all exposed groups of male and female mice were similar to those of the chamber controls by the end of the study. Dark-stained urine was observed in all exposed groups of male mice and in females exposed to 60 or 120 ppm. Creatinine-adjusted levels of all urinary metabolites increased with increasing exposure concentration in males and females.

The incidence of hemangiosarcoma of the spleen was increased in 120 ppm females and exceeded the historical control range for inhalation studies.

The incidences of olfactory epithelium atrophy, respiratory metaplasia, glandular hyperplasia, and suppurative inflammation in exposed groups of mice were significantly greater than those in the chamber controls. Transitional epithelium cytoplasmic eosinophilic granules were present in the urinary bladder of all exposed mice. The incidence of corneal mineralization in 120 ppm females was significantly increased.

Genetic Toxicology

Tetralin was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 or in *E. coli* strain WP2 *uvrA*, with or without exogenous metabolic activa-

tion. No significant increases in the frequencies of micronucleated normochromatic erythrocytes, indicators of chromosomal damage, were observed in peripheral blood samples from male or female mice exposed to tetralin for 3 months.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of tetralin in male F344/N rats based on the increased incidence of cortical renal tubule adenoma. The increased incidence of testicular interstitial cell adenoma may have been related to tetralin exposure. There was *some evidence of carcinogenic activity* of tetralin in female F344/N rats based on the increased incidences of hepatocellular neoplasms and uterine stromal polyp. There was *no evidence of carcinogenic activity* of tetralin in male B6C3F1 mice exposed to 30, 60, or 120 ppm. There was *equivocal evidence of carcinogenic activity* of tetralin in female B6C3F1 mice based on the increased incidence of splenic hemangiosarcoma.

Exposure to tetralin resulted in nonneoplastic lesions of the nose in male and female rats and mice, kidney and testis in male rats, uterus in female rats, and urinary bladder in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13.

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in air	0, 30, 60, or 120 ppm	0, 30, 60, or 120 ppm	0, 30, 60, or 120 ppm	0, 30, 60, or 120 ppm
Body weights	120 ppm group 6% less than the chamber control group after week 29	120 ppm group 5% less than the chamber control group after week 29	120 ppm group 9% less than the chamber control group after week 29	Exposed groups generally similar to the chamber control group
Survival rates	20/50, 29/50, 25/50, 28/50	31/50, 36/50, 31/50, 38/50	36/50, 35/50, 38/50, 36/50	31/50, 38/50, 42/50, 43/50
Nonneoplastic effects	Kidney: renal tubule, hyperplasia (standard evaluation - 1/50, 2/50, 0/50, 3/50; standard and extended evaluations combined - 1/50, 2/50, 1/50, 7/50); severity of nephropathy (2.6, 3.0, 3.0, 3.4); pelvis, transitional epithelium, hyperplasia (1/50, 1/50, 0/50, 7/50) Testis: germinal epithelium, atrophy (32/50, 42/50, 34/50, 45/50) Nose: glands, dilatation (0/50, 3/50, 3/49, 16/50); olfactory epithelium, degeneration (1/50, 40/50, 43/49, 42/50); olfactory epithelium, hyperplasia, basal cell (0/50, 38/50, 48/49, 48/50); olfactory epithelium, metaplasia (0/50, 17/50, 31/49, 37/50); olfactory epithelium, inflammation, suppurative (0/50, 12/50, 8/49, 10/50); olfactory epithelium, mineralization (0/50, 5/50, 12/49, 17/50); respiratory epithelium, inflammation, chronic (4/50, 4/50, 18/49, 16/50)	Uterus: endometrium, hyperplasia (2/50, 5/50, 7/50, 11/50) Nose: glands, dilatation (0/50, 6/50, 10/50, 16/50); olfactory epithelium, degeneration (0/50, 47/50, 50/50, 46/50); olfactory epithelium, hyperplasia, basal cell (0/50, 48/50, 50/50, 49/50); olfactory epithelium, metaplasia (0/50, 41/50, 43/50, 49/50); olfactory epithelium, inflammation, suppurative (0/50, 16/50, 15/50, 19/50); olfactory epithelium, mineralization (0/50, 2/50, 8/50, 13/50); respiratory epithelium, inflammation, chronic (1/50, 7/50, 11/50, 12/50)	Nose: glands, olfactory epithelium, hyperplasia (14/49, 49/49, 50/50, 49/50); olfactory epithelium, atrophy (2/49, 49/49, 50/50, 50/50); olfactory epithelium, metaplasia, respiratory (2/49, 47/49, 50/50, 49/50); inflammation, suppurative (2/49, 26/49, 45/50, 45/50) Urinary bladder: transitional epithelium, eosinophilic granules, cytoplasmic (0/49, 47/47, 50/50, 48/48)	Nose: glands, olfactory epithelium, hyperplasia (17/50, 50/50, 50/50, 49/49); olfactory epithelium, atrophy (1/50, 50/50, 50/50, 49/49); olfactory epithelium, metaplasia, respiratory (1/50, 49/50, 50/50, 49/49); inflammation, suppurative (3/50, 28/50, 48/50, 46/49) Urinary bladder: transitional epithelium, eosinophilic granules, cytoplasmic (0/49, 50/50, 49/49, 49/49)
Neoplastic effects	Kidney: cortical renal tubule adenoma (standard evaluation - 0/50, 1/50, 1/50, 2/50; standard and extended evaluations combined - 0/50, 3/50, 2/50, 6/50)	Liver: hepatocellular adenoma (0/50, 0/50, 0/50, 3/50); hepatocellular adenoma or carcinoma (0/50, 0/50, 1/50, 4/50) Uterus: stromal polyp (6/50, 10/50, 9/50, 17/50)	None	None

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Equivocal findings	Testis: adenoma, interstitial cell (29/50, 39/50, 31/50, 41/50)	None	None	Spleen: hemangiosarcoma (1/50, 0/50, 1/50, 4/50)
Level of evidence of carcinogenic activity	Some evidence	Some evidence	No evidence	Equivocal evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:		Negative in strains TA97, TA98, TA100, and TA1535 and in *Escherichia coli* strain WP2 *uvrA*/pKM101, with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative in males and females		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on tetralin on February 25, 2009, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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Watertown, MA

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 25, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of tetralin received public review by the National Toxicology Program's Board of Scientific Counselors Technical Report Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of tetralin by describing the uses, structure, and metabolism of the chemical, the design of the short- and long-term studies, the body weights, clinical signs, and nonneoplastic lesions in the short-term studies, and neoplasms and nonneoplastic lesions in the two-year study. The proposed conclusions were:

> Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of tetralin in male F344/N rats based on the increased incidence of cortical renal tubule adenoma. The increased incidence of testicular interstitial cell adenoma may have been related to tetralin exposure. There was *some evidence of carcinogenic activity* of tetralin in female F344/N rats based on the increased incidences of hepatocellular neoplasms and uterine stromal polyp. There was *no evidence of carcinogenic activity* of tetralin in male B6C3F1 mice exposed to 30, 60, or 120 ppm. There was *equivocal evidence of carcinogenic activity* of tetralin in female B6C3F1 mice based on the increased incidence of splenic hemangiosarcoma.
>
> Exposure to tetralin resulted in nonneoplastic lesions of the nose in male and female rats and mice, kidney and testis in male rats, uterus in female rats, and urinary bladder in male and female mice.

Dr. Nagarkatti asked if the elevation in aspartate transaminase activity indicated liver toxicity rather than kidney toxicity. Dr. G. Travlos, NIEHS, replied that the aspartate transaminase measurements in this study were from urine, not serum.

Dr. Portier, the first primary reviewer, asked if the somewhat lower survival in the control female mice reduced the power of the study and impacted the ability to formulate a conclusion. He also inquired about the justification for the statement that testicular interstitial cell adenomas in male rats may have been related to tetralin administration. Dr. Chan explained that the statement 'may have been related' corresponded to an equivocal finding. Dr. Portier felt the incidences for this lesion represented background variation, as the concurrent control value was extremely low. Dr. Grace Kissling, NIEHS, said the statistical significance for the testicular interstitial cell adenomas would have remained even if all the control animals had survived to the end of the study.

Dr. Sherley, the second primary reviewer, inquired if the presence of decalin contamination in the tetralin test material could have contributed to the observed neoplasms. He suggested that more discussion be provided regarding the concentration of decalin in the exposure chamber. He also noted that for male rats, there were significant trends for increases in some skin lesions, although none were significant by pairwise comparison, and suggested these might constitute equivocal evidence of carcinogenic activity. He suggested this finding be added to the results section and addressed in the discussion section.

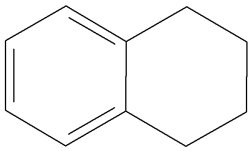
Dr. Chan replied that further analysis indicated less than 0.1% of the test material in the 2-year studies of tetralin was decalin, and in the NTP studies of decalin there were no neoplastic responses from exposure to 25 ppm. Regarding the skin neoplasms in male rats, Dr. Chan noted that when the squamous cell neoplasms and the basal cell neoplasms were combined, the statistical significance of the trend disappeared.

Dr. Cattley, the third primary reviewer, inquired why the extended evaluation of the kidney was performed in male rats but not in females. He noted that the stromal polyps were benign, with no evidence of progression to stromal sarcoma. Dr. Ronald Herbert, NIEHS, explained that step sections normally are performed when there is some hint of lesions in the initial examination. Although there was little indication of kidney lesions in the female rats, step sections of the female kidneys were also performed more recently and confirmed there was no effect. Those data would be added to the final report.

Dr. Portier suggested that the statement about testicular tumors in male rats be removed from the conclusions.

Dr. Paul Foster, NIEHS, explained that the observed atrophy of the seminal tubules could be linked to the occurrence of the testicular tumors. Dr. Portier agreed and withdrew his suggestion. Dr. Sherley again raised the question of whether the skin lesions might be considered equivocal evidence. Dr. Kissling provided the historical background rates for the skin lesions and said the papillomas fell into the historical range for inhalation studies. After further discussion it was agreed that the skin lesions would be mentioned in the results text but not in the conclusions. Dr. Cattley suggested that the liver neoplasms in female rats be identified as adenomas and adenomas or carcinomas combined. Dr. Eastmond moved and Dr. Pino seconded that the conclusions be accepted with the proposed revision. The motion was approved with 7 yes votes, 1 no vote (Dr. Sherley), and 0 abstentions. Dr. Sherley voted no because he thought that squamous cell papillomas might be related to tetralin exposure and that this statement should be added to the conclusions.

INTRODUCTION



TETRALIN

CAS No. 119-64-2

Chemical Formula: $C_{10}H_{12}$ Molecular Weight: 132.21

Synonyms: Benzocyclohexane; $\Delta^{5,7,9}$-naphthalene; naphthalene 1,2,3,4-tetrahydride; tetrahydronaphthalene; 1,2,3,4-tetrahydronaphthalene; tetraline

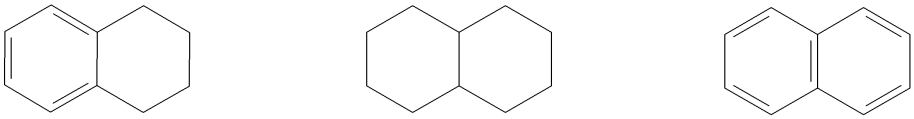
Trade name: Tetranap

Chemical and Physical Properties

Tetralin is a liquid with an odor resembling that of a mixture of benzene and menthol. It has a boiling point of 207.2° C at 760 mm Hg and a melting point of –31° C. It is insoluble in water, soluble in methanol at 50.6% (w/w), and miscible with petroleum ether, chloroform, decalin, ethanol, butanol, acetone, benzene, and ether (Merck, 1996). It has a vapor pressure of 1 mm Hg at 38° C (Sax and Lewis, 1989), flash points of 77° C (open cup) and 82° C (closed cup), a specific gravity of 0.9702 at 20° C, and a log octanol/water partition coefficient of 3.52 (Merck, 1996). Tetralin reacts with oxidizing materials; in prolonged, direct contact with air, it forms tetralin peroxide, which may lead to explosion; tetralin peroxide formation is prevented by addition of an antioxidant such as hydroquinone (Merck, 1996). Tetralin is combustible when exposed to heat or flame and emits acrid smoke and irritating fumes when heated to decomposition (Sax and Lewis, 1989).

Tetralin, decalin, and naphthalene each contain 10 carbon atoms and are composed of two fused, six-membered rings (Figure 1). However, the structural and electronic character of the molecules differ. Structurally, the aromatic ring of tetralin causes that part of the molecule to be planar, while the aliphatic portion of the molecule remains nonplanar. The *cis* and *trans* isomers of decalin are each composed of two fused cyclohexane rings that exist in nonplanar chair configurations. Electronically, the aromatic ring of tetralin will activate the α-carbons toward oxidation.

While Tetralin® and Decalin® are trade names (E.I. du Pont de Nemours & Company, Wilmington, DE) for tetrahydro- and decahydronaphthalene products,



Tetralin Decalin Naphthalene

FIGURE 1
Chemical Structures of Tetralin, Decalin, and Naphthalene

respectively, the names are commonly used for any tetrahydro- and decahydronaphthalene. This Technical Report follows common usage.

PRODUCTION, USE, AND HUMAN EXPOSURE

Tetralin occurs naturally in petroleum and coal and is released into the environment in emissions from petroleum refining, coal tar distillation, and gasoline and diesel fuel combustion; it also is released in waste streams in the disposal of crude oil, refined petroleum products, and products containing the compound as a solvent (e.g., paints and waxes).

Tetralin is manufactured by hydrogenation of naphthalene in the presence of a nickel or modified nickel catalyst. Because these catalysts are sensitive to sulfur, naphthalene with a low sulfur content is used. The primary naphthalene-sulfur compound formed during catalysis is thionaphthene, and it is removed by sodium treatment and a catalytic hydrodesulfurization process. Tetralin marketed by Union Carbide contains 98% tetrahydronaphthalene with naphthalene making up most of the remainder (CRCS, 1984). DuPont's Tetralin® contains 97% tetrahydronaphthalene, 2% decahydronaphthalene, and 1% naphthalene (DuPont, 2005). The Aldrich Chemical Company offers tetralin at 99% purity (*Aldrich*, 1990).

Tetralin is in high demand with annual usage in the millions of pounds, but a quantitative estimate of current domestic production of tetralin was not found in a search of the literature.

Tetralin is used as an industrial solvent primarily for naphthalene, fats, resins, oils, and waxes; as a solvent and stabilizer for shoe polishes and floor waxes; as a solvent for pesticides, rubber, asphalt, and aromatic hydrocarbons (e.g., anthracene); as a dye solvent carrier in the textile industry; as a substitute for turpentine in lacquers, paints, and varnishes; in paint thinners and as a paint remover; in alkali-resistant lacquers for cleaning printing ink from rollers and type; as a constituent of motor fuels and lubricants; for the removal of naphthalene in gas distribution systems; as an insecticide for clothes moths and a larvicide for mosquitoes; and as an intermediate in the manufacture of certain agricultural chemicals such as carbaryl, napropamide, and 1-naphthoxyacetic acid. Tetralin mixed with decalin is used for certain applications where a synergistic solvency is desired (Sandmeyer, 1981; Longacre, 1987; Sax and Lewis, 1989).

The most probable human exposure to tetralin is through dermal contact or inhalation during manufacture or use. Potential occupational exposures are controlled by the use of engineering controls (for example, the threshold limit value 8-hour time-weighted average concentration for the reactant naphthalene is 10 ppm; ACGIH, 2007) and the routine use of personal protective equipment. DuPont, the major manufacturer, also recommends that the compound be handled in closed systems where possible or in work areas with good ventilation (DuPont, 2005). Based on data collected from 1972 to 1974, the National Occupational Hazard Survey (NOHS) estimated that 2,237 workers were potentially exposed to tetralin (NIOSH, 1976). The 1981 to 1983 National Occupational Exposure Survey (NOES) reported 504 workers potentially exposed to tetralin (NIOSH, 1990). The NOES estimate represents actual observations only (i.e., the surveyor observed the use of the specific compound), whereas the NOHS estimate is made up of actual observations, trade name observations (the surveyor observed the use of a trade name product known to contain the compound), and generic observations (the surveyor observed a product in some type of general use which led the National Institute for Occupational Safety and Health to suspect that the compound might be contained in that product).

A study at a small pilot-scale direct coal liquefaction facility in British Columbia detected tetralin at a mean concentration of 0.07 mg/m^3 in 11/58 samples of workplace air. The limit of detection was 0.05 mg/m^3 (Leach *et al.*, 1987).

Consumers may be exposed to tetralin used as a solvent in paints, varnishes, lacquers, waxes, and shoe polishes and in finished petroleum products (gasoline, motor oils). In addition, nonoccupational exposure to tetralin may occur in urban atmospheres, through contaminated drinking water supplies, and during recreational activities at contaminated waterways. Tetralin has been detected at 100 ppb in a pond water sample obtained in an uninhabited, forested area in central New Brunswick in May 1977 (CRCS, 1984).

Regulatory Status

No standards or guidelines have been set for occupational exposures or environmental levels of tetralin.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Tetralin undergoes hydroxylation at the nonaromatic portion of the molecule in rats and rabbits. Tetralin metabolites were identified in the urine of male Fischer 344 rats administered 48.5 mg tetralin/kg body weight intragastrically on alternate days for 2 weeks (Servé *et al.*, 1989). The metabolites were monoalcohols (1-tetralol and 2-tetralol), hydroxyketones (2-hydroxy-1-tetralone and 4-hydroxy-1-tetralone), and diols (tetralin-1,4-diol and tetralin-1,2-diol). These metabolites were excreted as glucuronic acid or sulfate conjugates. In rats administered 45 mg tetralin/kg body weight intraperitoneally, biliary excretion amounted to 13% of the administered dose mainly as the glucuronide of tetralin-1,2-diol. Information on metabolism of tetralin in female rats was not found in a search of the literature.

A study on the hydroxylation of tetralin by rat liver homogenates indicated that hydroperoxide was an intermediate in the conversion of tetralin to tetralol (Chen and Lin, 1968).

In rabbits administered 3.4 mmol/kg ^{14}C-tetralin via a stomach tube, 87% to 90% of the dose was excreted in the urine within 2 days, 0.6% to 1.8% in feces, and less than 0.2% in expired breath (Elliott and Hanam, 1968). The radioactive residue in tissues amounted to 0.07% of the administered dose. The major metabolites were conjugates of 1-tetralol (52.4%) and 2-tetralol (25.3%). Minor metabolites were *cis*-tetralin-1,2-diol (0.4%), *trans*-tetralin-1,2-diol (0.6%), and 4-hydroxy-1-tetralone (6.1%). Traces of mercapturic acids were found that did not appear to originate from the tetralin that was administered (Longacre, 1987).

Humans

Unchanged tetralin, 1-tetralol, and the glucuronides of 1-tetralol and 2-tetralol were identified in the urine of a woman who had ingested 250 mL of an ectoparasiticide containing 31.5% tetralin (Longacre, 1987).

Hansen and Andersen (1988) estimated the affinity of tetralin in several biological materials using solubility parameter techniques. Results were reported as relative energy difference (RED) numbers; values approaching zero indicated strongest affinity; values less than 1.0 indicated a strong affinity, and progressively higher values indicated increasingly lower affinities. The RED was 0.65 in lard at 37° C, 0.52 in lard at 23° C, 1.36 in a 1% solution of tetralin in water, 1.73 in blood serum, 1.78 in sucrose, 1.49 in urea, and 0.90 in psoriasis scales.

Toxicity

Experimental Animals

The oral LD_{50} for tetralin in rats is 2.86 g/kg; the dermal LD_{50} in rabbits is 17.3 g/kg, and the LC_{10} in guinea pigs for 17 8-hour exposures is 275 ppm (CRCS, 1984; Longacre, 1987). Exposure to saturated vapor for 8 hours was not lethal to rats (Sandmeyer, 1981). Clinical signs of acute toxicity are loss of weight, tremors, paralysis of the hindquarters, and difficult respiration (Longacre, 1987).

Intragastric administration of 485 mg tetralin/kg body weight on alternate days for 2 weeks induced nephrotoxicity in male Fischer 344 rats (Servé *et al.*, 1989). The dose was considered the highest dose tolerated without lethal side effects. Toxicity in female rats was not studied. At necropsy, increased amounts of cytoplasmic hyaline droplets were found in proximal convoluted tubule epithelial cells and foci of cellular degeneration were found in the proximal convoluted tubules.

Administration of tetralin to rats and guinea pigs caused a green coloration of the urine (Longacre, 1987). The significance of green urine was not clear. Other toxic effects of tetralin reported in animals include methemoglobinemia in cats and anemia, leucopenia, hyperemia, and fatty degeneration and centrilobular atrophy of the liver in guinea pigs.

Humans

Tetralin is irritating to the eyes, skin, and mucous membranes and is known to produce nausea, vomiting, intragastric discomfort, transient liver damage, green-gray urine, and some clinical and enzymatic changes. It is also a central nervous system depressant at high concentrations and has been reported to cause dermatitis in painters (Sandmeyer, 1981).

Several case studies on the acute effects of tetralin have been reported. Tetralin has been associated with restlessness of babies sleeping in a room recently treated with a tetralin-based varnish and asthenia in persons sleeping in rooms that had been waxed with a tetralin-containing polish. Temporary liver and kidney damage has been reported following ingestion of approximately 250 mL of Cuprex®, an ectoparasiticide containing 31.5% tetralin, 0.03% copper oleate, 52.7% paraffin oil, and 15.7% acetone (Longacre, 1987).

Oral ingestion of tetralin by humans has resulted in kidney damage (Sandmeyer, 1981). The mechanism of inducing kidney damage is not known. No hyaline droplets have been reported in patients exposed to tetralin or decalin.

Reproductive and Developmental Toxicity

No information on the reproductive or developmental toxicity of tetralin in experimental animals or humans was found in a review of the literature.

Carcinogenicity

Experimental Animals

No studies of the carcinogenicity of tetralin in experimental animals were found in a review of the literature.

The NTP conducted a 2-year inhalation study of naphthalene at 0, 10, or 30 ppm in B6C3F1 mice (NTP, 1992). In this study, there was no evidence of carcinogenicity in male mice and some evidence of carcinogenicity in female mice based on occurrences of alveolar/bronchiolar adenoma. The NTP subsequently conducted a 2-year inhalation study of naphthalene in F344/N rats exposed to 0, 10, 30, or 60 ppm, and there was clear evidence of carcinogenicity in males and females based on increased incidences of respiratory epithelial adenoma and olfactory neuroblastoma of the nose (NTP, 2000).

The NTP conducted 2-year inhalation studies of decalin in F344/N rats at 0, 25, 50 (males only), 100, or 400 ppm and in B6C3F1 mice at 0, 25, 100, or 400 ppm (NTP, 2005a). In these studies, there was clear evidence of carcinogenic activity in male F344/N rats based on increased incidences of renal tubule neoplasms, no evidence of carcinogenic activity in female F344/N rats or in male B6C3F1 mice, and equivocal evidence of carcinogenic activity in female B6C3F1 mice based on marginally increased incidences of hepatocellular and uterine neoplasms.

Humans

No epidemiology studies of tetralin were found in a review of the literature.

Genetic Toxicity

No information on the genetic toxicity of tetralin was found in a review of the literature.

Study Rationale

Tetralin is widely used in solvents and as a substitute for turpentine in the manufacture of paints, lacquers, waxes, and polishes. In addition, it has specific secondary uses. It is found in indoor and outdoor air, workplaces, homes, fuels, exhaust air, drinking water, waterways, and recreational facilities. A high potential for human inhalation or dermal exposure to tetralin exists as a result of contact with naturally occurring crude oil, cigarette smoke, or other combustion products; during manufacturing or solvent uses; or because of environmental releases. Tetralin was studied by the NTP because of its structural similarity to decalin, high production volume, and high potential for worker and consumer exposure.

MATERIALS AND METHODS

Procurement and Characterization of Tetralin

Tetralin was obtained from Sigma Aldrich Fluka Bulk Chemicals (St. Louis, MO) in two lots (00822JG and 07808LG) and from Advanced Aromatics, L.P. (Baytown, TX), in one lot (139699). Lots 00822JG and 07808LG were used in the 2-week and 3-month studies as a mixture combined by Research Triangle Institute (Research Triangle Park, NC) and assigned lot number 8359-80-01; lot 139699 was used during the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute, and the study laboratory, Battelle Toxicology Northwest (Richland, WA). The study laboratory also performed stability testing; additional testing was performed by Chemir/Polytech Laboratories, Inc. (St. Louis, MO), Chemir Analytical Services (Maryland Heights, MO), and Galbraith Laboratories, Inc. (Knoxville, TN) (Appendix J). Reports on analyses performed in support of the tetralin studies are on file at the National Institute of Environmental Health Sciences.

All lots of the chemical, a clear, colorless liquid, were identified as tetralin using infrared and proton nuclear magnetic resonance spectroscopy and gas chromatography coupled with mass spectroscopy (GC/MS). The purity of each lot was determined by elemental analyses, GC/MS, and GC with flame ionization detection (FID). Elemental analysis showed good agreement between theoretical and actual percentages for carbon and hydrogen; oxygen, nitrogen, and sulfur content were determined to be less than 0.5%. For lot 139699, Karl Fischer titration indicated a water content of 52 ppm. For lots 00822JG and 07808LG, GC/MS by one system indicated one major peak and no impurities greater than 0.1% of the major peak area; the purity of each lot was determined to be greater than 97%. For combined lot 8359-80-01, GC/FID by one system indicated a major peak and three impurities 0.1% or greater of the total peak area. The overall purity for lots 00822JG, 07808LG, and/or combined lot 8359-80-01 was determined to be greater than 97%. For lot 139699, GC/MS by one system indicated one major peak and six impurities greater than 0.1% of the total peak area. GC/FID by one system indicated a major peak and four impurities greater than 0.1% of the total peak area. GC/FID by another system indicated one major peak and three impurities greater than 0.1% of the total peak area. The overall purity for lot 139699 was determined to be greater than 98%. Potentiometric titration was used to determine the peroxide content of each lot: 7.02 mEq/kg (lot 00822JG), 8.79 mEq/kg (lot 07808LG), and 2.62 mEq/kg (lot 139699). To prevent the formation of hydroperoxides, 4-*tert*-butylcatechol was added to lot 139699 at a concentration of 50 ppm. The concentration was monitored every 6 months during the 2-year studies using high-performance liquid chromatography. When the concentration of 4-*tert*-butylcatechol fell below 30 ppm, it was refortified to approximately 50 ppm.

To ensure stability, the bulk chemical was stored in the original shipping containers (55-gallon metal drums) under a nitrogen headspace at 18° to 23° C. Stability was monitored by the study laboratory during the 2-week, 3-month, and 2-year studies using GC/FID. No degradation of the chemical occurred.

Vapor Generation and Exposure System

Preheated tetralin was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor out of the generator. Generator output was controlled by the delivery rate of the chemical metering pump.

Vapor leaving the generator was transported to the exposure room at an elevated temperature to prevent condensation. In the exposure room, the vapor was mixed with additional heated compressed air before entering a short vapor distribution manifold. Concentration in the mani-

fold was determined by the chemical pump rate, generator nitrogen flow rate, and dilution air flow rate. The pressure in the distribution manifold was kept fixed to ensure constant flows through the manifold and into the chambers.

An electronically actuated metering valve controlled the flow to each chamber; a pneumatically operated chamber exposure shutoff valve in line with the metering valve stopped flow to the chamber. Until the generation system was stable and exposures were ready to proceed, all chamber exposure valves were closed and vapor was directed to the exposure chamber exhaust. When exposures started, the chambers' exposure valves were opened to allow the vapor to flow through the metering valves and then through temperature-controlled delivery lines to each exposure chamber. The vapor was then injected into the chamber inlet duct where it was further diluted with conditioned chamber air to achieve the desired concentrations.

The study laboratory designed the inhalation exposure chamber (Lab Products, Inc., Seaford, DE) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A condensation particle counter [Type CN, Gardner Associates, Schenectady, NY (2-week and 3-month studies) or Model 3022A, TSI Inc., St. Paul, MN (2-year studies)] was used to count the particles in all chambers before and during generation to determine whether tetralin vapor, and not aerosol, was produced. No particle counts greater than 200 particles/cm^3 were detected.

Vapor Concentration Monitoring

The tetralin concentrations in the exposure chambers were monitored by an online gas chromatograph. Samples were drawn from each exposure chamber approximately every 24 (2-week and 3-month studies) or 26 (2-year studies) minutes during each 6-hour exposure period. A 12- (2-week and 3-month studies) or a 16-port (2-year studies) stream select valve (VALCO Instruments Company, Houston, TX) directed a continuous stream of sampled atmosphere to a 6-port sampling valve (VALCO Instruments Company) with a 1.0 mL sample loop housed in a dedicated valve oven at 280° (2-week and 3-month studies) or 150° C (2-year studies). A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow. Summaries of the chamber vapor concentrations are given in Tables J2, J3, and J4.

The online gas chromatograph was checked throughout the day for instrument drift against an online standard of tetralin in nitrogen supplied by a diffusion standard generator (Kin-Tek Model 491, Precision Calibration Systems, La Marque, TX). The online GC was calibrated monthly by a comparison of chamber concentration data to data from grab samples, which were collected with charcoal sampling tubes (ORBO^{TM}-101, Supelco, Bellefonte, PA). The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. These samples were extracted with toluene containing 1-phenylhexane as an internal standard and analyzed using an offline gas chromatograph. The offline gas chromatograph was calibrated with gravimetrically prepared standard solutions of tetralin containing 1-phenylhexane as an internal standard in toluene.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined without and with animals present in the chambers. At a chamber airflow rate of 15 cfm, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) was approximately 12.5 minutes. A T_{90} value of 12 minutes was selected for all studies.

The uniformity of tetralin vapor concentration in the inhalation exposure chambers without and with animals present in the chambers was measured once during the 2-week and 3-month studies and every 3 months during the 2-year studies using the online gas chromatograph. Chamber concentration uniformity was maintained throughout the studies.

The persistence of tetralin in the chamber after vapor delivery ended was determined by monitoring the concentration without and with animals present in the 120 ppm chambers. In the 2-week and 3-month studies, the concentration decreased to 1% of the target concentration in approximately 118 minutes without animals present and in 106 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of

the target concentration within 48 minutes (rats) and 97 minutes (mice) without animals present and in approximately 164 minutes (rats) and approximately 113 minutes (mice) with animals present.

Stability studies of tetralin in the generation and delivery system were performed. No evidence of degradation was detected, and no impurities were found that were not present in the bulk material. The stability of tetralin in the generator reservoir was monitored during the studies. Tetralin was stable in the generator reservoir for at least 6 months. Stability testing of tetralin in the generator reservoir was performed during the 2-year studies; no evidence of degradation of the test chemical was found. All measurements of 4-*tert*-butylcatechol concentration in exposure chambers and generator reservoir samples were within the required specifications.

2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), and male and female NCI Black Reiter (NBR) rats were obtained from the Frederick Cancer Research and Development Center (Frederick, MD). Male NBR rats do not produce α2u-globulin; the NBR rats were included to study the relationship of α2u-globulin and renal lesion induction. On receipt, the F344/N rats and the mice were approximately 4 weeks old; NBR rats were approximately 5 weeks old. Animals were quarantined for 38 (F344/N rats), 40 (NBR rats), or 12 (mice) days and were approximately 9 (F344/N rats), 10 (NBR rats), or 5 to 6 (mice) weeks old on the first day of the studies. Approximately 4 weeks after receipt and before the studies began, five male F344/N rats and five female NBR rats were selected for parasite evaluation and gross observation for evidence of disease; serum was collected, and serologic analyses were performed using the protocols of the NTP Sentinel Animal Program (Appendix L). Five male and five female mice were selected for parasite evaluation and gross observation for evidence of disease at study termination; serum was collected, and serologic analyses were performed using the protocols of the NTP Sentinel Animal Program.

Groups of five male (F344/N and NBR) and five female (F344/N) rats and groups of five male and five female mice were exposed to tetralin at concentrations of 0, 7.5, 15, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 12 (rats) or 13 (mice) exposures. The 120 ppm concentration was the maximum possible without generating an aerosol. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings for rats and mice were recorded on days 6 and 13 and at terminal sacrifice. The animals were weighed initially, on days 6 and 13, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all animals. The right kidney, liver, and lung 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 5 µm, and stained with hematoxylin and eosin. Histopathologic examinations were performed on all chamber control and 120 ppm animals. In addition to gross lesions, the nose, lung, liver, and kidney were examined to a no-effect level. Table 1 lists the tissues and organs routinely examined.

The right kidney from male F344/N rats was collected and stored at approximately –70° C. Each right kidney was thawed; a volume of sodium/potassium phosphate buffer (pH ~7.2) equivalent to twice the recorded fresh weight of the sample was added, and the sample was homogenized for 30 to 60 seconds using a tissue homogenizer. The homogenate was centrifuged at approximately 3,000 g for 15 minutes. The protein content of each supernatant was measured in a 1:50 dilution in phosphate-buffered saline (PBS)-Tween using the Pyrogallol Red Assay. Analysis of α2u-globulin concentrations in 1:10,000 dilutions in PBS-Tween of the kidney homogenates was conducted using a validated enzyme-linked immunosorbent assay (ELISA) method. The amount of α2u-globulin was measured by comparing the relative fluorescent signal intensity in the study samples to that observed with known amounts of α2u-globulin present in calibration standards. Calibration standards and ELISA control standards (negative and positive) were plated in predetermined wells on 96-well microtiter plates. Calibration standards were assayed in triplicate; study samples were assayed in quadruplicate. Results were reported as ng α2u-globulin/µg soluble protein.

For cell proliferation studies, sections of the left kidney of all male rats were fixed with 10% neutral buffered for-

malin for approximately 24 hours. After fixation, the tissues were processed and embedded in paraffin. A cross-section of duodenum was included in the embedding paraffin as a positive control. The kidney sections were stained with Mallory-Heidenhain or proliferating cell nuclear antigen (PCNA). The slides stained with PCNA were assessed qualitatively for adequate labeling. Evaluation was done using a 40× objective and an ocular grid. Approximately 2,000 proximal tubule nuclei were counted. Counts of labeled nuclei and total nuclei counted were recorded. The labeling index was calculated as the percentage of labeled nuclei/total number of nuclei counted.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetralin and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 14 (males) or 15 (females) days and were approximately 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Serologic analyses were performed on five male and five female sentinel rats and mice during week 1 and on five male and five female chamber control rats and mice at terminal sacrifice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female rats and 10 male and 10 female mice were exposed to tetralin at concentrations of 0, 7.5, 15, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. The same exposure concentrations were given to additional groups of 10 male and 10 female clinical pathology study rats for up to 6 weeks and five male renal toxicity rats for 2 weeks. All animals had at least two consecutive days of exposure before terminal sacrifice. Feed was available *ad libitum* except during exposure and urine collection periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly for core study animals. The core study animals were weighed initially, weekly, and at the end of the studies, and renal toxicity rats were weighed at necropsy. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 23 and from core study rats at the end of the study for hematology and clinical chemistry analyses; blood was collected from the retroorbital sinus of mice at the end of the study for hematology analyses. Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Erythrocyte, leukocyte, and platelet counts; hemoglobin concentrations; packed cell volume; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using a Roche Cobas Helios hematology analyzer (Roche Diagnostics, Branchburg, NJ). Manual hematocrit values were determined using a Damon/IEC MB microcentrifuge (International Equipment Company, Needham Heights, MA) and a Damon/IEC capillary reader (International Equipment Company) for comparison to Cobas values for packed cell volume. Blood smears for rats and mice were stained with Wright-Giemsa stain in a Wescor 7100 Aerospray Slide Stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts for rats and mice were based on classifying a minimum of 100 white cells. Reticulocytes were stained with new methylene blue and enumerated as a reticulocyte:erythrocyte ratio using the Miller disc method (Brecher and Schneiderman, 1950). Blood samples for clinical chemistry analyses were placed in tubes containing separator gel and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Roche Cobas Fara (Roche Diagnostics). Table 1 lists the parameters measured.

During week 12, core study rats were placed in metabolism cages, and urine was collected over ice for 16 hours. During collection, the animals had access to water but not to food. After collection, the volume and specific gravity of the samples were determined and recorded. The urine samples were then centrifuged, and aliquots were collected and analyzed using a Roche Cobas Fara (Roche Diagnostics). Table 1 lists the parameters measured.

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 exposed to 0, 30, 60, or 120 ppm. 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, the left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core and renal toxicity animals. The heart, right kidney, liver, lung, right testis, and thymus of core study animals 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 chamber control and 120 ppm animals. Additional tissues examined in core study animals included the nose in all groups of rats (except 7.5 ppm males) and 30 and 60 ppm mice, the kidney in all male rats, the urinary bladder in all mice, the ovary in 30 and 60 ppm female mice, and the uterus in all female mice; the remaining tissues were examined to a no-effect level in the remaining exposed groups. Table 1 lists the tissues and organs routinely examined.

The right and left kidneys were removed from renal toxicity male rats at 2 weeks, five male clinical pathology study rats at 6 weeks, and five male core study rats at terminal sacrifice for renal toxicity study. The processing of kidneys, tissue staining, determination of α2u-globulin concentrations, and qualitative assessment for adequate labeling for cell proliferation were performed as described for the 2-week studies.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were exposed to tetralin at concentrations of 0, 30, 60, or 120 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks.

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 and mice were quarantined for 12 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 and mice were approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure and urine collection periods; water was available *ad libitum*. Cages, racks, and chambers were changed weekly. Cages were rotated weekly in chambers. Further details of animal

maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies. Animals were weighed initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies.

Five male and five female rats and mice per exposure group were randomly selected for urine collection at 12 months. The animals were placed in metabolism cages, and urine was collected over ice for 16 hours. During collection, the animals had access to water but not to food. After collection, the volume and specific gravity of the samples were determined and recorded. Glucose, bilirubin, ketones, blood, pH, protein, urobilinogen, nitrites, and leukocytes were measured using Bayer Multistix® 9 Reagent Strips (Bayer, Inc., Tarrytown, NY); confirmation of the presence of bilirubin was performed using the Bayer Ictotest® (Bayer, Inc.). The urine samples were then centrifuged, and aliquots were collected and analyzed for creatinine using a Roche Hitachi 911 (Roche Diagnostic Systems, Basel, Switzerland) automated chemistry analyzer. Tetralin metabolites were analyzed using a GC/MS method. Briefly, 50 µL of urine samples were spiked with an internal standard (2.5 µg 1-decalone) and 10 µL β-glucuronidase/arylsulfatase, followed by 0.1 mL acetate buffer (pH 4). Samples were incubated overnight at 37° C. Samples were extracted with ~1 mL methylene chloride, vortexed, and centrifuged; the methylene chloride layer was transferred to an automated liquid sampler vial for analysis by GC/MS. Table 1 lists the parameters measured.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (eyes were fixed in Davidson's solution for up to 72 hours and then transferred to 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 for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. For extended evaluation of renal proliferative lesions, kidneys of male rats were step-sectioned at 1 mm intervals to obtain three to four additional sections from each kidney. Tissues examined microscopically are listed in Table 1.

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

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. 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 Inhalation Studies of Tetralin

	2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory	Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	F344/N rats NBR rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source	Taconic Farms, Inc. (Germantown, NY) (F344/N rats and B6C3F1 mice) Frederick Cancer Research and Development Center (Frederick, MD) (NBR rats)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	Rats: 38 (F344/N) or 40 (NBR) days Mice: 12 days	14 (males) or 15 (females) days	12 days
Age When Studies Began	Rats: 9 to 10 (F344/N) or 11 (NBR) weeks Mice: 5 to 6 weeks	6 weeks	5 to 6 weeks
Date of First Exposure	April 7, 1996	August 19 (males) or 20 (females), 1996	Rats: June 16, 2003 Mice: June 23, 2003
Duration of Exposure	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 12 (rats) or 13 (mice) exposures	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 14 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure	Rats: April 22, 1996 Mice: April 23, 1996	Rats: November 18 (males) or 19 (females), 1996 Mice: November 20 (males) or 21 (females), 1996	Rats: June 15, 2005 Mice: June 23, 2005
Necropsy Dates	Rats: April 23, 1996 Mice: April 24, 1996	Rats: November 19 (males) or 20 (females), 1996 Mice: November 21 (males) or 22 (females), 1996	Rats: June 13-16, 2005 Mice: June 20-24, 2005
Age at Necropsy	Rats: 11 to 12 (F344/N) or 13 (NBR) weeks Mice: 7 to 8 weeks	19 weeks	109 to 111 weeks
Size of Study Groups	Rats: 5 males and 5 females (F344/N) or 5 males (NBR) Mice: 5 males and 5 females	Rats: 10 males and 10 females (core study) 10 males and 10 females (clinical pathology study) 5 males (renal toxicity) Mice: 10 males and 10 females	50 males and 50 females
Method of Distribution	Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetralin

	2-Week Studies	3-Month Studies	2-Year Studies
Animals per Cage	1	1	1
Method of Animal Identification	Tail tattoo	Tail tattoo	Tail tattoo
Diet	NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum* (except during animal exposure periods); changed weekly	Same as 2-week studies, except also not available during urine collection	Same as 3-month studies
Water	Tap water (Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI); available *ad libitum*; changed weekly	Same as 2-week studies	Same as 2-week studies
Cages	Stainless steel, wire bottom (Hazleton Systems, Inc., Aberdeen, MD); changed weekly	Same as 2-week studies	Same as 2-week studies except manufacturer is Lab Products, Inc., Seaford, DE
Chamber Air Supply Filters	Single HEPA (Northland Filter System International, Mechanicville, NY), new at study start; charcoal (RSE, Inc., New Baltimore, MI), new at study start; Purafil (Environmental Systems, Lynnwood, WA), new at study start	Same as 2-week studies	Same as 2-week studies, except single HEPA is open stock
Chambers	Stainless steel, with excreta pan below each cage unit (Lab Products, Inc., Harford Division, Aberdeen, MD); chambers and excreta pans changed weekly	Same as 2-week studies	Same as 2-week studies
Chamber Environment	Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetralin

	2-Week Studies	3-Month Studies	2-Year Studies
Exposure Concentrations	0, 7.5, 15, 30, 60, or 120 ppm	0, 7.5, 15, 30, 60, or 120 ppm	0, 30, 60, or 120 ppm
Type and Frequency of Observation	Observed twice daily; clinical findings were recorded on days 6 and 13 and at terminal sacrifice for rats and mice. The animals were weighed initially, on days 6 and 13, and at the end of the studies.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies, and clinical findings were recorded weekly; renal toxicity rats were weighed at necropsy.	Observed twice daily; clinical findings were recorded every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies. Animals were weighed initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies.
Method of Sacrifice	Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy	Necropsies were performed on all animals. Organs weighed were right kidney, liver, and lung.	Necropsies were performed on core and renal toxicity animals. The heart, right kidney, liver, lung, right testis, and thymus of core study animals were weighed.	Necropsies were performed on all animals.
Clinical Pathology	None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 23 and from core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology. ***Hematology:*** hematocrit; packed cell volume; hemoglobin; erythrocyte, reticulocyte, and platelet counts; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte counts and differentials; and hemolysis (rats) ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetralin

	2-Week Studies	3-Month Studies	2-Year Studies
Histopathology	Histopathology was performed on all chamber control and 120 ppm animals. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: nose, kidney, liver, and lung.	Complete histopathology was performed on core study chamber control and 120 ppm rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to the no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with mainstem bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), 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 following tissues were also examined in core study animals: nose in the remaining groups of rats (except 7.5 ppm males) and 30 and 60 ppm mice, the kidney in all male rats, the urinary bladder in all mice, the ovary in 30 and 60 ppm female mice, and the uterus in all female mice.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with mainstem bronchus), lymph nodes (mandibular, mesenteric, bronchial, mediastinal), 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 male animals in the 0, 30, 60, and 120 ppm groups for sperm motility evaluations. 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 exposed to 0, 30, 60, or 120 ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetralin

	2-Week Studies	3-Month Studies	2-Year Studies
Urinalysis and Urinary Metabolites	None	See below	Five male and five female rats and mice per exposure group were placed in metabolism cages for 16-hour urine collection at 12 months. Urine samples were analyzed for creatinine, glucose, bilirubin, ketones, blood, pH, protein, urobilinogen, nitrites, leukocytes, specific gravity, volume, 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, and 4-hydroxy-1-tetralone
Renal Toxicity Study	At the end of the study, concentrations of α2u-globulin and soluble protein were measured in the right kidney of male F344/N rats; the left kidneys of male F344/N and NBR rats were used for assessment of cell proliferation indices.	At 2 (five male renal toxicity rats) and 6 (five male clinical pathology rats) weeks and the end (five male core study rats) of the study, concentrations of α2u-globulin and soluble protein were measured in the right kidney; the left kidneys were used for assessment of cell proliferation indices. Core study rats were placed in metabolism cages for 16-hour urine collection during week 12, and urine samples were analyzed for creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, γ-glutamyltransferase, *N*-acetyl-β-D-glucosaminidase, volume, and specific gravity.	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, A4, B1, B4, C1, C3, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., Harderian gland, intestine, mammary gland, and skin) before microscopic evalua-

tion, 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 exposed group with controls and a test for an overall exposure-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 exposed 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, urinalysis, renal toxicity, urine metabolites, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. Proportions of regular cycling females in each exposed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

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

Quality Assurance Methods

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

Genetic Toxicology

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

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

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

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (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

2-Week Studies

All rats survived to the end of the studies (Tables 2 and 3). The final mean body weight of female rats exposed to 120 ppm and mean body weight gains of female rats exposed to 30 ppm or greater were significantly less than those of the chamber controls. Final mean body weights of exposed groups of male NBR rats and mean body weight gains of all exposed groups of male rats were significantly less than those of the chamber controls. Dark-stained urine was observed in all 120 ppm rats. On one occasion, dark-stained urine was noted in the catch pans of one 7.5 ppm NBR rat and two 60 ppm NBR rats. Squinting, weeping, or matted fur around the eyes were noted in one NBR rat and the majority of F344/N rats exposed to 120 ppm.

Except in 30 ppm NBR rats, there were no significant differences in the labeling indices in the kidney between chamber control rats and exposed male rats (Table G1). In all exposed groups of male F344/N rats, the α2u-globulin concentrations in the kidney were significantly greater than that in the chamber control group (Tables 4 and G1). The concentration of α2u-globulin was not measured in NBR rats because this strain does not produce appreciable amounts of α2u-globulin.

The absolute kidney weight of 60 ppm females and the relative kidney weights of male F344/N rats exposed to 30 ppm or greater, NBR rats exposed to 7.5 ppm, and female rats exposed to 15 ppm or greater were significantly greater than those of the chamber controls (Tables H1 and H2). The absolute liver weight of 120 ppm NBR male rats and the relative liver weights of male and female rats exposed to 60 or 120 ppm were

TABLE 2
Survival and Body Weights of F344/N Rats in the 2-Week Inhalation Study of Tetralin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	197 ± 6	242 ± 6	45 ± 2	
7.5	5/5	204 ± 3	241 ± 4	38 ± 2*	100
15	5/5	200 ± 3	236 ± 4	37 ± 2*	98
30	5/5	203 ± 3	241 ± 5	38 ± 3*	99
60	5/5	202 ± 3	232 ± 4	30 ± 3**	96
120	5/5	208 ± 4	233 ± 6	26 ± 2**	96
Female					
0	5/5	142 ± 1	161 ± 1	18 ± 0	
7.5	5/5	140 ± 1	157 ± 3	17 ± 2	98
15	5/5	141 ± 1	155 ± 4	15 ± 3	96
30	5/5	140 ± 2	153 ± 1	13 ± 1*	95
60	5/5	142 ± 3	155 ± 4	13 ± 1*	96
120	5/5	140 ± 2	144 ± 2**	4 ± 1**	89

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** P≤0.01
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

TABLE 3
Survival and Body Weights of Male NBR Rats in the 2-Week Inhalation Study of Tetralin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
0	5/5	252 ± 4	281 ± 5	30 ± 2	
7.5	5/5	244 ± 3	265 ± 4*	21 ± 2**	94
15	5/5	246 ± 5	262 ± 5**	16 ± 2**	93
30	5/5	246 ± 3	258 ± 4**	13 ± 3**	92
60	5/5	244 ± 4	258 ± 5**	14 ± 3**	92
120	5/5	247 ± 2	261 ± 4**	14 ± 3**	93

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** P≤0.01
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

significantly greater than those of the chamber controls. The relative lung weights of male F344/N rats exposed to 30 ppm only and of females exposed to 120 ppm were significantly greater than those of the chamber controls.

The incidences of mononuclear cell cellular infiltration in the nose of all exposed groups of rats, except male F344/N rats exposed to 7.5 ppm, were significantly greater than those in the chamber controls; in general, the severity increased with increasing exposure concentration (Table 4). The incidences of glandular hypertrophy and olfactory epithelium degeneration were significantly increased in male F344/N rats exposed to 120 ppm, and the incidence of olfactory epithelium necrosis was slightly increased in this group. In male F344/N rats, the severity of renal tubule hyaline droplet accumulation increased with increasing exposure concentration. No renal tubule hyaline droplet accumulation was observed in male NBR rats (included for comparison to renal lesion development in male F344/N rats) exposed to 0 or 120 ppm tetralin. Olfactory epithelial degeneration and necrosis were minimal lesions that involved the olfactory epithelium lining the dorsal portion of the nasal septum of Level III. Degeneration was characterized by disorganization of the layers of neuronal cell nuclei, with a decrease in the number of cells. Necrosis consisted of focal epithelial cell hyperchromasia, disruption, and loss. Glands underlying the olfactory epithelium were more prominent, as were their ducts due to a slight increase in the size of glandular epithelial cells (glandular hypertrophy). Mononuclear cell infiltrates consisted of focal to diffuse aggregates of primarily lymphocytes mixed with low numbers of macrophages in the lamina propria of all three nasal sections.

Exposure Concentration Selection Rationale: Tetralin had no effect on survival in male or female rats. The body weight and histopathology changes were not considered severe enough to limit selection of 120 ppm as the highest exposure concentration. Therefore, the exposure concentrations selected for the 3-month inhalation study in rats were 7.5, 15, 30, 60, and 120 ppm.

3-Month Study

All rats survived to the end of the study (Table 5). During the first 4 weeks of exposure, dark-stained urine was occasionally observed in the catch pans of rats exposed to 30, 60, or 120 ppm.

Hematology and clinical chemistry data for rats in the 3-month study of tetralin are listed in Tables 6 and F1. On day 93, exposure-related effects in the erythron were observed. These effects were characterized by small decreases in the hemoglobin values (≤4%) and/or erythrocyte counts (≤8%) in 60 and 120 ppm males and in females exposed to 15 ppm or greater; hematocrit values were unaffected. Mean cell volume values were slightly increased in 60 and 120 ppm males and in females exposed to 30 ppm or greater and probably reflect the increased numbers of larger reticulocytes (120 ppm males and females exposed to 30 ppm or greater) in the circulation. Thus, it appears that, with time, exposure to

TABLE 4
Incidences of Selected Nonneoplastic Lesions and α2u-Globulin Concentrations in Rats in the 2-Week Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male (F344/N)						
Nose[a]	5	5	5	5	5	5
Infiltration Cellular, Mononuclear Cell[b]	0	3 (1.0)[c]	5** (1.0)	5** (1.8)	5** (2.0)	5** (2.8)
Glands, Hypertrophy	0	0	0	0	0	5** (1.2)
Olfactory Epithelium, Degeneration	0	0	0	0	0	5** (1.6)
Olfactory Epithelium, Necrosis	0	0	0	0	0	3 (1.3)
Kidney	5	5	5	5	5	5
Renal Tubule, Accumulation, Hyaline Droplet	5 (1.2)	5 (1.6)	5 (2.0)	5 (2.0)	5 (2.6)	5 (3.6)
α2u-Globulin (ng/µg soluble protein)[d]	55.7 ± 6.4	104.3 ± 25.0▲	119.7 ± 25.3▲	99.3 ± 9.2▲	144.3 ± 32.2▲	164.2 ± 20.1▲▲
Male (NBR)						
Nose	5	5	5	5	5	5
Infiltration Cellular, Mononuclear Cell	0	5** (1.4)	5** (1.4)	5** (1.8)	5** (2.0)	5** (2.8)
Kidney	5	0	0	0	0	5
Renal Tubule, Accumulation, Hyaline Droplet	0					0
Female (F344/N)						
Nose	5	5	5	5	5	5
Infiltration Cellular, Mononuclear Cell	0	5** (1.0)	5** (1.0)	5** (1.2)	5** (1.6)	5** (2.0)

** Significantly different (P≤0.01) from the chamber control group by the Fisher exact test
▲ Significantly different (P≤0.05) from the chamber control group by Shirley's test
▲▲ P≤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] Data are presented as mean ± standard error.

tetralin induced a minimal decrease in the erythron that resulted in a hematopoietic response; the effect was more pronounced in females. The mechanism for the erythron decrease was not apparent. Platelet counts were slightly increased (≤21%) in 60 and 120 ppm males and all exposed groups of females. The mechanism for the increased platelet counts was unknown but may, in part, reflect a generalized increase in hematopoietic activity in response to changes in the erythron.

At all time points, serum alanine aminotransferase activity demonstrated exposure-related decreases (Table F1). On day 3, all exposed groups were affected. This effect diminished over time and by day 93 was apparent only in 60 ppm males and 120 ppm males and females; sorbitol dehydrogenase activity was also decreased at this time point. The significance of decreases in these serum markers of hepatocellular injury was unknown. There was evidence that the liver was affected by tetralin exposure, characterized by increases in relative liver weights in 120 ppm males and 60 and 120 ppm females. Thus, it could be suggested that the changes in these serum biomarkers could be related to some alteration in liver metabolism.

At 12 weeks, there were significant urine chemistry changes consistent with renal injury in males and females (Tables 6 and G3). The urine aspartate aminotransferase/creatinine ratios demonstrated exposure concentration-related increases, of twofold or greater, in

TABLE 5
Survival and Body Weights of Rats in the 3-Month Inhalation Study of Tetralin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	90 ± 3	294 ± 8	205 ± 7	
7.5	10/10	89 ± 3	301 ± 7	212 ± 7	102
15	10/10	85 ± 3	299 ± 6	214 ± 5	102
30	10/10	89 ± 3	301 ± 8	212 ± 9	102
60	10/10	85 ± 3	289 ± 8	204 ± 7	98
120	10/10	87 ± 3	276 ± 5	190 ± 3	94
Female					
0	10/10	86 ± 2	183 ± 4	98 ± 5	
7.5	10/10	86 ± 3	190 ± 4	104 ± 6	104
15	10/10	85 ± 3	184 ± 3	99 ± 4	101
30	10/10	86 ± 3	180 ± 4	94 ± 4	98
60	10/10	84 ± 2	178 ± 4	94 ± 4	97
120	10/10	82 ± 3	173 ± 3	91 ± 3	95

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

males and females exposed to 30 ppm or greater. Additionally, the urine lactate dehydrogenase/creatinine ratio was increased in 60 and 120 ppm males by up to 60%. Increased activity of these enzymes in the urine would be consistent with increased cell membrane leakage of the renal tubule epithelial cells. The urine glucose/creatinine ratio was minimally increased (16%) in 120 ppm males; increased urinary glucose excretion could be consistent with a loss of proximal tubule function. However, other than a mild increased severity in hyaline droplet accumulation and chronic progressive nephropathy in male rats (there were no morphological changes in the kidney of exposed females), there was no strong morphological evidence to support the urinary enzyme or glucose increases.

The absolute kidney weights of 60 and 120 ppm females and the relative kidney weights of all groups of males and females exposed to 15 ppm or greater were significantly greater than those of the chamber controls (Table H3). The absolute and relative liver weights of male rats exposed to 15 ppm and the relative liver weights of 60 ppm females and 120 ppm males and females were significantly greater than those of the chamber controls.

No significant differences in reproductive organ weights or in sperm parameters or estrous cyclicity were observed between exposed and chamber control groups of male and female rats (Tables I1 and I2).

In general, concentrations of α2u-globulin in the kidney of male rats were higher in the exposed groups than in the chamber control group (Table G2). The concentrations at week 2 (8 weeks of age) were less than those at weeks 6 and 14 (12 and 20 weeks of age, respectively); this was due in part to the age- and androgen-dependent production of α2u-globulin. The production of α2u-globulin is generally relatively low at 8 weeks of age. Concentrations of α2u-globulin increased between weeks 2 and 6 but decreased significantly between weeks 6 and 14. At week 2, α2u-globulin concentrations (nmol/g kidney and ng/μg soluble protein) were significantly increased in male rats exposed to 60 or 120 ppm. At week 14, α2u-globulin concentrations of all exposed groups of male rats were significantly greater than those of the chamber control group; however, the increases were not exposure concentration dependent. The labeling indices in 60 and 120 ppm male rats at week 6 and all exposed groups of male rats at week 14 were significantly greater than those of the chamber control group.

TABLE 6
Selected Hematology and Urinalysis Data for Rats in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 3	43.9 ± 0.5	45.0 ± 0.9	45.2 ± 0.5	44.1 ± 0.5	45.0 ± 0.7	44.4 ± 0.3
Day 23	47.6 ± 0.3	47.5 ± 0.4	47.8 ± 0.3	48.3 ± 0.6	48.5 ± 0.4	47.6 ± 0.3
Week 14	46.9 ± 0.1	46.9 ± 0.3	46.5 ± 0.3	46.4 ± 0.4	46.0 ± 0.3	46.2 ± 0.3
Packed cell volume (mL/dL)						
Day 3	43.2 ± 0.6	43.6 ± 0.8	44.3 ± 0.5	42.7 ± 0.5	43.9 ± 0.7	43.7 ± 0.5
Day 23	46.3 ± 0.5	45.9 ± 0.4	46.2 ± 0.3	47.3 ± 0.7	46.9 ± 0.4	46.4 ± 0.2
Week 14	46.2 ± 0.3	46.4 ± 0.2	45.8 ± 0.3	45.9 ± 0.5	45.9 ± 0.3	45.7 ± 0.3
Hemoglobin (g/dL)						
Day 3	13.3 ± 0.2	13.7 ± 0.3	14.0 ± 0.2	13.3 ± 0.1	13.6 ± 0.3	13.6 ± 0.2
Day 23	15.1 ± 0.1	15.1 ± 0.2	15.2 ± 0.1	15.4 ± 0.1	15.4 ± 0.2	15.2 ± 0.1
Week 14	15.3 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	15.1 ± 0.1	15.0 ± 0.1*
Erythrocytes (10^6/μL)						
Day 3	6.74 ± 0.11	6.83 ± 0.14	6.98 ± 0.07	6.74 ± 0.09	7.02 ± 0.13	6.95 ± 0.09
Day 23	7.50 ± 0.11	7.49 ± 0.09	7.49 ± 0.07	7.77 ± 0.11	7.66 ± 0.11	7.49 ± 0.06
Week 14	8.40 ± 0.06	8.40 ± 0.06	8.27 ± 0.08	8.29 ± 0.10	8.21 ± 0.04*	8.07 ± 0.06**
Reticulocytes (10^6/μL)						
Day 3	0.28 ± 0.02	0.24 ± 0.03	0.26 ± 0.04	0.37 ± 0.05	0.36 ± 0.05	0.28 ± 0.03
Day 23	0.29 ± 0.02	0.32 ± 0.02	0.34 ± 0.02	0.31 ± 0.02	0.32 ± 0.03	0.38 ± 0.03*
Week 14	0.09 ± 0.01	0.09 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.16 ± 0.02**
Mean cell volume (fL)						
Day 3	64.2 ± 0.4	63.7 ± 0.4	63.4 ± 0.3	63.4 ± 0.3	62.6 ± 0.3**	62.9 ± 0.2**
Day 23	61.8 ± 0.4	61.2 ± 0.9	61.6 ± 0.3	60.9 ± 0.4	61.2 ± 0.6	61.9 ± 0.5
Week 14	55.1 ± 0.2	55.1 ± 0.2	55.5 ± 0.2	55.3 ± 0.2	55.8 ± 0.2*	56.6 ± 0.2**
Platelets (10^3/μL)						
Day 3	876.1 ± 22.9	935.9 ± 13.3	880.3 ± 20.6	885.4 ± 25.3	883.7 ± 15.2	894.0 ± 16.2
Day 23	796.2 ± 13.9	773.2 ± 15.8	779.0 ± 11.4	786.3 ± 12.7	757.5 ± 11.5	763.8 ± 12.8
Week 14	560.7 ± 12.5	557.0 ± 10.5	582.8 ± 9.9	556.7 ± 20.5	608.5 ± 7.7**	631.3 ± 4.4**
Urinalysis at 12 Weeks						
n	10	10	10	10	10	10
Glucose/creatinine ratio	0.11 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01*
Aspartate aminotransferase/creatinine ratio	0.102 ± 0.011	0.13 ± 0.01	0.13 ± 0.01	0.18 ± 0.01**	0.22 ± 0.03**	0.35 ± 0.03**
Lactate dehydrogenase/creatinine ratio	0.48 ± 0.03	0.48 ± 0.03	0.48 ± 0.03	0.57 ± 0.05	0.63 ± 0.04*	0.78 ± 0.04**

TABLE 6
Selected Hematology and Urinalysis Data for Rats in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	9	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 3	46.1 ± 0.4	46.1 ± 0.6	46.1 ± 0.4	45.7 ± 0.5	46.3 ± 0.4	45.5 ± 0.4
Day 23	48.4 ± 0.2	48.1 ± 0.3	47.7 ± 0.2	48.2 ± 0.2	48.0 ± 0.4	46.9 ± 0.2**
Week 14	47.1 ± 0.4	47.1 ± 0.4	46.5 ± 0.5	46.0 ± 0.2	46.8 ± 0.3	46.2 ± 0.3
Packed cell volume (mL/dL)						
Day 3	44.5 ± 0.4	45.0 ± 0.8	45.3 ± 0.5	44.6 ± 0.6	45.1 ± 0.6	44.9 ± 0.6
Day 23	47.1 ± 0.3	47.2 ± 0.4	46.6 ± 0.3	46.9 ± 0.2	46.6 ± 0.4	46.3 ± 0.4
Week 14	47.2 ± 0.5	47.1 ± 0.4	46.0 ± 0.6	45.7 ± 0.3	46.4 ± 0.3	45.8 ± 0.3
Hemoglobin (g/dL)						
Day 3	14.1 ± 0.2	14.3 ± 0.2	14.3 ± 0.2	14.1 ± 0.2	14.3 ± 0.2	14.0 ± 0.2
Day 23	15.6 ± 0.1	15.8 ± 0.2	15.5 ± 0.1	15.6 ± 0.1	15.6 ± 0.1	15.3 ± 0.1
Week 14	15.4 ± 0.2	15.3 ± 0.1	15.0 ± 0.2	14.8 ± 0.1**	14.9 ± 0.1**	14.8 ± 0.1**
Erythrocytes (10^6/μL)						
Day 3	6.99 ± 0.08	7.08 ± 0.14	7.12 ± 0.11	7.01 ± 0.12	7.09 ± 0.12	7.09 ± 0.11
Day 23	7.62 ± 0.06	7.73 ± 0.07	7.55 ± 0.07	7.53 ± 0.05	7.57 ± 0.10	7.63 ± 0.07
Week 14	8.01 ± 0.10	7.94 ± 0.08	7.69 ± 0.11*	7.51 ± 0.05**	7.50 ± 0.04**	7.40 ± 0.07**
Reticulocytes (10^6/μL)						
Day 3	0.49 ± 0.03	0.47 ± 0.04	0.49 ± 0.04	0.45 ± 0.04	0.47 ± 0.04	0.47 ± 0.05
Day 23	0.22 ± 0.02	0.26 ± 0.01	0.27 ± 0.02	0.27 ± 0.01	0.22 ± 0.02	0.24 ± 0.02
Week 14	0.10 ± 0.01	0.12 ± 0.02	0.13 ± 0.03	0.15 ± 0.01*	0.19 ± 0.02**	0.16 ± 0.02*
Mean cell volume (fL)						
Day 3	63.6 ± 0.3	63.6 ± 0.5	63.7 ± 0.3	63.5 ± 0.3	63.9 ± 0.6	63.3 ± 0.2
Day 23	62.0 ± 0.5	61.0 ± 0.6	61.8 ± 0.4	62.3 ± 0.5	61.7 ± 0.5	60.5 ± 0.5
Week 14	59.0 ± 0.3	59.3 ± 0.4	59.8 ± 0.3	61.0 ± 0.3**	61.8 ± 0.3**	61.8 ± 0.3**
Platelets (10^3/μL)						
Day 3	828.0 ± 17.1	822.5 ± 16.4	804.5 ± 12.0	825.4 ± 19.8	842.7 ± 23.3	833.1 ± 17.3
Day 23	755.2 ± 19.0	734.4 ± 9.5	742.0 ± 20.5	736.8 ± 10.3	734.0 ± 17.6	764.6 ± 11.1
Week 14	504.8 ± 7.1	536.7 ± 10.8*	562.6 ± 9.0**	596.6 ± 9.8**	579.7 ± 10.2**	609.6 ± 16.6**
Urinalysis at 12 Weeks						
n	10	10	10	10	10	10
Glucose/creatinine ratio	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.00
Aspartate aminotransferase/creatinine ratio	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01**	0.09 ± 0.01**	0.20 ± 0.01**
Lactate dehydrogenase/creatinine ratio	0.32 ± 0.02	0.38 ± 0.03	0.37 ± 0.04	0.36 ± 0.03	0.38 ± 0.03	0.45 ± 0.05

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

Histologically, α2u-globulin manifests as accumulation of hyaline droplets within the renal tubule epithelial cells. At week 2, the severity of hyaline droplet accumulation in exposed rats was not significantly different than that of the chamber control group (Table 7). However, in the exposed groups, the droplets tended to be larger or occurred as aggregates. At weeks 6 and 14, the severity of hyaline droplet accumulation in male rats exposed to 15 ppm or greater was slightly greater than that of the chamber control group. Chamber control males had fine, brightly eosinophilic, pink cytoplasmic globules in the proximal tubule epithelium; the hyaline droplets in exposed males tended to be larger or conglomerates of several droplets and easily distinguished from the chamber controls with the Mallory-Heidenhain stain.

There were significantly increased incidences of olfactory epithelium necrosis in rats exposed to 30 ppm or greater (Table 7). Incidences of olfactory epithelium regeneration were significantly increased in 60 and 120 ppm rats. Olfactory epithelium necrosis and regeneration were generally minimal to mild lesions confined to Level III of the nasal cavity and affected the olfactory epithelium lining the dorsal meatus, the dorsal nasal septum, and adjacent areas of the ethmoid turbinates. Necrosis consisted of segmental epithelial cell hyperchromasia and disruption and loss of the olfactory epithelium. In some areas, rafts of necrotic epithelium, separated from the underlying lamina propria, were noted within the adjacent nasal passages. Regeneration was diagnosed when segments of the olfactory epithelium were replaced by disorganized layers of squamous to cuboidal epithelial cells.

Exposure Concentration Selection Rationale: Tetralin had no effect on survival in male or female rats, and body weight effects in males were minimal. The clinical pathology and histopathology changes were not considered severe enough to limit selection of 120 ppm as the highest exposure concentration. Therefore, the exposure concentrations selected for the 2-year inhalation study in rats were 30, 60, and 120 ppm.

TABLE 7
Incidences of Selected Nonneoplastic Lesions in F344/N Rats in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male						
Kidney						
Week 2[a]	5	5	5	5	5	5
Week 6	5	5	5	5	5	5
Week 14	10	10	10	10	10	10
Accumulation, Hyaline Droplet						
Week 2[b]	2 (1.0)[c]	2 (1.0)	3 (1.0)	4 (1.8)	5 (1.8)	5 (1.6)
Week 6	5 (1.4)	5 (1.2)	5 (1.6)	5 (2.0)	5 (2.4)	5 (2.4)
Week 14	10 (1.0)	10 (1.1)	10 (2.0)	10 (2.0)	10 (2.0)	10 (2.0)
Nose[a]	10	0	10	10	10	10
Olfactory Epithelium, Necrosis[b]	0		0	4* (1.8)	10** (2.0)	10** (2.0)
Olfactory Epithelium, Regeneration	0		0	0	7** (1.6)	10** (1.8)
Female						
Nose	10	10	10	10	10	10
Olfactory Epithelium, Necrosis	0	0	1 (1.0)	6** (1.2)	10** (1.9)	10** (2.0)
Olfactory Epithelium, Regeneration	0	0	0	0	9** (1.6)	10** (1.8)

* Significantly different ($P \leq 0.05$) from the chamber 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 rats are shown in Table 8 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of rats was similar to that of the chamber controls.

Body Weights and Clinical Findings

Mean body weights of 120 ppm females were less than those of the chamber controls after week 29; mean body weights of exposed groups of males were similar to those of the chamber controls throughout the study (Figure 3; Tables 9 and 10). Although more prevalent in females, dark-stained urine was observed in all exposed groups of rats, and the incidences increased with increasing exposure concentration (males: 0 ppm, 0/50; 30 ppm, 20/50; 60 ppm, 33/50; 120 ppm, 50/50; females: 2/50, 40/50, 48/50, 50/50). Clonic seizures of short duration occurred in a few males (5/50, 2/50, 2/50, 4/50) and females (7/50, 5/50, 7/50, 10/50). They were most frequently observed and recorded during daily animal care activities. No evidence of brain lesions was found to account for the cause or effect of the clonic seizures.

Similar, sporadic seizures have been observed in F344/N rats in six other NTP inhalation or dermal studies at three different laboratories. In all of these studies, the single common factor was that the animals were housed individually. No such episodes have been observed in concurrent dosed feed, gavage, or drinking water studies in which animals are group housed. In the individually housed animals, most seizures were observed early in the day, when technical and maintenance activities were commencing following the animals' dark cycle period. No deaths were associated with the seizures, and there were no correlations with body weight, feed consumption or composition, or histopathological lesions in this or the other studies. Thus, these transient events were not considered to have affected the toxicologic or carcinogenic evaluations of this study.

TABLE 8
Survival of Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	24	14	20	19
Natural deaths	6	7	5	3
Animals surviving to study termination	20	29[a]	25	28
Percent probability of survival at end of study[b]	40	58	50	56
Mean survival (days)[c]	668	688	674	685
Survival analysis[d]	P = 0.234N	P = 0.097N	P = 0.432N	P = 0.116N
Female				
Animals initially in study	50	50	50	50
Moribund	16	11	15	11
Natural deaths	3	3	4	1
Animals surviving to study termination	31	36	31	38
Percent probability of survival at end of study	62	72	62	76
Mean survival (days)	682	690	693	706
Survival analysis	P = 0.245N	P = 0.381N	P = 1.000N	P = 0.177N

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

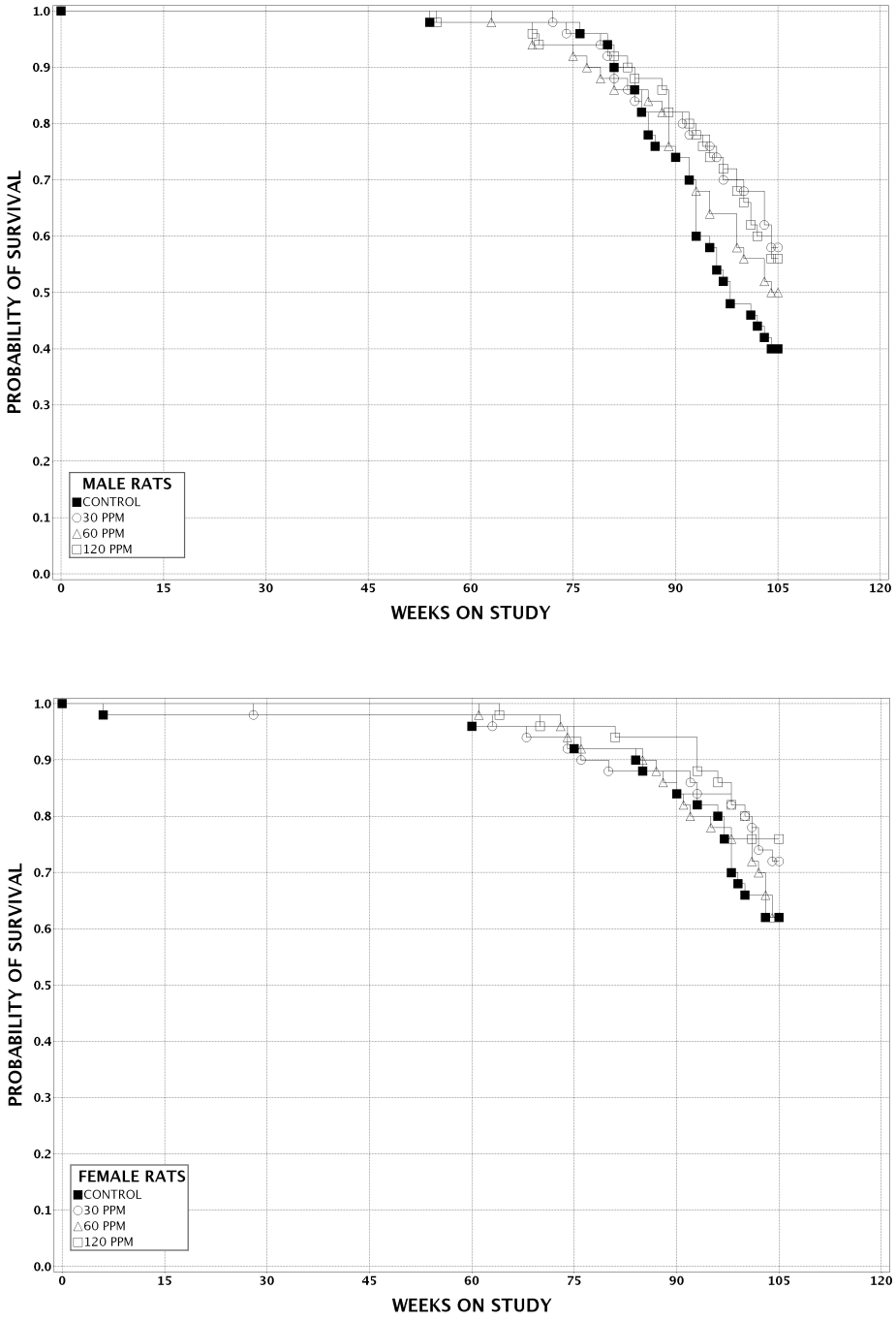


FIGURE 2
Kaplan-Meier Survival Curves for Rats Exposed to Tetralin by Inhalation for 2 Years

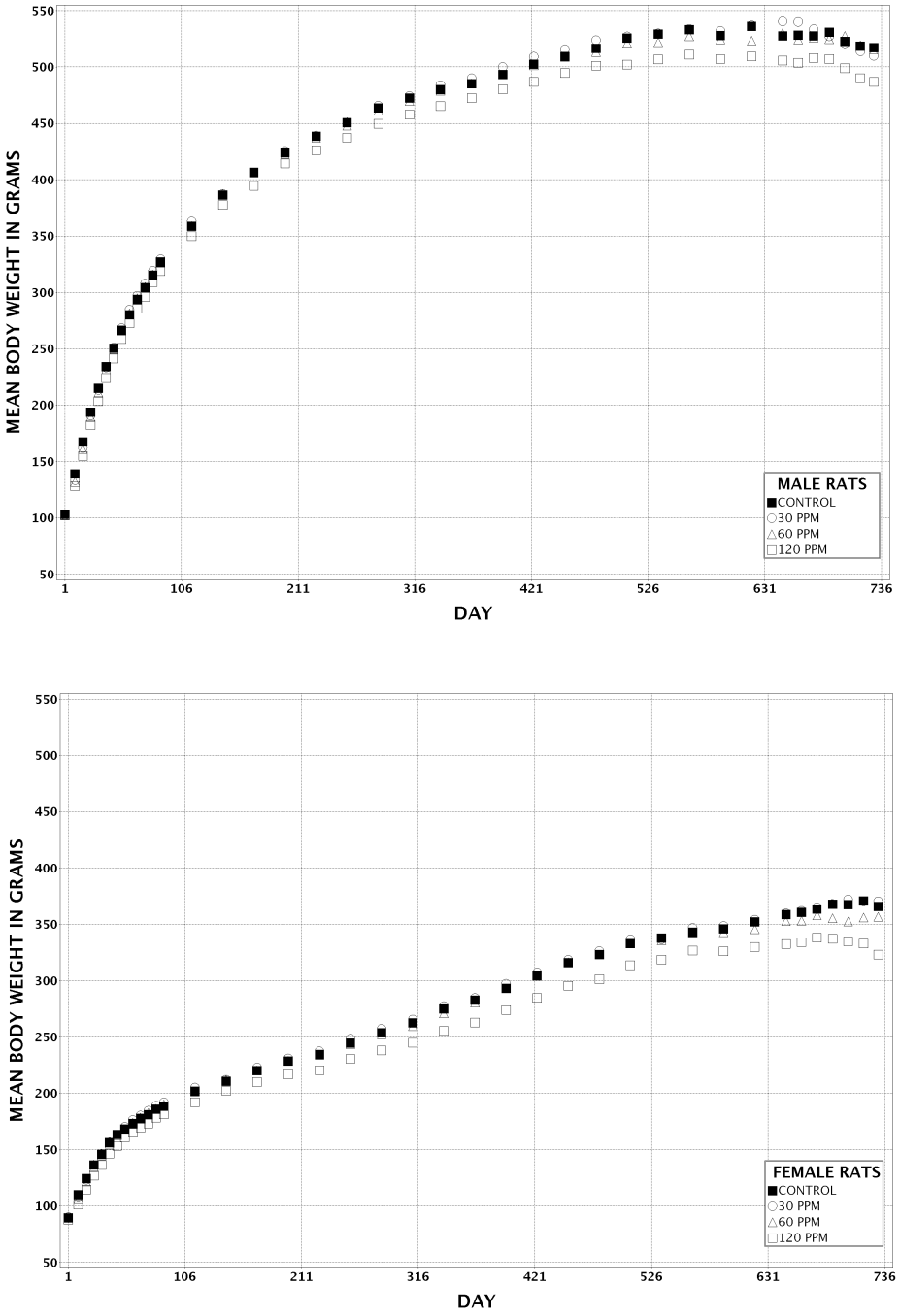


FIGURE 3
Growth Curves for Rats Exposed to Tetralin by Inhalation for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Tetralin

Days on Study	Chamber Control		30 ppm			60 ppm			120 ppm		
	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	103	50	103	99	50	103	99	50	102	99	50
10	139	50	134	96	50	132	95	50	129	92	50
17	168	50	162	97	50	161	96	50	155	93	50
24	194	50	191	99	50	189	98	50	183	94	50
31	215	50	214	99	50	211	98	50	204	95	50
38	234	50	234	100	50	232	99	50	224	96	50
45	251	50	251	100	50	250	100	50	241	96	50
52	267	50	269	101	50	266	100	50	259	97	50
59	280	50	285	102	50	282	100	50	273	97	50
66	294	50	297	101	50	295	100	50	286	97	50
73	304	50	308	101	50	305	100	50	296	97	50
80	316	50	319	101	50	316	100	50	309	98	50
87	327	50	330	101	50	326	100	50	319	98	50
115	359	50	363	101	50	359	100	50	350	98	50
143	387	50	388	100	50	388	100	50	378	98	50
171	407	50	406	100	50	406	100	50	395	97	50
199	424	50	426	100	50	423	100	50	414	98	50
227	439	50	439	100	50	437	100	50	426	97	50
255	451	50	451	100	50	448	99	50	437	97	50
283	464	50	466	100	50	461	100	50	450	97	50
311	473	50	474	100	50	470	99	50	458	97	50
339	480	50	484	101	50	479	100	50	465	97	50
367	485	50	490	101	50	486	100	50	472	97	50
395	494	49	500	101	50	494	100	50	481	97	49
423	503	49	509	101	50	502	100	50	487	97	49
451	509	49	516	101	50	509	100	49	495	97	49
479	516	49	524	102	50	513	99	49	501	97	49
507	526	49	527	100	49	521	99	47	502	96	47
535	529	48	530	100	48	522	99	46	507	96	47
563	533	45	534	100	44	527	99	43	511	96	47
591	528	42	532	101	41	525	99	43	507	96	44
619	536	38	537	100	41	524	98	40	510	95	41
647	528	32	541	102	39	530	101	34	506	96	39
661	528	30	540	102	39	525	99	34	504	95	38
675	528	27	534	101	36	526	100	32	508	96	36
689	531	24	527	99	35	525	99	30	507	96	34
703	523	23	521	100	34	528	101	28	499	96	31
717	519	22	514	99	31	519	100	28	490	95	30
Mean for weeks											
1-13	238		238	100		236	99		229	96	
14-52	431		433	100		430	100		419	97	
53-103	520		524	101		517	99		499	96	

TABLE 10
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Tetralin

Days on Study	Chamber Control		30 ppm			60 ppm			120 ppm		
	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	90	50	90	101	50	90	100	50	88	98	50
10	110	50	108	98	50	106	96	50	101	92	50
17	124	50	123	99	50	122	98	50	115	92	50
24	137	50	137	100	50	135	99	50	127	93	50
31	146	50	147	100	50	145	99	50	137	94	50
38	156	49	157	100	50	155	99	50	147	94	50
45	164	49	164	100	50	163	100	50	154	94	50
52	169	49	170	101	50	169	100	50	161	95	50
59	174	49	177	102	50	174	100	50	165	95	50
66	178	49	181	101	50	179	100	50	170	96	50
73	181	49	185	102	50	183	101	50	173	96	50
80	186	49	189	102	50	187	100	50	179	96	50
87	189	49	192	102	50	190	101	50	182	96	50
115	202	49	205	102	50	202	100	50	192	95	50
143	211	49	212	101	50	212	101	50	202	96	50
171	220	49	223	101	50	221	100	50	210	95	50
199	229	49	231	101	49	229	100	50	217	95	50
227	235	49	238	101	49	234	100	50	221	94	50
255	245	49	249	102	49	244	100	50	231	94	50
283	254	49	257	101	49	253	99	50	238	94	50
311	263	49	266	101	49	260	99	50	245	93	50
339	275	49	278	101	49	271	99	50	256	93	50
367	283	49	285	101	49	281	99	50	263	93	50
395	293	49	297	101	49	293	100	50	274	93	50
423	304	48	308	101	49	304	100	50	285	94	50
451	316	48	319	101	48	317	100	49	296	94	49
479	323	48	326	101	47	323	100	49	301	93	49
507	333	48	337	101	47	333	100	49	314	94	48
535	338	46	337	100	45	336	99	46	319	94	48
563	343	46	347	101	44	343	100	46	327	95	47
591	346	44	349	101	44	343	99	45	326	94	47
619	352	44	354	101	44	346	98	43	330	94	47
647	359	42	360	100	43	353	98	40	333	93	45
661	361	41	362	100	42	353	98	40	335	93	44
675	364	39	365	101	42	358	98	39	338	93	43
689	368	35	368	100	41	356	97	38	337	92	41
703	368	33	372	101	39	353	96	38	335	91	38
717	371	31	370	100	37	356	96	33	333	90	38
Mean for weeks											
1-13	154		155	101		154	100		146	95	
14-52	237		240	101		236	100		224	95	
53-103	339		341	101		334	99		315	93	

Urinary Metabolites and Urinalysis at 12 Months

Creatinine-adjusted levels of all urinary metabolites increased with increasing exposure concentration in male and female rats with the concentration of 2-hydroxy-1-tetralone > 4-hydroxy-1-tetralone ≈ 1-tetralol >>> 2-tetralol. Production of some metabolites exceeded dose proportionality in the 120 ppm groups as can be seen from the exposure concentration-adjusted values (Table G4). Creatinine-adjusted concentrations in male rats were higher than those in female rats, except for 2-tetralol where females had a higher concentration than males. No treatment-related effects were demonstrated by urinalysis evaluations performed at 12 months (Table G4).

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or non-neoplastic lesions of the kidney, liver, uterus, testis, nose, lung, heart, and eye. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Kidney: In the standard evaluation of the kidney, there were slightly increased incidences of cortical renal tubule adenoma in male rats (Tables 11 and A1). Although not statistically significant, the incidence in the 120 ppm group exceeded the historical control mean from inhalation studies and all study routes (Tables 11 and A3a). One renal tubule adenoma and one renal tubule carcinoma occurred in female rats exposed to 120 ppm (Tables 11 and B1). In the standard evaluation, a single section of each kidney was examined microscopically. Because the incidence of cortical renal

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Male				
Single Sections (Standard Evaluation)				
Number Examined Microscopically	50	50	50	50
Cortex, Renal Tubule, Hyperplasia[a]	1 (2.0)[b]	2 (3.0)	0	3 (3.7)
Pelvis, Transitional Epithelium, Hyperplasia	1 (1.0)	1 (2.0)	0	7* (1.7)
Nephropathy, Chronic	48 (2.6)	50 (3.0)	48 (3.0)	50 (3.4)
Cortex, Renal Tubule, Adenoma[c]				
Overall rate[d]	0/50 (0%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate[e]	0.0%	2.3%	2.4%	4.6%
Terminal rate[f]	0/20 (0%)	1/29 (3%)	1/25 (4%)	0/28 (0%)
First incidence (days)	—[h]	729 (T)	729 (T)	701
Poly-3 test[g]	P = 0.169	P = 0.516	P = 0.506	P = 0.256
Step Sections (Extended Evaluation)				
Number Examined Microscopically	50	50	50	50
Cortex, Renal Tubule, Hyperplasia	0	1 (4.0)	1 (2.0)	5* (2.6)
Cortex, Renal Tubule, Adenoma	0	2	1	5*
Single and Step Sections (Combined)				
Number Examined Microscopically	50	50	50	50
Cortex, Renal Tubule, Hyperplasia	1 (2.0)	2 (3.0)	1 (2.0)	7* (2.9)
Cortex, Renal Tubule, Adenoma (includes multiple)				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	7.0%	4.9%	13.9%
Terminal rate	0/20 (0%)	2/29 (7%)	2/25 (8%)	2/28 (7%)
First incidence (days)	—	715	729 (T)	674
Poly-3 test	P = 0.014	P = 0.134	P = 0.244	P = 0.020

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Female				
Single Sections (Standard Evaluation)				
Number Examined Microscopically	50	50	50	50
Cortex, Renal Tubule, Adenoma[i]	0	0	0	1
Cortex, Renal Tubule, Carcinoma[j]	0	0	0	1
Cortex, Renal Tubule, Carcinoma or Adenoma[k]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	0.0%	4.3%
Terminal rate	0/31 (0%)	0/36 (0%)	0/31 (0%)	1/38 (3%)
First incidence (days)	—	—	—	442
Poly-3 test	P = 0.048	—[l]	—	P = 0.255
Step Sections (Extended Evaluation)				
Number Examined Microscopically	50	50	50	50
Cortex, Renal Tubule, Adenoma	1	1	0	0
Cortex, Renal Tubule, Hyperplasia	0	1 (1)	1 (1)	0
Single and Step Sections (Combined)				
Number Examined Microscopically	50	50	50	50
Cortex, Renal Tubule, Adenoma and Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Adjusted rate	2.3%	2.3%	0%	4.3%
Terminal rate	1/31 (3%)	1/36 (3%)	0/31 (0%)	1/38 (3%)
First incidence (days)	730 (T)	730 (T)	—	442
Poly-3 test	P = 0.383	P = 0.755N	P = 0.497N	P = 0.528

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 2/349 (0.6% ± 1.0%), range 0%-2%; all routes: 8/1,394 (0.6% ± 1.0%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with kidney examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the chamber control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for inhalation studies: 0/348; all routes: 1/1,340 (0.1% ± 0.4%), range 0%-2%
[j] Historical incidence for inhalation studies: 1/348 (0.3% ± 0.8%), range 0%-2%; all routes: 1/1,340 (0.1% ± 0.4%), range 0%-2%
[k] Historical incidence for inhalation studies: 1/348 (0.3% ± 0.8%), range 0%-2%; all routes: 2/1,340 (0.2% ± 0.5%), range 0%-2%
[l] Value of statistic cannot be computed.

tubule adenoma indicated the possibility of a treatment-related carcinogenic effect, an extended evaluation of the kidney was performed in male rats to explore this possibility.

In the extended evaluation, additional incidences of cortical renal tubule adenoma and cortical renal tubule hyperplasia were identified (Table 11). In the combined analysis, the incidences of cortical renal tubule adenoma were increased in all exposed male groups, and the incidence was significantly increased in the 120 ppm group. In the combined analysis, there was also a significantly increased incidence of cortical renal tubule hyperplasia in the 120 ppm group.

In the standard evaluation, the severity of chronic nephropathy was increased in 120 ppm males, but the incidences were similar among all groups, including the chamber controls (Tables 11 and A4). The incidence of transitional epithelium hyperplasia in the renal pelvis of 120 ppm male rats was significantly increased.

Cortical renal tubule adenoma was a discrete, highly cellular, proliferative lesion and larger than focal hyperplasia (generally greater than the combined diameter of five normal-sized renal tubules). Adenomas tended to be more complex in structure than hyperplasias and were characterized by closely packed tubules and solid nests composed of a mixture of cells with large, vesicular nuclei and abundant, pale, eosinophilic cytoplasm and vacuolated cells. Cortical renal tubule hyperplasia was considered a preneoplastic lesion distinguished from regenerative epithelial changes that commonly occur as a component of age-related nephropathy. Hyperplasias were single or multiple expanded cortical tubules composed of increased numbers of tubule epithelial cells arranged in multiple layers that partially or completely filled the tubule. Transitional epithelial hyperplasia of the pelvis was characterized by an increased thickening of the transitional epithelium lining the renal pelvis, often forming papillary projections into the urinary space. This lesion, commonly associated with nephropathy, was generally of minimal to mild severity and occurred mostly in rats with moderate to severe chronic nephropathy.

Liver: Three hepatocellular adenomas occurred in 120 ppm females, and one hepatocellular carcinoma each was observed in the 60 and 120 ppm groups (Tables 12, B1, and B2).

Uterus: Incidences of stromal polyp and endometrium hyperplasia in 120 ppm female rats were significantly greater than those in the chamber controls, and the severity of endometrium hyperplasia was increased in 120 ppm females (Tables 13, B1, B2, and B4). However, the incidences of endometrial epithelial neoplasms were not increased. Endometrial polyps were single, sessile or pedunculated masses that protruded into the uterine lumen. They were composed of loosely arranged spindle-shaped or stellate endometrial stromal cells surrounding numerous, small, thin-walled blood vessels and entrapped endometrial glands and were lined by low cuboidal endometrial epithelium.

Testis: Incidences of interstitial cell adenoma and germinal epithelial atrophy in 30 and 120 ppm males were significantly greater than those in the chamber controls (Tables 14, A1, A2, and A4). Interstitial cell adenomas were discrete nodular masses that varied in size but generally were equal to or larger than the diameter of three adjacent seminiferous tubules with varying compression of adjacent tubules. They were composed of a relatively uniform population of small to medium sized cells that had abundant, finely vacuolated eosinophilic cytoplasm and a single centrally located nucleus. Interstitial cell adenomas are commonly observed spontaneous testicular neoplasms in control and treated F344/N rats, occurring at rates of 58% to 84% (inhalation studies; mean, 72%) and 58% to 98% (all routes; mean, 84%). The concurrent chamber control incidence is the lowest observed among historical chamber controls for inhalation studies.

Skin: There was a positive trend in the incidences of squamous cell papillomas of the skin in male rats (0/50, 1/50, 1/50, 4/50; Tables A1 and A2). However these incidences were within the range of historical controls and there were no significant increases in the incidences of any other related skin neoplasms (Tables A1 and A2). Thus the occurrence of these papillomas was not considered related to tetralin exposure.

TABLE 12
Incidences of Hepatocellular Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Number Examined Microscopically	50	50	50	50
Adenoma[a]				
Overall rate[b]	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[c]	0.0%	0.0%	0.0%	6.5%
Terminal rate[d]	0/31 (0%)	0/36 (0%)	0/31 (0%)	2/38 (5%)
First incidence (days)	—[e]	—	—	685
Poly-3 test[f]	P = 0.012	—[g]	—	P = 0.131
Carcinoma[h,i]	0	0	1	1
Adenoma or Carcinoma[j]				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	2.3%	8.6%
Terminal rate	0/31 (0%)	0/36 (0%)	1/31 (3%)	3/38 (8%)
First incidence (days)	—	—	730 (T)	685
Poly-3 test	P = 0.006	—	P = 0.503	P = 0.069

(T) Terminal sacrifice
[a] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 0/350; all routes: 16/1,350 (1.2% ± 2.6%), range 0%-12%
[b] Number of animals with neoplasm per number of animals with liver examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Not applicable; no neoplasms in animal group
[f] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[g] Value of statistic cannot be computed
[h] Number of animals with neoplasm
[i] Historical incidence for inhalation studies: 1/350 (0.3% ± 0.8%), range 0%-2%; all routes: 1/1,350 (0.1% ± 0.4%), range 0%-2%
[j] Historical incidence for inhalation studies: 1/350 (0.3% ± 0.8%), range 0%-2%; all routes: 17/1,350 (1.3% ± 2.6%), range 0%-12%

Nose: The incidences of olfactory epithelium degeneration, basal cell hyperplasia, metaplasia, and suppurative inflammation in all exposed groups of male and female rats were significantly greater than those in the chamber controls (Tables 15, A4, and B4). There were significantly increased incidences of olfactory epithelium mineralization in all exposed groups of males and in 60 and 120 ppm females. The incidences of glandular dilatation (minimal to mild) were significantly increased in 120 ppm males and all exposed groups of females. The incidences of respiratory epithelium chronic inflammation were significantly increased in males exposed to 60 or 120 ppm and all exposed groups of females.

Microscopically, normal olfactory epithelium appears multilayered because the nuclei of the various cell types composing the epithelium occur at different levels (Plate 1). Degeneration of the olfactory epithelium consisted of focal areas of loss and disorganization of the normal olfactory epithelium with accompanying atrophy of the submucosal olfactory nerve bundles (Plate 2). This lesion was most commonly located in the dorsal

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Number Necropsied	50	50	50	50
Endometrium, Hyperplasia[a]	2 (1.0)[b]	5 (1.2)	7 (1.4)	11[**] (2.3)
Stromal Polyp, Bilateral	0	2	0	0
Stromal Polyp (includes bilateral)[c]				
Overall rate[d]	6/50 (12%)	10/50 (20%)	9/50 (18%)	17/50 (34%)
Adjusted rate[e]	13.8%	22.2%	19.8%	36.5%
Terminal rate[f]	5/31 (16%)	7/36 (19%)	4/31 (13%)	15/38 (40%)
First incidence (days)	697	644	591	647
Poly-3 test[g]	P = 0.008	P = 0.228	P = 0.318	P = 0.011
Stromal Sarcoma[h]	0	1	1	0
Stromal Polyp or Stromal Sarcoma[i]				
Overall rate	6/50 (12%)	11/50 (22%)	10/50 (20%)	17/50 (34%)
Adjusted rate	13.8%	24.4%	22.0%	36.5%
Terminal rate	5/31 (16%)	8/36 (22%)	4/31 (13%)	15/38 (40%)
First incidence (days)	697	644	591	647
Poly-3 test	P = 0.010	P = 0.160	P = 0.234	P = 0.011

** Significantly different (P≤0.01) from the chamber control group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 66/350 (18.9% ± 5.9%), range 12%-26%; all routes: 241/1,350 (17.9% ± 6.6%), range 4%-32%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence for 2-year inhalation studies: 4/350 (1.1% ± 1.2%), range 0%-4%; all routes: 9/1,350 (0.7% ± 1.2%), range 0%-4%
[i] Historical incidence for 2-year inhalation studies: 70/350 (20.0% ± 6.4%), range 12%-28%; all routes: 250/1,350 (18.5% ± 7.0%), range 6%-34%

region of Level III. Olfactory epithelial respiratory metaplasia consisted of replacement of the normal olfactory epithelium by ciliated respiratory epithelium in Levels II and III; increasing severity was represented by increasing coverage by the metaplastic epithelium (Plate 3). Basal cell hyperplasia consisted of focal to multifocal irregular proliferation of the epithelial cells lining the base of the olfactory epithelium and often the Bowman's glands (Plate 4); increasing extent of the hyperplastic cells represented increasing severity. Frequently, Bowman's glands subjacent to the affected olfactory epithelium were dilated and contained a mixture of inflammatory cells. Small laminated basophilic foci of mineral were present in the sites of olfactory epithelial hyperplasia and metaplasia and basal epithelium cell hyperplasia. Suppurative inflammation consisted of accumulation of neutrophils and proteinaceous debris in the lumen and occasionally small numbers of neutrophils within the mucosa and submucosa. Chronic inflammation of the respiratory epithelium consisted of

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Testis in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Number Examined Microscopically	50	50	50	50
Germinal Epithelium, Atrophy[a]	32 (2.2)[b]	42* (2.4)	34 (2.4)	45** (2.3)
Interstitial Cell, Adenoma, Bilateral	18	20	17	30**
Interstitial Cell Adenoma (includes bilateral)[c]				
Overall rate[d]	29/50 (58%)	39/50 (78%)	31/50 (62%)	41/50 (82%)
Adjusted rate[e]	67.0%	83.7%	69.6%	87.9%
Terminal rate[f]	16/20 (80%)	26/29 (90%)	19/25 (76%)	27/28 (96%)
First incidence (days)	563	513	523	566
Poly-3 test[g]	P = 0.025	P = 0.038	P = 0.487	P = 0.008

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 250/349 (71.7% ± 8.5%), range 58%-84%; all routes: 1,170/1,399 (83.6% ± 11.5%), range 58%-98%
[d] Number of animals with neoplasm per number of animals with testis examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

small aggregates of lymphocytes in the mucosa and submucosa of nasal and maxillary turbinates in Levels I and II.

Lung: The incidence of minimal histiocytic cellular infiltration of the alveolus was significantly increased in 120 ppm females (21/50, 30/50, 24/50, 34/50); the incidence of minimal chronic inflammation was also increased in this group (12/50, 16/50, 14/50, 21/50), but the increase was not statistically significant (Table B4).

Microscopically, histiocytic cellular infiltration consisted of focal accumulations of foamy macrophages within alveolar lumens. Chronic inflammation consisted of small interstitial infiltrates of mostly macrophages and lymphocytes and low numbers of neutrophils; these infiltrates were occasionally accompanied by minimal increases in fibrous connective tissue.

Heart: The incidences of cardiomyopathy in 60 and 120 ppm females were significantly increased (0 ppm, 22/50; 30 ppm, 24/50; 60 ppm, 32/50; 120 ppm, 34/50; Table B4).

Eye: The incidence of lens cataract in 120 ppm females was significantly increased (2/49, 6/50, 7/49, 11/50; Table B4).

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia in all exposed groups of males and females were significantly less than those in the chamber controls (male: 25/50, 16/50, 5/50, 2/50; female: 17/50, 2/50, 0/50, 4/50; Tables A2 and B2).

TABLE 15
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Male				
Number Examined Microscopically	50	50	49	50
Glands, Dilatation[a]	0	3 (1.3)[b]	3 (1.3)	16** (1.8)
Olfactory Epithelium, Degeneration	1 (1.0)	40** (1.9)	43** (1.6)	42** (2.0)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	38** (1.8)	48** (1.9)	48** (2.0)
Olfactory Epithelium, Metaplasia	0	17** (1.6)	31** (1.6)	37** (1.8)
Olfactory Epithelium, Inflammation, Suppurative	0	12** (1.1)	8** (1.4)	10** (1.9)
Olfactory Epithelium, Mineralization	0	5* (1.2)	12** (1.5)	17** (1.3)
Respiratory Epithelium, Inflammation, Chronic	4 (1.0)	4 (1.3)	18** (1.2)	16** (1.1)
Female				
Number Examined Microscopically	50	50	50	50
Glands, Dilatation	0	6* (1.0)	10** (1.5)	16** (1.8)
Olfactory Epithelium, Degeneration	0	47** (1.6)	50** (1.6)	46** (1.5)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	48** (1.6)	50** (1.7)	49** (1.6)
Olfactory Epithelium, Metaplasia	0	41** (1.2)	43** (1.2)	49** (1.6)
Olfactory Epithelium, Inflammation, Suppurative	0	16** (1.2)	15** (1.1)	19** (1.3)
Olfactory Epithelium, Mineralization	0	2 (1.0)	8** (1.1)	13** (1.1)
Respiratory Epithelium, Inflammation, Chronic	1 (2.0)	7* (1.0)	11** (1.2)	12** (1.2)

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

MICE

2-Week Study

All mice survived to the end of the study (Table 16). Final mean body weights and mean body weight gains of exposed groups of mice were similar to those of the chamber controls. Dark-stained urine was observed in most of the exposed mice.

The absolute and relative liver weights of 60 and 120 ppm males and 30 and 120 ppm females and the relative liver weight of 60 ppm females were significantly greater than those of the chamber controls (Table H4).

In the nose, the incidences of olfactory epithelium atrophy were significantly increased in 60 and 120 ppm males and females (Table 17). The incidences of glandular dilatation were increased in 120 ppm males and females, and the increase was significant in females. The incidences of glandular hyperplasia were significantly increased in 120 ppm males and females. Mononuclear cell cellular infiltration occurred in two 120 ppm females. Atrophy involved the olfactory epithelium lining the dorsal meatus of Levels I, II, and III and the dorsal one-third of the nasal septum of Level III and consisted of thinning of the olfactory epithelium due to loss of neuronal cells with disorganization of the olfactory epithelium. The Bowman's glands underlying the olfactory epithelium were hypercellular and more basophilic and, in some mice, were dilated and contained inflammatory cells and proteinaceous fluid. Mononuclear cell infiltrates consisted of focal aggregates of primarily lymphocytes and low numbers of macrophages in the lamina propria of Level II of the nasal cavity.

Exposure Concentration Selection Rationale: Tetralin had no effect on survival or body weights in male or female mice. Histopathological changes in the nose did not preclude selection of 120 ppm as the highest exposure concentration. Therefore, the exposure concentrations selected for the 3-month inhalation study in mice were 7.5, 15, 30, 60, and 120 ppm.

TABLE 16
Survival and Body Weights of Mice in the 2-Week Inhalation Study of Tetralin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	20.8 ± 0.3	25.2 ± 0.2	4.4 ± 0.2	
7.5	5/5	21.0 ± 0.4	25.3 ± 0.8	4.3 ± 0.5	100
15	5/5	21.0 ± 0.3	25.4 ± 0.3	4.3 ± 0.5	101
30	5/5	20.5 ± 0.4	24.3 ± 0.6	3.7 ± 0.3	96
60	5/5	20.8 ± 0.2	25.2 ± 0.6	4.5 ± 0.5	100
120	5/5	20.9 ± 0.5	24.2 ± 0.5	3.3 ± 0.4	96
Female					
0	5/5	17.6 ± 0.4	21.3 ± 0.4	3.7 ± 0.3	
7.5	5/5	18.0 ± 0.4	22.0 ± 0.4	4.0 ± 0.3	103
15	5/5	18.5 ± 0.3	22.0 ± 0.3	3.5 ± 0.3	103
30	5/5	18.1 ± 0.1	22.3 ± 0.2	4.1 ± 0.3	105
60	5/5	18.2 ± 0.1	21.4 ± 0.2	3.1 ± 0.2	100
120	5/5	18.1 ± 0.3	21.2 ± 0.4	3.1 ± 0.3	100

[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test.

TABLE 17
Incidences of Selected Nonneoplastic Lesions in the Nose of Mice in the 2-Week Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male						
Number Examined Microscopically	5	0	0	5	5	5
Glands, Dilatation[a]	0			0	0	2 (1.5)[b]
Glands, Hyperplasia	0			0	0	5[**] (2.0)
Olfactory Epithelium, Atrophy	0			0	4[**] (1.0)	5[**] (2.2)
Female						
Number Examined Microscopically	5	0	0	5	5	5
Glands, Dilatation	0			0	0	5[**] (1.8)
Glands, Hyperplasia	0			0	0	5[**] (2.4)
Olfactory Epithelium, Atrophy	0			0	5[**] (1.0)	5[**] (2.2)
Infiltration Cellular, Mononuclear Cell	0			0	0	2 (1.0)

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

3-Month Study

All mice survived to the end of the study (Table 18). Final mean body weights and mean body weight gains of 120 ppm male mice were significantly less than those of the chamber controls. Dark-stained urine was frequently observed during the first month of the study in the catch pans of mice exposed to 30, 60, or 120 ppm.

The hematology data for mice in the 3-month study of tetralin are listed in Table F2. Similar to what occurred in the 3-month rat study, exposure-related effects in the erythron were observed in exposed mice. There were minimal decreases in hematocrit values (≤6%) and erythrocyte counts (≤10%) that occurred in 120 ppm males and females. An apparent response to the decreased erythron was evidenced by an increase in reticulocyte counts in 60 and 120 ppm males and females and 30 ppm females. The mean cell volume was minimally increased (≤4%) in those exposed groups, reflecting the increased circulating numbers of the larger, immature erythrocytes. Platelet counts were slightly increased (≤14%) in 60 and 120 ppm males and females, possibly reflecting a generalized increase in hematopoietic activity in response to the changes in the erythron.

The relative liver weights of 120 ppm males and 30 ppm or greater females were significantly greater than those of the chamber controls (Table H5). The absolute and relative heart weights of 120 ppm male mice were significantly less than those of the chamber control group. The absolute and relative kidney weights of 60 and 120 ppm male mice were significantly less than those of the chamber controls.

No significant differences in reproductive organ weights or in sperm parameters were observed between exposed and chamber control groups of male mice (Table I3). In female mice, the length of the estrous cycle was significantly longer in the 120 ppm group than in the chamber control group (4.6 ± 0.1 days versus 4.0 ± 0.0 days, respectively; Table I4).

Incidences of olfactory epithelium metaplasia were significantly increased in 60 and 120 ppm males and females (Table 19). Incidences of respiratory epithelium hyaline droplet accumulation were significantly increased in 120 ppm males and 60 and 120 ppm females. Metaplasia of the olfactory epithelium consisted of replacement of the normal multilayered olfactory epithelium (lining the dorsal meatuses of Levels II

TABLE 18
Survival and Body Weights of Mice in the 3-Month Inhalation Study of Tetralin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	22.9 ± 0.5	38.0 ± 1.2	15.1 ± 0.9	
7.5	10/10	23.0 ± 0.4	39.5 ± 0.8	16.4 ± 0.5	104
15	10/10	23.0 ± 0.5	37.7 ± 0.8	14.7 ± 0.5	99
30	10/10	22.9 ± 0.4	38.2 ± 1.2	15.3 ± 0.9	101
60	10/10	22.5 ± 0.5	35.9 ± 0.8	13.4 ± 0.6	94
120	10/10	22.5 ± 0.4	34.6 ± 0.8*	12.1 ± 0.7**	91
Female					
0	10/10	19.3 ± 0.2	31.4 ± 1.0	12.1 ± 1.1	
7.5	10/10	19.0 ± 0.4	30.8 ± 0.9	11.8 ± 0.7	98
15	10/10	19.2 ± 0.3	31.8 ± 0.9	12.7 ± 0.9	101
30	10/10	19.2 ± 0.3	30.9 ± 1.1	11.6 ± 0.9	98
60	10/10	19.4 ± 0.3	30.8 ± 0.7	11.5 ± 0.6	98
120	10/10	19.2 ± 0.4	29.2 ± 0.9	10.0 ± 0.9	93

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

TABLE 19
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male						
Nose[a]	10	0	2	10	10	10
Olfactory Epithelium, Metaplasia[b]	0	0	0	0	9** (1.0)[c]	10** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	0	0	0	0	9** (1.0)
Urinary Bladder	10	10	10	10	10	10
Transitional Epithelium, Eosinophilic Granules, Cytoplasmic	0	10** (1.8)	10** (2.0)	10** (2.5)	10** (2.9)	10** (3.0)
Female						
Nose	10	4	1	10	10	10
Olfactory Epithelium, Metaplasia	0	0	0	0	10** (1.4)	10** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	0	0	0	8** (1.0)	10** (1.0)
Urinary Bladder	10	10	10	10	10	10
Transitional Epithelium, Eosinophilic Granules, Cytoplasmic	0	10** (1.7)	10** (2.0)	10** (2.6)	10** (3.0)	10** (3.0)
Ovary	10	0	0	10	10	10
Atrophy	0	0	0	0	4* (2.0)	8** (2.0)
Uterus	10	10	10	10	10	10
Atrophy	0	0	2 (2.0)	2 (2.0)	6** (2.0)	8** (2.0)

* Significantly different ($P \leq 0.05$) from the chamber 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

and III) by tall, ciliated, columnar epithelial cells, morphologically consistent with respiratory epithelial cells. Hyaline droplet accumulation of the respiratory epithelium consisted of homogenous, brightly eosinophilic droplets in the cytoplasm of the respiratory epithelial cells primarily at the interface of the respiratory and olfactory epithelia in Level II of the nasal cavity.

Incidences of cytoplasmic eosinophilic granules within the transitional epithelium lining the urinary bladder were significantly increased in all exposed groups of males and females; in general, there was an exposure concentration-related increase in the severity of this lesion in both sexes (Table 19). The change was characterized by prominent accumulation of numerous, small, refractile, spherical, brightly eosinophilic granules within the apical cytoplasm of the most superficial layer of the transitional epithelial cells. The granules were iron and PAS negative but stained faintly orange-red with the Mallory-Heidenhain stain. The biological significance of this change is not clear but could represent a pinocytosed metabolite of tetralin.

Incidences of ovarian atrophy and uterine atrophy were significantly increased in 60 and 120 ppm females (Table 19). Affected ovaries were smaller than ovaries of the chamber control group, more basophilic, and had decreased numbers of follicles and larger corpora lutea. Uterine atrophy was characterized by uteri that were smaller than those of the chamber controls with concomitant decreased prominence of endometrial glands and stroma.

Exposure Concentration Selection Rationale: Tetralin had no effect on survival in male or female mice. The body weight and histopathology changes were not considered severe enough to limit selection of 120 ppm as the highest exposure concentration. Therefore, the exposure concentrations selected for the 2-year inhalation study in mice were 30, 60, and 120 ppm.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 20 and in the Kaplan-Meier survival curves (Figure 4). Survival of 60 and 120 ppm female mice was significantly greater than that of the chamber controls. Survival of exposed groups of male mice was similar to that of the chamber controls.

Body Weights and Clinical Findings

Although the mean body weights of 60 and 120 ppm mice were up to 10% less than those of the chamber controls during portions of the study, the mean body weights of all exposed groups of mice were similar to those of the chamber controls at the end of the study (Tables 21 and 22; Figure 5).

Dark-stained urine was observed in all exposed groups of male mice and in females exposed to 60 or 120 ppm but was more frequent in males (males: 0 ppm, 0/50; 30 ppm, 8/50; 60 ppm, 36/50; 120 ppm, 45/50; females: 0/50, 0/50, 3/50, 8/50). The incidences of dark-stained urine in males increased with increasing exposure concentration.

Urinary Metabolites and Urinalysis at 12 Months

Creatinine-adjusted levels of all urinary metabolites increased with increasing exposure concentration in male and female mice: in male mice, 4-hydroxy-1-tetralone > 1-tetralol > 2-tetralol > 2-hydroxy-1-tetralone; in female mice, 4-hydroxy-1-tetralone > 1-tetralol > 2-hydroxy-1-tetralone > 2-tetralol. Production of some metabolites exceeded dose proportionality in the 120 ppm groups as can be seen from the exposure concentration-adjusted values (Table G5).

TABLE 20
Survival of Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	9	8	7	11
Natural deaths	5	7	5	3
Animals surviving to study termination	36	35	38	36
Percent probability of survival at end of study[a]	72	70	76	72
Mean survival (days)[b]	695	700	703	693
Survival analysis[c]	P = 1.000N	P = 1.000	P = 0.784N	P = 1.000
Female				
Animals initially in study	50	50	50	50
Moribund	17	9	6	6
Natural deaths	2	3	2	1
Animals surviving to study termination	31	38	42	43
Percent probability of survival at end of study	62	76	84	86
Mean survival (days)	663	698	725	723
Survival analysis	P = 0.004N	P = 0.157N	P = 0.012N	P = 0.006N

[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 chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.

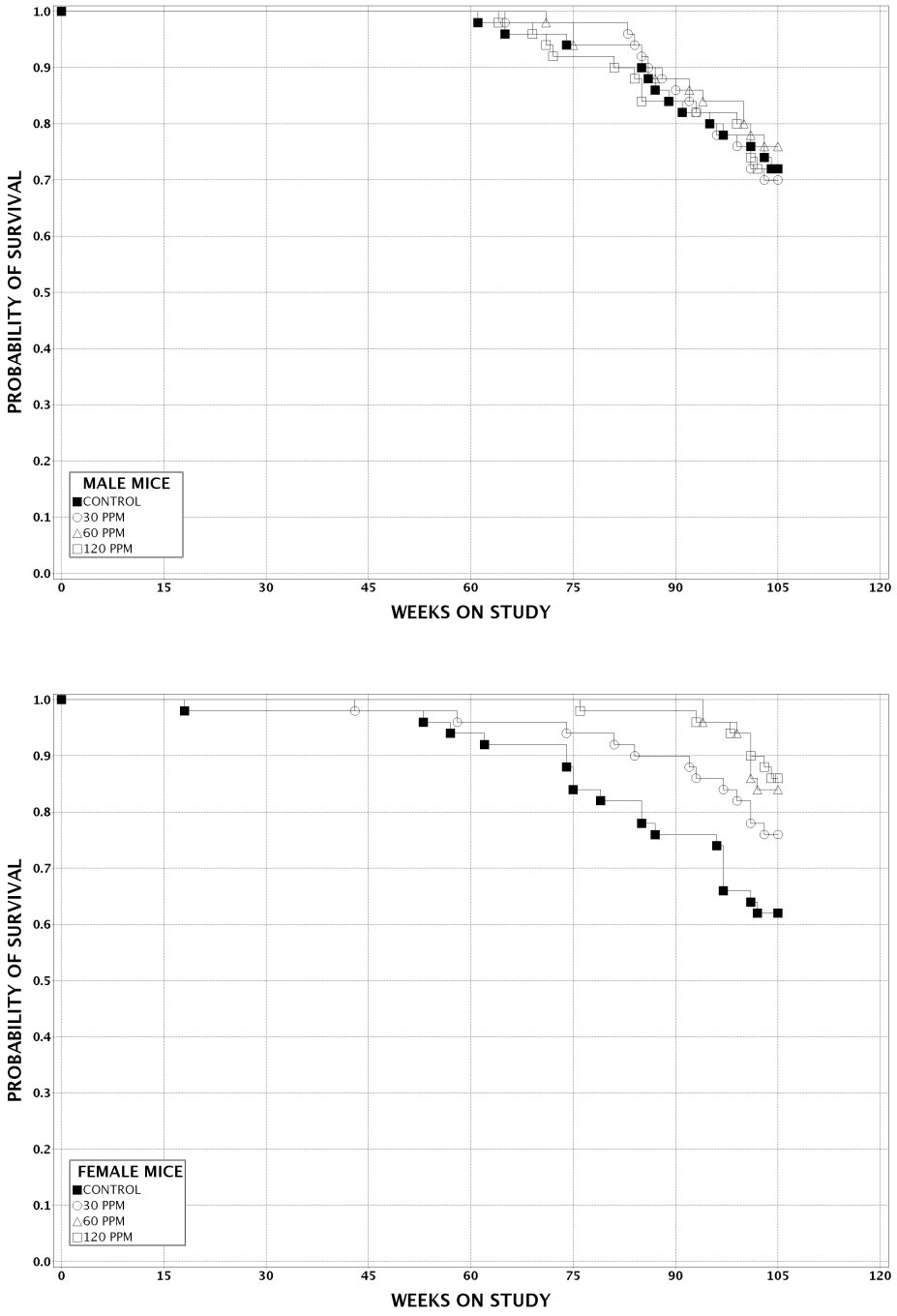


FIGURE 4
Kaplan-Meier Survival Curves for Mice Exposed to Tetralin by Inhalation for 2 Years

TABLE 21
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Tetralin

Days on Study	Chamber Control		30 ppm			60 ppm			120 ppm		
	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	23.6	50	23.6	100	50	23.5	99	50	23.4	99	50
11	25.6	50	25.3	99	50	25.3	99	50	25.1	98	50
18	26.9	50	26.7	99	50	26.4	98	50	26.1	97	50
25	27.8	50	27.5	99	50	27.3	98	50	26.8	97	50
32	29.0	50	28.5	99	50	28.4	98	50	27.9	96	50
39	29.8	50	29.6	99	50	29.4	99	50	28.7	96	50
46	30.8	50	30.4	99	50	30.1	98	50	29.6	96	50
53	31.8	50	31.0	97	50	30.8	97	50	30.3	95	50
60	32.7	50	31.9	97	50	31.6	97	50	31.0	95	50
67	33.4	50	32.5	97	50	32.5	97	50	31.8	95	50
74	34.7	50	33.8	97	50	33.4	96	50	32.7	94	50
81	35.7	50	34.9	98	50	34.3	96	50	33.6	94	50
88	36.9	50	35.8	97	50	35.3	96	50	34.4	93	50
116	39.7	50	38.4	97	50	37.5	94	50	36.5	92	50
144	42.2	50	40.9	97	50	39.6	94	50	38.7	92	50
172	44.4	50	43.2	97	50	41.7	94	50	40.7	92	50
200	46.3	50	44.9	97	50	43.4	94	50	42.2	91	50
228	47.4	50	46.3	98	50	44.6	94	50	43.6	92	50
256	49.2	50	48.5	99	50	46.7	95	50	45.8	93	50
284	50.0	50	49.7	99	50	48.0	96	50	47.3	95	50
312	50.3	50	50.4	100	50	48.8	97	50	48.4	96	50
340	50.5	50	51.1	101	50	49.6	98	50	49.3	98	50
367	50.8	50	51.5	101	50	50.3	99	50	49.9	98	50
396	51.6	50	52.9	102	50	52.6	102	50	52.1	101	50
424	51.6	49	53.0	103	50	52.6	102	50	52.3	101	50
452	52.4	48	53.7	103	50	53.3	102	50	53.2	102	49
480	52.7	48	54.3	103	49	54.3	103	50	54.1	103	48
508	52.8	48	54.5	103	49	54.2	103	49	54.3	103	46
536	52.7	47	54.6	104	49	54.3	103	47	54.6	104	46
564	52.7	47	54.9	104	49	54.8	104	47	55.4	105	45
592	51.8	46	54.4	105	46	54.8	106	47	55.8	108	42
620	52.9	42	54.2	103	44	54.9	104	44	55.7	105	42
648	52.5	41	55.0	105	41	54.8	104	43	55.1	105	42
662	51.9	41	54.4	105	41	54.7	105	42	55.0	106	41
676	52.0	40	55.0	106	39	54.5	105	42	54.4	105	41
690	51.3	39	54.7	107	38	53.1	104	42	52.9	103	40
704	51.4	39	54.6	106	38	53.5	104	40	53.1	103	39
718	50.7	38	54.5	108	35	53.4	105	38	53.6	106	36
Mean for weeks											
1-13	30.7		30.1	98		29.9	97		29.3	95	
14-52	46.7		45.9	98		44.4	95		43.6	93	
53-103	52.0		54.1	104		53.8	103		53.8	103	

TABLE 22
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Tetralin

Days on Study	Chamber Control		30 ppm			60 ppm			120 ppm		
	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	19.8	50	19.5	99	50	19.5	98	50	19.4	98	50
11	21.6	50	21.5	100	50	21.2	98	50	21.6	100	50
18	22.4	50	22.6	101	50	22.5	101	50	22.5	100	50
25	23.7	50	23.5	99	50	23.0	97	50	23.4	98	50
32	24.6	50	24.7	101	50	24.3	99	50	24.5	100	50
39	25.1	50	25.6	102	50	25.4	101	50	25.3	101	50
46	25.9	50	26.4	102	50	26.0	100	50	25.9	100	50
53	26.9	50	27.0	100	50	26.5	99	50	26.6	99	50
60	27.3	50	27.5	101	50	27.4	101	50	27.4	101	50
67	28.1	50	28.3	101	50	28.0	100	50	28.1	100	50
74	28.9	50	28.9	100	50	28.6	99	50	28.4	98	50
81	29.6	50	29.7	100	50	29.5	100	50	29.5	100	50
88	30.3	50	30.3	100	50	30.2	100	50	30.1	100	50
116	33.1	50	32.9	100	50	32.2	97	50	32.1	97	50
144	36.3	49	35.9	99	50	34.6	95	50	34.5	95	50
172	38.5	49	37.7	98	50	36.0	94	50	36.6	95	50
200	41.0	49	39.5	96	50	37.7	92	50	38.3	94	50
228	42.9	49	41.4	96	50	39.2	91	50	39.9	93	50
256	45.4	49	43.6	96	50	41.3	91	50	42.4	94	50
284	47.2	49	45.9	97	50	43.2	92	50	44.6	94	50
312	49.6	49	47.2	95	49	44.5	90	50	45.7	92	50
340	49.5	49	48.3	98	49	45.2	91	50	47.0	95	50
367	51.7	48	49.6	96	49	46.5	90	50	47.7	92	50
396	54.9	47	53.0	96	49	49.8	91	50	51.0	93	50
424	55.6	47	53.7	97	48	50.2	90	50	51.8	93	50
452	58.2	46	56.0	96	48	52.2	90	50	53.6	92	50
480	59.7	46	57.6	96	48	53.6	90	50	54.9	92	50
508	60.4	46	58.8	97	48	54.7	91	50	56.0	93	50
536	61.2	42	60.6	99	47	55.8	91	50	56.3	92	49
564	62.1	41	61.3	99	46	56.2	90	50	57.4	92	49
592	61.8	39	61.2	99	45	55.9	90	50	56.9	92	49
620	60.9	38	61.0	100	45	56.7	93	50	57.2	94	49
648	59.9	38	60.5	101	43	56.3	94	50	57.0	95	48
662	58.3	38[a]	59.9	103	43	56.6	97	48	56.6	97	48
676	58.4	34	59.6	102	42	55.8	96	48	56.0	96	48
690	56.8	33	58.5	103	41	54.7	96	47	54.8	97	47
704	55.8	33	58.4	105	40	54.9	98	44	54.3	97	47
718	55.0	31	57.3	104	39	54.4	99	42	53.8	98	45
Mean for weeks											
1-13	25.7		25.8	100		25.5	99		25.6	100	
14-52	42.6		41.4	97		39.3	92		40.1	94	
53-103	58.2		57.9	99		54.0	93		54.7	94	

[a] The number of animals weighed was less than the number of animals surviving.

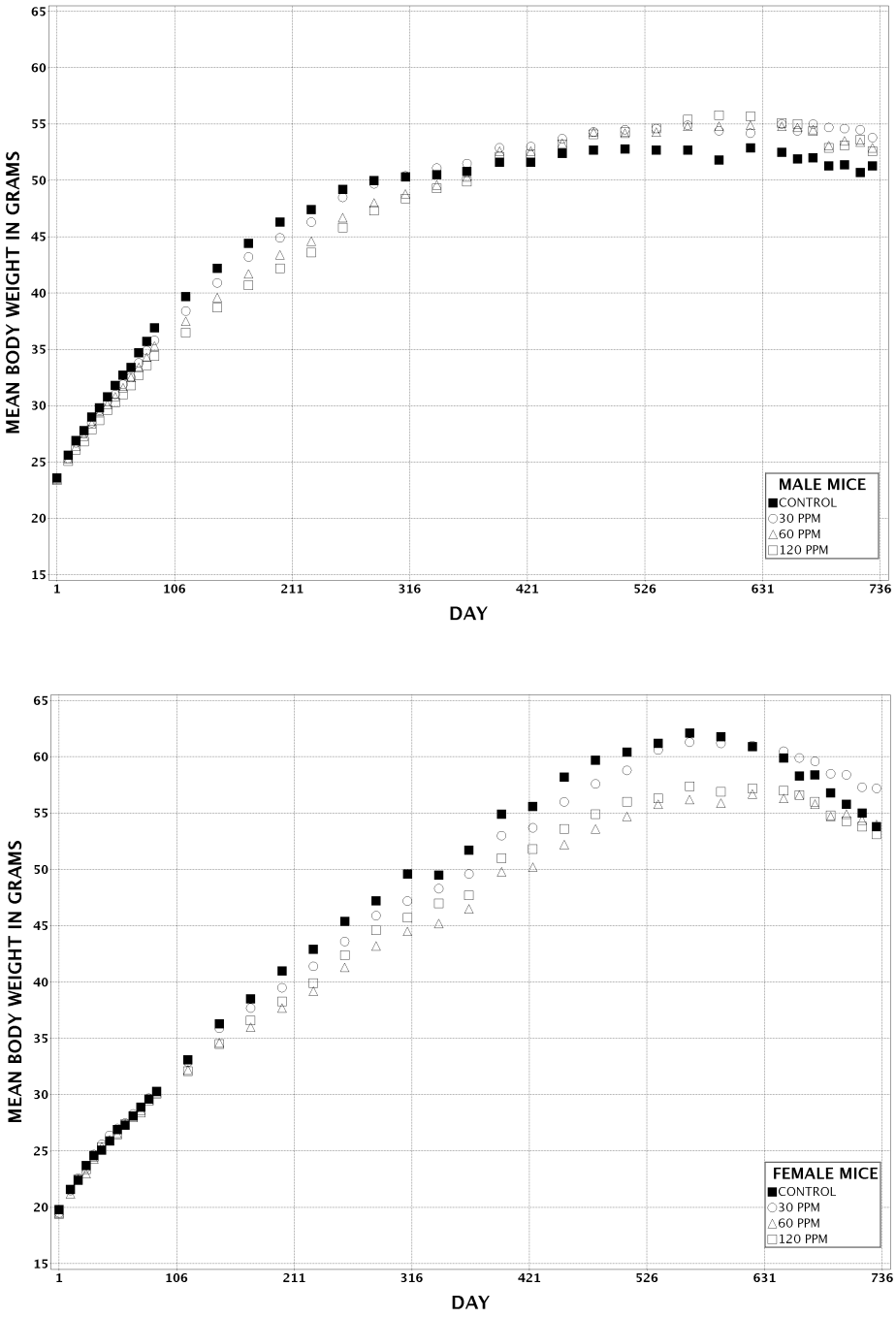


FIGURE 5
Growth Curves for Mice Exposed to Tetralin by Inhalation for 2 Years

Male mice generally produced higher concentrations of metabolites than female mice at all exposure concentrations. No treatment-related effects were demonstrated by urinalysis evaluations performed at 12 months (Table G5).

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 spleen, nose, urinary bladder, and eye. 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 neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Spleen: There was a positive trend in the incidences of hemangiosarcoma in female mice, and the incidence in 120 ppm females exceeded the historical control range for inhalation studies but not for all routes of administration (Tables 23, D1, D2, and D3). Hemangiosarcomas were small, focal to focally expansive lesions that effaced the parenchyma and consisted of prominent, irregular, and variably sized vascular spaces that contained red blood cells. The vascular spaces were separated by variable amounts of stroma and lined by pleomorphic endothelial cells that had plump nuclei. Occasional mitoses were observed.

Nose: The incidences of glandular hyperplasia, olfactory epithelium atrophy, and respiratory metaplasia in exposed groups of mice were significantly greater than those in the chamber controls (Tables 24, C3, and D4). In general, the severities of the olfactory epithelium lesions increased with increasing exposure concentration. The incidences of suppurative inflammation were significantly increased in all exposed groups of male and female mice.

The microscopic changes in the olfactory epithelium occurred primarily in the dorsal meatus in Level II of the nasal cavity and the epithelium of the upper one-third of the nasal septum and adjacent turbinates in Level III. The epithelium had a gradation of changes ranging from thinning and disorganization of the epithelium (olfactory epithelium atrophy) due to segmental loss of the olfactory epithelial cells (Plate 5) to complete replacement of the multilayered epithelium by a single layer of tall columnar epithelial cells with extension into the underlying Bowman's glands (respiratory metaplasia and Bowman's gland hyperplasia; Plates 6 and 7). Bowman's glands were prominent, tortuous, and lined by profilerating cuboidal to tall columnar epithelial cells that were continuous with the metaplastic epithelium replacing the olfactory epithelium. Many Bowman's glands were dilated and contained inflammatory cells, secretory material, and eosinophilic debris. Minimal suppurative inflammation accompanied the olfactory epithelium changes and consisted of accumulations of neutrophils and proteinaceous debris

TABLE 23
Incidences of Hemangiosarcoma of the Spleen in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Overall rate[a,b]	1/50 (2%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate[c]	2.5%	0.0%	2.1%	8.2%
Terminal rate[d]	1/31 (3%)	0/38 (0%)	1/42 (2%)	3/43 (7%)
First incidence (days)	731 (T)	—[f]	731 (T)	705
Poly-3 test[e]	P = 0.041	P = 0.479N	P = 0.718N	P = 0.239

(T) Terminal sacrifice
a Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 6/398 (1.5% ± 1.4%), range 0%-4%; all routes: 27/1,478 (1.8% ± 2.4%), range 0%-10%
b Number of animals with neoplasm per number of animals with spleen examined microscopically
c Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
d Observed incidence at terminal kill
e Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by **N**.
f Not applicable; no neoplasms in animal group

TABLE 24
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Male				
Nose[a]	49	49	50	50
Glands, Olfactory Epithelium, Hyperplasia[b]	14 (1.0)[c]	49**(2.1)	50**(3.0)	49**(3.1)
Olfactory Epithelium, Atrophy	2 (1.0)	49**(2.6)	50**(3.3)	50**(3.8)
Olfactory Epithelium, Metaplasia, Respiratory	2 (1.0)	47**(1.6)	50**(2.3)	49**(2.4)
Inflammation, Suppurative	2 (1.5)	26**(1.0)	45**(1.3)	45**(1.6)
Urinary Bladder	49	47	50	48
Transitional Epithelium, Eosinophilic Granules, Cytoplasmic	0	47**(1.0)	50**(1.0)	48**(1.0)
Female				
Nose	50	50	50	49
Glands, Olfactory Epithelium, Hyperplasia	17 (1.0)	50**(2.1)	50**(3.0)	49**(3.0)
Olfactory Epithelium, Atrophy	1 (1.0)	50**(2.8)	50**(3.4)	49**(3.9)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	49**(1.4)	50**(2.9)	49**(3.3)
Inflammation, Suppurative	3 (1.0)	28**(1.0)	48**(1.3)	46**(1.2)
Urinary Bladder	49	50	49	49
Transitional Epithelium, Eosinophilic Granules, Cytoplasmic	0	50**(1.0)	49**(1.0)	49**(1.0)
Eye	49	49	49	49
Cornea, Mineralization	0	3 (1.0)	3 (1.0)	12**(1.1)

** Significantly different ($P \leq 0.01$) from the chamber control group by the Poly-3 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

in the mucosal epithelium, nasal passages, and olfactory epithelium glands.

Urinary Bladder: The incidences of minimal transitional epithelium cytoplasmic eosinophilic granules were significantly increased in all exposed groups of male and female mice (Tables 24, C3, and D4). The change was similar to that observed in the 3-month mouse study and consisted of numerous, small, refractile, spherical, brightly eosinophilic granules within the apical cytoplasm of the most superficial layer of the transitional epithelium cells (Plate 8). The biological significance of this change is not clear but could represent a pinocytosed metabolite of tetralin.

Eye: The incidence of minimal corneal mineralization was significantly increased in 120 ppm females (Tables 24 and D4). Corneal mineralization also occurred in 30 and 60 ppm females, but the differences from the chamber controls were not significant.

GENETIC TOXICOLOGY

Tetralin (0.3 to 333 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 when testing was conducted with or without induced rat or hamster liver metabolic activation enzymes (Table E1). A second bacterial mutagenicity assay conducted with the same lot of tetralin (2 to 500 µg/plate) used in the 2-year study showed no mutagenicity in *S. typhimurium* strains TA98 or TA100 or in *Escherichia coli* strain WP2 *uvrA*, with or without rat liver activation enzymes (Table E2). At the end of the 3-month study, no increase in the frequency of micronucleated normochromatic (mature) erythrocytes was seen in peripheral blood samples of male or female B6C3F1 mice (Table E3). In both male and female mice, the percentages of immature (polychromatic) erythrocytes generally increased with increasing tetralin concentration, suggesting possible stimulation of erythropoiesis as a response to exposure.

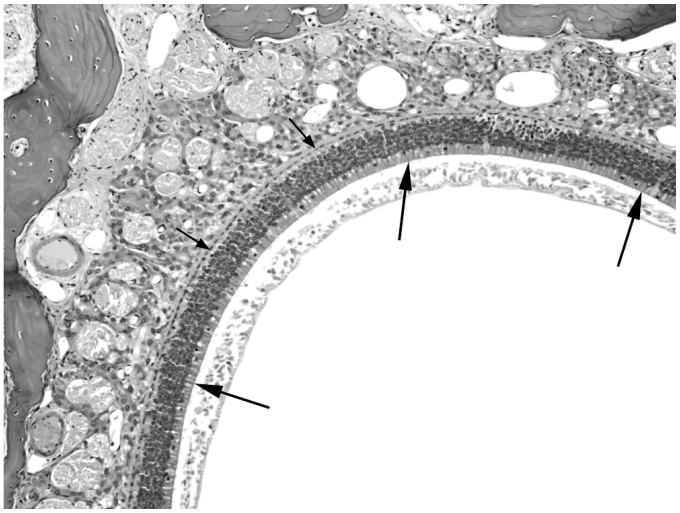


Plate 1
Normal olfactory epithelium lining the nasal passages (meatuses) in the Level III section of the nasal cavity of a male chamber control rat in the 2-year study of tetralin. The epithelium appears multilayered or pseudostratified (long arrows), and the basal border is lined by a single layer of flattened basal epithelial cells (short arrows). H&E

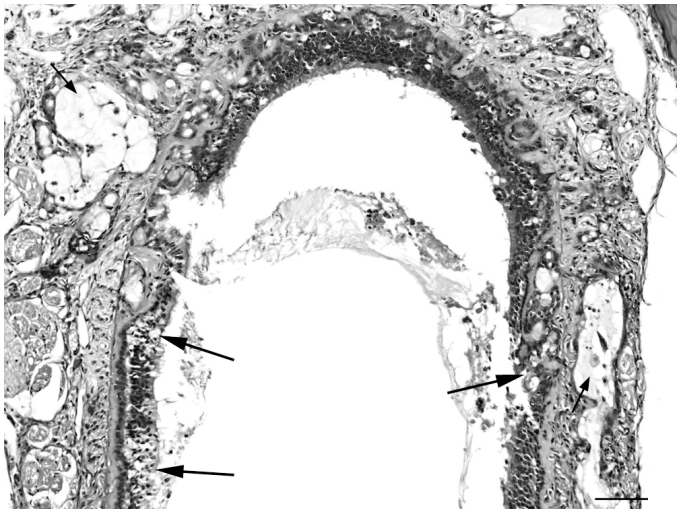


Plate 2
Olfactory epithelial degeneration in a male rat exposed to 120 ppm tetralin for 2 years. Note the focal loss and disorganization of the epithelium (long arrows) and the dilated Bowman's glands containing mucus, a few inflammatory cells, and cell debris (short arrows). The nasal passage contains proteinaceous material. H&E

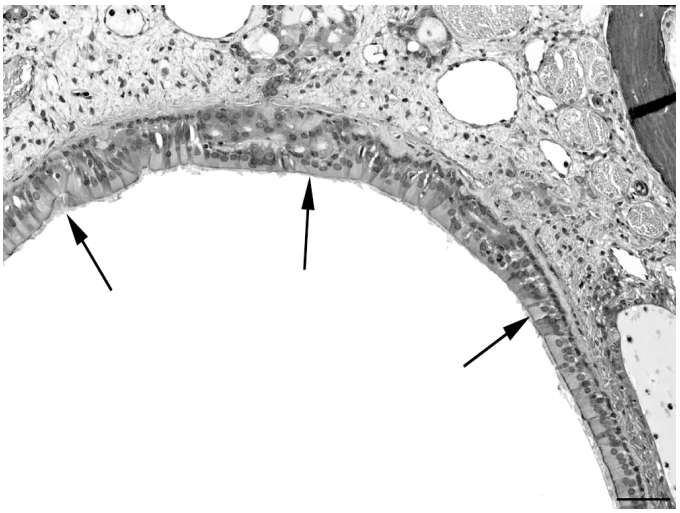


Plate 3
Respiratory epithelial metaplasia in a male rat exposed to 120 ppm tetralin for 2 years. The normal olfactory epithelium is completely replaced by a single layer of tall columnar epithelial cells (arrows). H&E

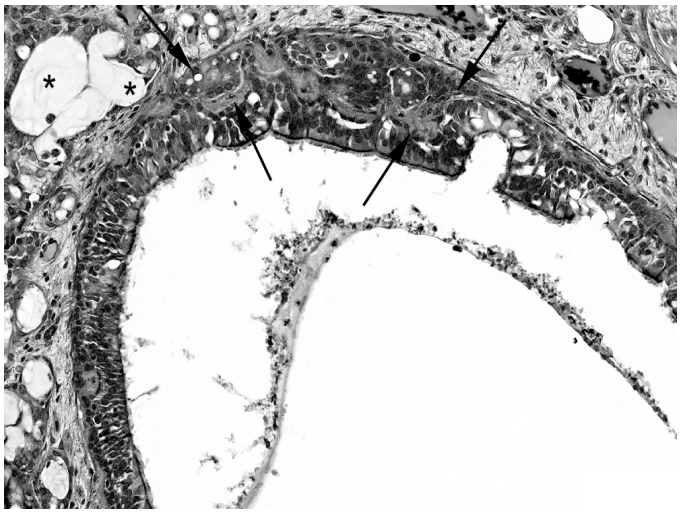


Plate 4
Olfactory epithelial basal cell hyperplasia in a male rat exposed to 120 ppm tetralin for 2 years. Basal cell hyperplasia consists of focal proliferation of the epithelial cells lining the base of the olfactory epithelium (arrows). Note dilated Bowman's glands (asterisks) and proteinaceous material in the nasal passage. H&E

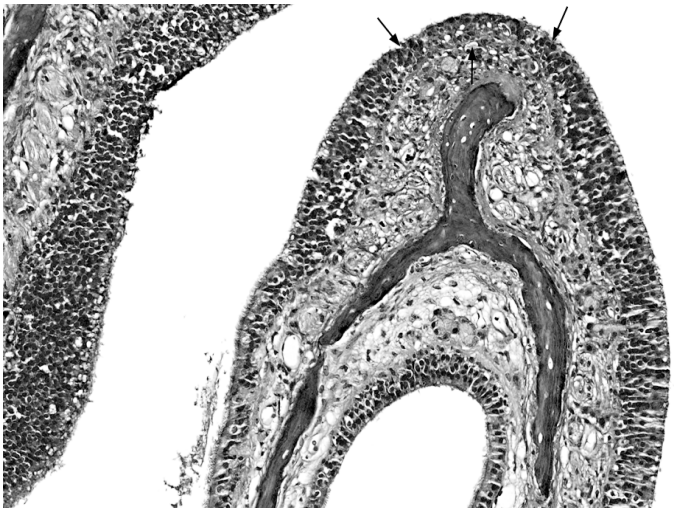


Plate 5
Olfactory epithelial atrophy in a male mouse exposed to 120 ppm tetralin for 2 years. Note segmental thinning of the epithelium due to loss of olfactory epithelial cells (arrows). H&E

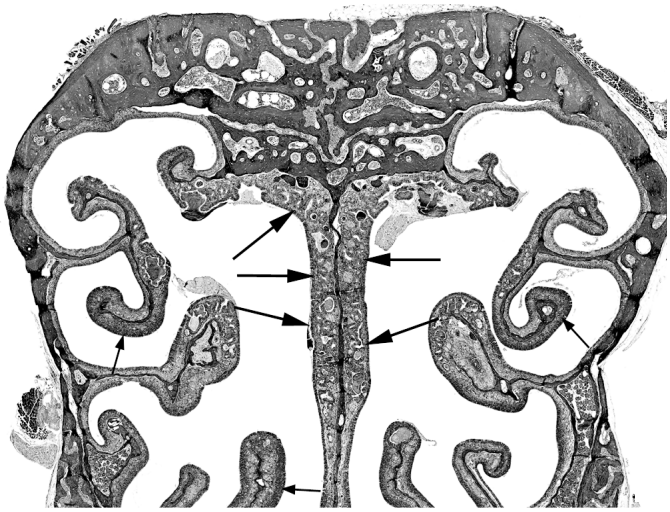


Plate 6
Olfactory epithelial respiratory metaplasia and Bowman's gland epithelial hyperplasia in a female mouse exposed to 120 ppm tetralin for 2 years. The normal olfactory epithelium lining the dorsal nasal passages (meatuses), adjacent turbinates, and dorsal one-third of the nasal septum and epithelium of the submucosal Bowman's glands are replaced by tall columnar epithelial cells (long arrows). Note the remnants of the normal olfactory epithelium (short arrows). H&E

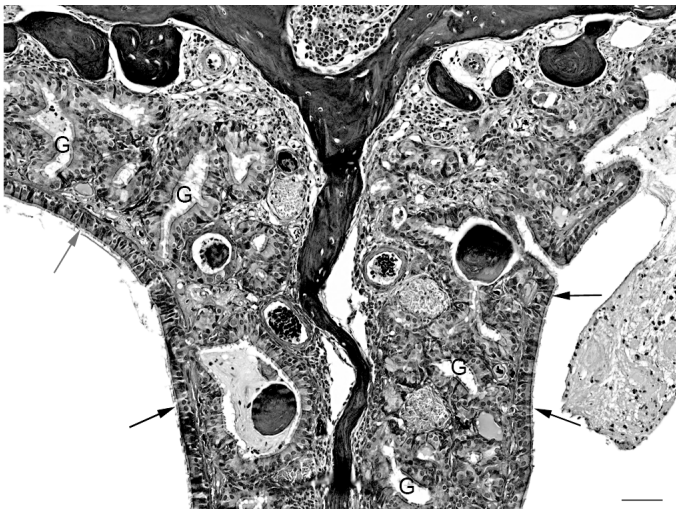


Plate 7
Higher magnification of Plate 6. Note tall ciliated columnar epithelial cells lining the dorsal nasal passages (arrows) and extending into the submucosal Bowman's glands (G) which are enlarged and tortuous and some of which contain mucous, proteinaceous secretory material. Infiltrates of neutrophils are scattered in the lamina propria around the glands. H&E

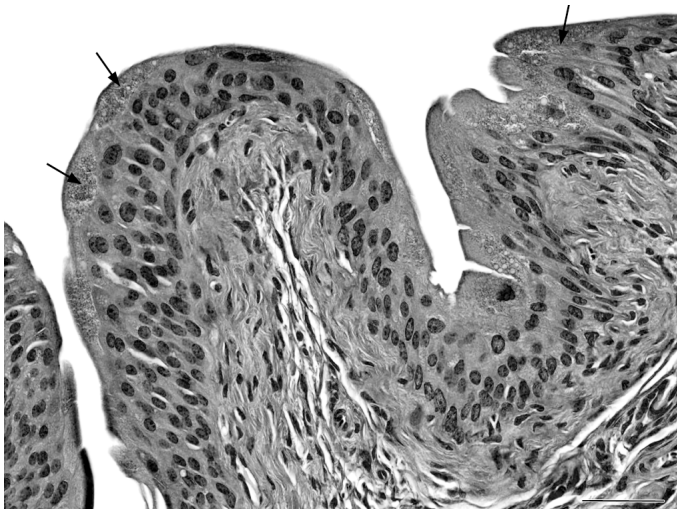


Plate 8
Transitional epithelium of the urinary bladder from a female mouse exposed to 120 ppm tetralin for 2 years. Note the accumulation of eosinophilic granules in the cytoplasm of the superficial transitional epithelial cells (arrows). H&E

DISCUSSION AND CONCLUSIONS

Tetralin was nominated by the National Cancer Institute for carcinogenicity studies because of its structure, high production volume, and high potential for worker and consumer exposure through its use as an industrial solvent in paints, waxes, and polishes. The most likely human exposure to tetralin is through dermal contact or inhalation during manufacture or use. Tetralin is structurally related to other rodent carcinogens including decalin and naphthalene (NTP 1992, 2000, 2005a). The present studies were designed to investigate the toxicity and carcinogenicity of tetralin in F344/N rats and B6C3F1 mice with special interest in the relationship between α2u-globulin accumulation, nephropathy, and renal carcinogenesis. The chemical structure of tetralin indicated a potential to induce α2u-globulin nephropathy, a renal syndrome in male rats characterized by the accumulation of hyaline droplets in the proximal tubule epithelium. In addition to F344/N rats, male NCI Black Reiter (NBR) rats were used in the 2-week study to determine if tetralin induces renal toxicity through α2u-globulin nephropathy. NBR rats do not develop this syndrome because they do not produce appreciable amounts of α2u-globulin and were exposed to tetralin concurrently with F344/N rats for comparison of renal lesion development. Strains of male rats, including Fischer rats, respond to α2u-globulin-inducing chemicals by first accumulating hyaline droplets in the renal tubules before developing renal toxicity and neoplasms (USEPA, 1991). For example, the structurally related chemical decalin induced renal toxicity and carcinogenicity in male F344/N rats by first inducing hyaline droplet accumulation (NTP, 2005a). NBR rats did not develop renal toxicity following exposure to decalin (Ridder *et al.*, 1990; NTP, 2005a).

The toxicity and carcinogenicity studies of tetralin were conducted in rats and mice by inhalation at exposure concentrations up to 120 ppm for 2 weeks, 3 months, or 2 years. The exposure concentration of 120 ppm was the maximum allowable vapor concentration generated without producing an aerosol by the inhalation exposure chamber system used.

Inhalation exposure to tetralin targeted the urinary tract of F344/N rats and B6C3F1 mice. In rats, the main target of tetralin toxicity was the kidney, whereas in mice, the urinary bladder was the target. Dark-stained urine was noted in many male and female rats and mice during the 2-week studies and occasionally in rats and mice exposed to 30 ppm or greater during the first 4 weeks of the 3-month studies. In the 2-year studies, the incidences of dark-stained urine generally increased with increasing exposure concentration. The cause and significance of the dark-stained urine are not clear (Tshala-Katumbay *et al.*, 2006) but appeared to be unrelated to kidney toxicity because histopathologic changes were not found in the urinary tracts of male NBR rats or female F344/N rats. Humans exposed to tetralin excreted dark, green-gray urine similar to that observed in rodents (Sandmeyer, 1981).

There were significant increases in kidney weights in male and female F344/N rats in the 2-week and 3-month studies; these were generally related to exposure concentration. In male NBR rats, only one exposure group (7.5 ppm) had increased kidney weights. In the 2-week and 3-month studies, the increases in kidney weights in male F344/N rats were accompanied by significant increases in α2u-globulin/soluble protein concentrations and significant increases in labeling indices in the proximal renal tubule epithelial cells that were not related to exposure concentration or duration. The latter finding is suggestive of a proliferative response secondary to tissue injury. In the 3-month study, the urinalysis data indicated that tetralin exposure caused significantly increased exposure concentration-related increases in urinary aspartate aminotransferase/creatinine ratios in male and female rats that were of similar magnitude (twofold or greater). In addition, urinary lactate dehydrogenase/creatinine ratios were significantly increased in male rats. These urinary changes are consistent with membrane injury and subsequent enzyme leakage.

The primary histologic changes observed in the 2-week and 3-month rat studies were increases in the severity of

hyaline droplet accumulation in the epithelium of the proximal renal tubules that manifested as changes in the size and character of the droplets. In the 2-week study, the severity increased with increasing exposure concentration. Hyaline droplet accumulation was observed in all male F344/N rats, including the chamber controls, but was not observed in female F344/N rats or male NBR rats.

In the 2-year rat study, marginal increases in the incidences of renal neoplasms (adenoma) were identified in males by standard single-section histopathologic evaluation of the kidney. Therefore, an extended histopathologic evaluation was conducted in additional sections (step-sections) from the kidneys of male rats. Additional proliferative lesions (hyperplasia and adenoma) were identified in the step-section analysis, primarily in the highest exposure concentration group (120 ppm). No renal tubule carcinomas were found. When the incidences of the single and step-sections were combined, there were increases in the incidences of renal tubule hyperplasia and adenoma primarily in 120 ppm males, and the increase in the incidence of renal tubule adenoma was considered to be some evidence of carcinogenic activity in the kidney of male rats. One adenoma and one carcinoma occurred in the 120 ppm female group.

In the 2-year rat study, chronic progressive nephropathy (CPN) was observed in almost all male rats, including chamber controls; however, average severity increased with increasing exposure concentration and was most severe in the 120 ppm exposure group. CPN is one of the most commonly observed spontaneous lesions in rats and as a syndrome is more prevalent and severe in male rats (Seely *et al.*, 2002). However, this syndrome can be exacerbated by chemical exposure, resulting in increased incidences and average severities (Lock and Hard, 2004). CPN also occurred in a large percentage of female rats, including chamber controls; however, the incidences and severities in exposed groups were generally similar to those in the chamber control group. The incidence of hyperplasia of the transitional epithelium lining the renal pelvis was also slightly increased in male rats at 120 ppm. Such hyperplasia frequently accompanies severe CPN (Montgomery and Seely, 1990), and in the present study, the increased incidence may reflect exacerbated CPN. Increased cell turnover associated with exacerbation of CPN is recognized as a kidney tumor risk factor (Swenberg *et al.*, 1989; Hard *et al.*, 1997; Hard, 1998).

The results of the tetralin studies in male rats are suggestive of α2u-globulin nephropathy and include increased α2u-globulin levels, increased renal cortex tubule cell labeling indices and hyaline droplet accumulation in renal tubule epithelial cells in the 2-week and 3-month studies, and evidence of exacerbated CPN in the 2-year study. In male rats, α2u-globulin is first detectable by 5 to 6 weeks of age, reaches maximum levels by approximately 2 to 4 months of age, and gradually declines thereafter (Motwani *et al.*, 1984; MacInnes *et al.*, 1986; Richardson *et al.*, 1987). NBR rats, female F344/N rats, and male and female B6C3F1 mice do not produce α2u-globulin and thus do not develop α2u-globulin nephropathy (MacInnes, *et al.*, 1986; Chatterjee *et al.*, 1989; Lehman-McKeeman and Caudill, 1992). In α2u-globulin nephropathy, renal toxicity is associated with the accumulation of a protein, α2u-globulin, in the form of brightly eosinophilic hyaline droplets in the cytoplasm of the proximal tubule epithelium (Swenberg *et al.*, 1989; Hard *et al.*, 1993; Swenberg and Lehman-McKeeman, 1999). The proposed sequence of events in the pathogenesis of α2u-globulin nephropathy involves binding of a chemical or its metabolites to α2u-globulin, which changes the conformation of the protein and decreases the rate of or prevents its degradation, ultimately resulting in accumulation within phagolysosomes of the renal tubule epithelial cells. The accumulation of α2u-globulin is thought to cause lysosomal dysfunction and subsequent release of lysosomal enzymes into the cytoplasm resulting in a cycle of cytotoxicity, cell death, and a compensatory increase in cell proliferation that, if chronic, may lead to the promotion of neoplastic lesions (Swenberg *et al.*, 1989; Borghoff *et al.*, 1990). Alternatively, it has been proposed that α2u-globulin may serve as a vector to increase the delivery of a toxicant or protoxicant to proximal tubule cells, so that nephrotoxicity occurs not from the abnormal accumulation and degradation of α2u-globulin, but because chemical levels are elevated in the renal tubules (Melnick, 1992). In either scenario, it is thought that cell proliferation in response to chronic cell injury and loss may increase the likelihood of fixing DNA damage into heritable mutations or promoting clonal expansion of initiated cells, resulting in carcinogenesis (Swenberg *et al.*, 1989; Borghoff *et al.*, 1990; Melnick, 1992; Lehman-McKeeman, 1993).

In a review of several prechronic and chronic NTP studies of compounds that induced α2u-globulin nephropathy, several lesions were identified as generally associated

with this syndrome (Doi *et al.*, 2007). These included α2u-globulin accumulation, increased cell proliferation, hyaline droplet formation, tubule regeneration, and granular cast formation in prechronic studies; exacerbated CPN, linear mineralization in the renal papilla, and renal tubule hyperplasia in chronic studies. Exacerbated CPN and linear mineralization, both indicators of sustained injury, were the best predictors of neoplasm outcome. In the current study, the observation of some of these features suggests that an α2u-globulin-dependent mechanism may have played a role in the development of the histopathologic lesions and the observed renal neoplasm outcome in male rats. However, several lines of evidence suggest that tetralin might cause nephrotoxicity by a mechanism other than or in addition to α2u-globulin nephropathy. Granular casts and papillary linear mineralization, considered key histopathologic features of α2u-globulin nephropathy, were not observed in the current 2-year study. Increased kidney weights and altered urine chemistry parameters (increased enzyme levels) indicative of renal injury were also observed in female rats and male and female mice. Tetralin-induced nephrotoxicity has been reported in various animal species (Longacre, 1987) in addition to male rats. Furthermore, human cases of kidney damage have been reported following oral ingestion of tetralin (Sandmeyer, 1981; Longacre, 1987). Whether tetralin exerts kidney toxicity in humans similar to that in rats is not clear.

Tetralin appeared to be a less potent renal toxicant than decalin (NTP, 2005a). Higher incidences of renal lesions, including renal tubule carcinoma, were induced by decalin than by tetralin at equivalent exposure concentrations. Decalin also induced intratubular casts and inflammation not seen in the present tetralin study. This could be due to the aromatic ring structure of tetralin resulting in formation of water soluble metabolites and more rapid elimination from the kidney compared to decalin (Servé *et al.*, 1989).

Although there were no changes in the mouse kidney epithelia, the urinary tract was also a target in the 2-year mouse study. An exposure concentration-related increase in the incidences of refractile eosinophilic granules were observed in the apical cytoplasm of the transitional epithelial cells lining the urinary bladder of most male and female mice exposed to tetralin for 3 months or 2 years. In the 3-month study, the severity increased with increasing exposure concentration. Similar changes were not observed in the transitional epithelium of the urinary bladder of rats exposed to tetralin. Since the incidences of these granules increased with increasing exposure concentration, they are presumably related to metabolism and/or excretion of tetralin or its metabolites. This unique change was observed in male and female mice exposed to anthraquinone in the feed for 2 years (NTP, 2005b). Anthraquinone induced low incidences of hyperplasia and rare benign (papilloma) and malignant (carcinoma) neoplasms in the transitional epithelium of the urinary bladder in exposed male and female rats but not in mice. In the current 2-year study of tetralin, a papilloma of the transitional epithelium of the urinary bladder occurred in a female rat exposed to the lowest exposure concentration (30 ppm).

In the 2-year studies, the male and female reproductive systems were also targets of tetralin exposure. Significant increases in the incidences of seminiferous tubule atrophy were accompanied by significant increases in the incidences of testicular interstitial cell adenoma in 30 and 120 ppm male rats. The increased incidences of testicular interstitial cell adenoma may have been related to tetralin exposure. This is a commonly observed spontaneous neoplasm in control and treated F344/N male rats. Although the incidences were statistically significant compared to the chamber control group, they were within the historical control range of 58%-84% for 2-year inhalation studies. In addition, the concurrent chamber control incidence is the lowest in the current NTP historical control database for inhalation studies. While large interstitial cell neoplasms can result in tubule compression, leading to atrophy, it is also well known that damage to the tubule compartment can result in an increased incidence of Leydig cell interstitial tumors, a response believed to be due to an elevation in gonadotrophin levels (Cook *et al.*, 1999). No testicular lesions were noted in the 3-month rat study.

In female rats, there was an increase in the incidences of uterine neoplasms and endometrial hyperplasia in the 2-year study that was statistically significant at 120 ppm. No adverse findings in the uterus of female rats were noted in the 3-month study. Ovarian atrophy occurred in the 3-month mouse study, and uterine atrophy also occurred, probably as a result of a secondary hormonal effect. This latter finding was accompanied by an elongated estrous cycle in 120 ppm female mice. Ovarian and uterine neoplasms were not observed in the 2-year mouse study. Inhalation administration of decalin and naphthalene had no effect on the reproductive organs of

male or female rats, whereas there were marginally increased incidences of uterine stromal polyps or stromal sarcoma in female mice exposed to decalin (NTP, 2000, 2005a).

Hepatocellular neoplasms were observed in female rats in the 2-year study. Increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were observed in the 120 ppm group. Although the incidences were not statistically significant compared to the chamber control group, these are rare neoplasms in female rats; the historical incidence of hepatocellular adenoma in chamber control female rats for 2-year inhalation studies is 0/350. One carcinoma each was observed in the 60 and 120 ppm groups. Based on these data, it was concluded that there was some evidence of carcinogenicity in the liver of female rats.

In the 2-year rat study, incidences of mononuclear cell leukemia were significantly decreased in all exposed groups of males and females. The significance of these decreases is not clear. The difference in body weights between exposed groups and the chamber control groups could not account for the reduction in incidences. Splenic toxicity has often been correlated with reduced incidences of mononuclear cell leukemia (Elwell *et al.*, 1996). However, it is unlikely that the mild nature of the splenic lesions that occurred in the current study could account for the dramatic decrease in the incidences of mononuclear cell leukemia. This suggests that the reduction was due to a direct effect of tetralin or its metabolites on the development of mononuclear cell leukemia. Similar decreases have been observed in the 2-year studies of 1-amino-2,4-dibromoanthraquinone, emodin, and anthraquinone (NTP, 1996, 2001, 2005b).

In the 2-year mouse study, there was a positive trend in the incidences of splenic hemangiosarcoma in female mice, and the incidence in the 120 ppm group exceeded the historical control range for inhalation studies. The increased incidence of splenic hemangiosarcoma may have been related to tetralin exposure. Splenic hemangiosarcoma was not associated with exposure to either decalin or naphthalene (NTP, 1992, 2005a).

Nonneoplastic lesions associated with inhalation exposure to tetralin were primarily found in the nasal passages of male and female rats and mice. The lesions included olfactory epithelium atrophy, hyperplasia, metaplasia, and suppurative inflammation. Increases in incidences and severities of these lesions in exposed rats and mice were exposure concentration-related. Lesions of the olfactory epithelium observed in rats and mice were consistent with the irritant potential of tetralin (*Merck*, 1996). The effects showed that tetralin is a strong irritant detrimental to the olfactory epithelial cells and, to a lesser extent, the respiratory epithelium. These nasal lesions are commonly observed in inhalation studies of irritating chemicals. They appear to be adaptive responses to the irritation and injury and are rarely accompanied by neoplastic changes. Metaplasia is the result of the development of a modified and more resistant epithelial barrier to the inhaled toxicant (Harkema *et al.*, 2006).

As noted above, tetralin is structurally related to naphthalene and decalin. Like tetralin, naphthalene is an irritant to the nasal cavity, inducing a spectrum of nonneoplastic lesions in the respiratory and olfactory epithelia (NTP, 1992, 2000). In addition, naphthalene induced nasal respiratory epithelium adenoma and olfactory epithelium neuroblastoma in male and female rats. The relationship between the nonneoplastic lesions in the respiratory and olfactory epithelia and the development of the respective neoplasms is not clear. Naphthalene also induced respiratory epithelium hyperplasia and focal inflammation and metaplasia of the olfactory epithelium of the nose in male and female mice but no nasal neoplasms. It was postulated that naphthalene is metabolized in the nasal epithelium by CYP2F2 to an epoxide, the active intermediate, which alkylates DNA, ultimately leading to carcinogenesis (Wang *et al.*, 1998; Schultz *et al.*, 1999). Tetralin is not metabolized into an active intermediate that can react with DNA; thus, tetralin does not induce tumor formation in the nasal cavity. Decalin did not cause any nasal lesions in male or female rats or mice (NTP, 2005a).

Urinary metabolites of tetralin were characterized in the 2-year studies; metabolites have not previously been characterized in mice. In the current 2-year studies, four chemically stable urinary metabolites, 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, and 4-hydroxy-1-tetralone, were quantified in both sexes of rats and mice. Concentrations of all urinary metabolites increased with increasing exposure concentration in both species and sexes; production of some metabolites exceeded dose proportionality in the 120 ppm exposure groups. When metabolite concentrations normalized to creatinine concentration and exposure were compared, mice generally produced greater concentrations of metabolites than rats, and male mice generally produced greater concentrations

of metabolites than female mice, although the effect was not significant in all cases. Concentrations of all metabolites in male rats were greater than or equal to female rats with one exception; the concentration of 2-tetralol in 120 ppm female rats was significantly greater than that in 120 ppm males. A single 6-hour whole body inhalation exposure to tetralin at 15, 60, or 120 ppm showed bi-exponential blood elimination kinetics in mice and rats with a rapid initial phase followed by a slower terminal phase (Appendix M). Significant increases in the area under the curve (AUC) normalized to exposure concentration were observed with increasing exposure concentration in both species, with males having higher AUCs than females. This indicates that elimination pathways of the parent compound from the blood are saturated at higher exposure concentrations. Whether similar differences were present following chronic exposure in the current studies is unknown, although it is apparent that the metabolite profile for chronically exposed males generally showed higher concentrations of metabolites than exposed females.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of tetralin in male F344/N rats based on the increased incidence of cortical renal tubule adenoma. The increased incidence of testicular interstitial cell adenoma may have been related to tetralin exposure. There was *some evidence of carcinogenic activity* of tetralin in female F344/N rats based on the increased incidences of hepatocellular neoplasms and uterine stromal polyp. There was *no evidence of carcinogenic activity* of tetralin in male B6C3F1 mice exposed to 30, 60, or 120 ppm. There was *equivocal evidence of carcinogenic activity* of tetralin in female B6C3F1 mice based on the increased incidence of splenic hemangiosarcoma.

Exposure to tetralin resulted in nonneoplastic lesions of the nose in male and female rats and mice, kidney and testis in male rats, uterus in female rats, and urinary bladder in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS
IN THE 2-YEAR INHALATION STUDY OF TETRALIN

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	24	14	20	19
Natural deaths	6	7	5	3
Survivors				
Died last week of study		1		
Terminal sacrifice	20	28	25	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, duodenum	(49)	(47)	(48)	(50)
Carcinoma				1 (2%)
Intestine small, ileum	(44)	(46)	(45)	(48)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)			
Mesentery	(15)	(13)	(12)	(11)
Oral mucosa	(1)		(3)	
Pancreas	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(3)	(3)	(1)
Squamous cell papilloma		1 (33%)		
Tooth	(3)	(2)	(1)	
Adamantinoma malignant	1 (33%)			
Cardiovascular System				
Blood vessel				(1)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Adenoma		1 (2%)	3 (6%)	
Adrenal medulla	(50)	(50)	(49)	(50)
Pheochromocytoma benign	7 (14%)	8 (16%)	12 (24%)	9 (18%)
Pheochromocytoma complex			1 (2%)	
Pheochromocytoma malignant	3 (6%)	2 (4%)	1 (2%)	
Bilateral, pheochromocytoma benign	2 (4%)	2 (4%)		
Bilateral, pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	4 (8%)	4 (8%)	1 (2%)
Carcinoma	1 (2%)	4 (8%)	3 (6%)	4 (8%)
Pituitary gland	(50)	(49)	(49)	(49)
Adenoma	36 (72%)	35 (71%)	36 (73%)	33 (67%)
Carcinoma	1 (2%)		1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)	1 (2%)	
C-cell, adenoma	5 (10%)	3 (6%)	4 (8%)	4 (8%)
C-cell, carcinoma	2 (4%)	4 (8%)	2 (4%)	2 (4%)
Follicular cell, adenoma		1 (2%)	2 (4%)	1 (2%)
Follicular cell, carcinoma		1 (2%)		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
General Body System				
Peritoneum		(2)		(3)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(49)
Adenoma			1 (2%)	
Carcinoma			1 (2%)	2 (4%)
Prostate gland	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (36%)	20 (40%)	17 (34%)	30 (60%)
Interstitial cell, adenoma	11 (22%)	19 (38%)	14 (28%)	11 (22%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(8)	(2)	(7)	(1)
Deep cervical, carcinoma, metastatic, thyroid gland			1 (14%)	
Lymph node, bronchial	(7)	(5)	(4)	(4)
Lymph node, mandibular		(1)	(3)	
Lymph node, mediastinal	(33)	(36)	(36)	(31)
Carcinoma, metastatic, thyroid gland	1 (3%)			
Carcinoma, metastatic, Zymbal's gland				1 (3%)
Fibrous histiocytoma, metastatic, skin			1 (3%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(47)	(43)	(44)	(43)
Thymoma benign			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)	2 (4%)	1 (2%)
Fibroadenoma	1 (2%)	1 (2%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Basal cell adenoma, multiple	1 (2%)			
Basal cell carcinoma	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Keratoacanthoma		1 (2%)	2 (4%)	2 (4%)
Keratoacanthoma, multiple	1 (2%)			
Osteosarcoma		1 (2%)		
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma		1 (2%)	1 (2%)	4 (8%)
Pinna, neural crest tumor		1 (2%)		
Sebaceous gland, adenoma	2 (4%)			2 (4%)
Subcutaneous tissue, fibroma	3 (6%)	4 (8%)	4 (8%)	5 (10%)
Subcutaneous tissue, fibrosarcoma	3 (6%)	1 (2%)	1 (2%)	
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, lipoma			1 (2%)	1 (2%)
Subcutaneous tissue, osteosarcoma, metastatic, bone	1 (2%)			
Subcutaneous tissue, sarcoma			1 (2%)	
Subcutaneous tissue, schwannoma benign		1 (2%)		
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Subcutaneous tissue, schwannoma malignant, multiple		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Osteosarcoma	2 (4%)			
Cranium, osteosarcoma			1 (2%)	1 (2%)
Maxilla, osteosarcoma	1 (2%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	1 (2%)		1 (2%)	
Osteosarcoma, metastatic, bone			1 (2%)	
Pineal gland, carcinoma				1 (2%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	2 (4%)	1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)			
Pheochromocytoma complex, metastatic, adrenal medulla			1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla	2 (4%)			
Nose	(50)	(50)	(49)	(50)
Adenoma, tubular			1 (2%)	
Pleura	(2)	(1)	(3)	(10)
Special Senses System				
Eye	(50)	(49)	(48)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland			(1)	(1)
Carcinoma			1 (100%)	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cortex, renal tubule, adenoma		1 (2%)	1 (2%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		1 (2%)
Leukemia mononuclear	25 (50%)	16 (32%)	5 (10%)	2 (4%)
Mesothelioma malignant		2 (4%)	1 (2%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	49	50
Total primary neoplasms	137	142	134	133
Total animals with benign neoplasms	48	49	48	49
Total benign neoplasms	91	104	107	109
Total animals with malignant neoplasms	33	29	21	20
Total malignant neoplasms	46	37	27	24
Total animals with metastatic neoplasms	6	1	5	1
Total metastatic neoplasms	6	1	5	2
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 Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	0/50 (0%)	1/50 (2%)	3/49 (6%)	0/50 (0%)
Adjusted rate[b]	0.0%	2.3%	7.5%	0.0%
Terminal rate[c]	0/20 (0%)	1/29 (3%)	3/24 (13%)	0/28 (0%)
First incidence (days)	—[e]	729 (T)	729 (T)	—
Poly-3 test[d]	P = 0.604	P = 0.516	P = 0.119	—[f]
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	9/50 (18%)	10/50 (20%)	12/49 (24%)	9/50 (18%)
Adjusted rate	21.5%	23.2%	28.8%	20.3%
Terminal rate	3/20 (15%)	8/29 (28%)	5/24 (21%)	4/28 (14%)
First incidence (days)	563	715	561	566
Poly-3 test	P = 0.490N	P = 0.531	P = 0.303	P = 0.549N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/49 (2%)	0/50 (0%)
Adjusted rate	9.9%	4.6%	2.5%	0.0%
Terminal rate	2/20 (10%)	1/29 (3%)	1/24 (4%)	0/28 (0%)
First incidence (days)	586	513	729 (T)	—
Poly-3 test	P = 0.028N	P = 0.299N	P = 0.179N	P = 0.052N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	12/50 (24%)	12/50 (24%)	14/49 (29%)	9/50 (18%)
Adjusted rate	28.3%	27.4%	33.3%	20.3%
Terminal rate	4/20 (20%)	9/29 (31%)	6/24 (25%)	4/28 (14%)
First incidence (days)	563	513	561	566
Poly-3 test	P = 0.234N	P = 0.557N	P = 0.397	P = 0.265N
Bone: Osteosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	7.4%	0.0%	2.4%	2.3%
Terminal rate	1/20 (5%)	0/29 (0%)	0/25 (0%)	0/28 (0%)
First incidence (days)	563	—	718	708
Poly-3 test	P = 0.300N	P = 0.108N	P = 0.302N	P = 0.285N
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
Adjusted rate	0.0%	4.6%	2.4%	11.6%
Terminal rate	0/20 (0%)	1/29 (3%)	1/25 (4%)	2/28 (7%)
First incidence (days)	—	715	729 (T)	674
Poly-3 test	P = 0.016	P = 0.256	P = 0.506	P = 0.038
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	7.0%	4.9%	13.9%
Terminal rate	0/20 (0%)	2/29 (7%)	2/25 (8%)	2/28 (7%)
First incidence (days)	—	715	729 (T)	674
Poly-3 test	P = 0.014	P = 0.134	P = 0.244	P = 0.020
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)	1/50 (2%)
Adjusted rate	7.5%	9.3%	9.7%	2.3%
Terminal rate	0/20 (0%)	4/29 (14%)	2/25 (8%)	0/28 (0%)
First incidence (days)	642	729 (T)	619	701
Poly-3 test	P = 0.186N	P = 0.538	P = 0.515	P = 0.281N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Pancreatic Islets: Carcinoma				
Overall rate	1/50 (2%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	2.5%	9.3%	7.3%	9.3%
Terminal rate	1/20 (5%)	3/29 (10%)	2/25 (8%)	2/28 (7%)
First incidence (days)	729 (T)	716	724	695
Poly-3 test	P = 0.242	P = 0.204	P = 0.316	P = 0.203
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	7/50 (14%)	5/50 (10%)
Adjusted rate	9.9%	16.2%	16.9%	11.6%
Terminal rate	1/20 (5%)	6/29 (21%)	4/25 (16%)	2/28 (7%)
First incidence (days)	642	716	619	695
Poly-3 test	P = 0.557N	P = 0.300	P = 0.274	P = 0.544
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	36/50 (72%)	35/49 (71%)	36/49 (73%)	33/49 (67%)
Adjusted rate	75.5%	74.8%	78.4%	71.8%
Terminal rate	15/20 (75%)	20/28 (71%)	19/24 (79%)	19/27 (70%)
First incidence (days)	372	547	480	483
Poly-3 test	P = 0.394N	P = 0.567N	P = 0.464	P = 0.429N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	37/50 (74%)	35/49 (71%)	37/49 (76%)	33/49 (67%)
Adjusted rate	77.6%	74.8%	80.5%	71.8%
Terminal rate	16/20 (80%)	20/28 (71%)	20/24 (83%)	19/27 (70%)
First incidence (days)	372	547	480	483
Poly-3 test	P = 0.326N	P = 0.469N	P = 0.458	P = 0.336N
Skin: Squamous Cell Papilloma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
Adjusted rate	0.0%	2.3%	2.4%	9.2%
Terminal rate	0/20 (0%)	0/29 (0%)	0/25 (0%)	3/28 (11%)
First incidence (days)	—	722	718	619
Poly-3 test	P = 0.019	P = 0.517	P = 0.506	P = 0.071
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	6/50 (12%)
Adjusted rate	2.5%	4.6%	7.3%	13.9%
Terminal rate	1/20 (5%)	1/29 (3%)	2/25 (8%)	5/28 (18%)
First incidence (days)	729 (T)	722	718	619
Poly-3 test	P = 0.025	P = 0.529	P = 0.316	P = 0.070
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	6/50 (12%)
Adjusted rate	2.5%	4.6%	9.8%	13.9%
Terminal rate	1/20 (5%)	1/29 (3%)	3/25 (12%)	5/28 (18%)
First incidence (days)	729 (T)	722	718	619
Poly-3 test	P = 0.028	P = 0.529	P = 0.187	P = 0.070
Skin: Basal Cell Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	7.5%	2.3%	4.9%	6.9%
Terminal rate	2/20 (10%)	1/29 (3%)	0/25 (0%)	1/28 (4%)
First incidence (days)	684	729 (T)	662	577
Poly-3 test	P = 0.495	P = 0.277N	P = 0.485N	P = 0.623N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Skin: Basal Cell Adenoma or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	10.0%	2.3%	4.9%	6.9%
Terminal rate	2/20 (10%)	1/29 (3%)	0/25 (0%)	1/28 (4%)
First incidence (days)	684	729 (T)	662	577
Poly-3 test	P = 0.535N	P = 0.155N	P = 0.321N	P = 0.455N
Skin: Basal Cell Carcinoma or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	7.5%	2.3%	7.3%	6.9%
Terminal rate	2/20 (10%)	1/29 (3%)	1/25 (4%)	1/28 (4%)
First incidence (days)	684	729 (T)	662	577
Poly-3 test	P = 0.480	P = 0.277N	P = 0.648N	P = 0.623N
Skin: Squamous Cell Papilloma, Squamous Cell Carcinoma, Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	6/50 (12%)	9/50 (18%)
Adjusted rate	12.5%	7.0%	14.5%	20.6%
Terminal rate	3/20 (15%)	2/29 (7%)	3/25 (12%)	6/28 (21%)
First incidence (days)	684	722	662	577
Poly-3 test	P = 0.084	P = 0.314N	P = 0.523	P = 0.244
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)	5/50 (10%)
Adjusted rate	7.4%	9.2%	9.8%	11.3%
Terminal rate	0/20 (0%)	2/29 (7%)	4/25 (16%)	0/28 (0%)
First incidence (days)	642	633	729 (T)	577
Poly-3 test	P = 0.337	P = 0.539	P = 0.506	P = 0.404
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.5%	2.3%	2.4%	0.0%
Terminal rate	2/20 (10%)	1/29 (3%)	0/25 (0%)	0/28 (0%)
First incidence (days)	668	729 (T)	547	—
Poly-3 test	P = 0.071N	P = 0.278N	P = 0.290N	P = 0.106N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	7.5%	2.3%	7.2%	0.0%
Terminal rate	2/20 (10%)	1/29 (3%)	2/25 (8%)	0/28 (0%)
First incidence (days)	668	729 (T)	547	—
Poly-3 test	P = 0.125N	P = 0.278N	P = 0.645N	P = 0.106N
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	6/50 (12%)	5/50 (10%)	7/50 (14%)	5/50 (10%)
Adjusted rate	14.7%	11.5%	16.9%	11.3%
Terminal rate	2/20 (10%)	3/29 (10%)	6/25 (24%)	0/28 (0%)
First incidence (days)	642	633	547	577
Poly-3 test	P = 0.440N	P = 0.453N	P = 0.515	P = 0.444N
Testes (Interstitial Cell): Adenoma				
Overall rate	29/50 (58%)	39/50 (78%)	31/50 (62%)	41/50 (82%)
Adjusted rate	67.0%	83.7%	69.6%	87.9%
Terminal rate	16/20 (80%)	26/29 (90%)	19/25 (76%)	27/28 (96%)
First incidence (days)	563	513	523	566
Poly-3 test	P = 0.025	P = 0.038	P = 0.487	P = 0.008

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Thyroid Gland (C-cell): Adenoma				
Overall rate	5/50 (10%)	4/50 (8%)	5/50 (10%)	4/50 (8%)
Adjusted rate	12.4%	9.3%	12.0%	9.3%
Terminal rate	3/20 (15%)	3/29 (10%)	3/25 (12%)	3/28 (11%)
First incidence (days)	593	722	642	722
Poly-3 test	P = 0.442N	P = 0.458N	P = 0.613N	P = 0.461N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	5.0%	9.3%	4.8%	4.7%
Terminal rate	2/20 (10%)	3/29 (10%)	0/25 (0%)	2/28 (7%)
First incidence (days)	729 (T)	722	621	729 (T)
Poly-3 test	P = 0.424N	P = 0.375	P = 0.679N	P = 0.666N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	6/50 (12%)	8/50 (16%)	7/50 (14%)	6/50 (12%)
Adjusted rate	14.9%	18.5%	16.6%	14.0%
Terminal rate	4/20 (20%)	6/29 (21%)	3/25 (12%)	5/28 (18%)
First incidence (days)	593	722	621	722
Poly-3 test	P = 0.445N	P = 0.439	P = 0.533	P = 0.578N
All Organs: Mononuclear Cell Leukemia				
Overall rate	25/50 (50%)	16/50 (32%)	5/50 (10%)	2/50 (4%)
Adjusted rate	55.8%	34.9%	12.0%	4.6%
Terminal rate	8/20 (40%)	8/29 (28%)	1/25 (4%)	0/28 (0%)
First incidence (days)	557	500	621	641
Poly-3 test	P<0.001N	P = 0.032N	P < 0.001N	P < 0.001N
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	4.6%	2.4%	6.8%
Terminal rate	0/20 (0%)	0/29 (0%)	1/25 (4%)	1/28 (4%)
First incidence (days)	—	547	729 (T)	566
Poly-3 test	P = 0.121	P = 0.259	P = 0.506	P = 0.137
All Organs: Osteosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	7.4%	2.3%	2.4%	2.3%
Terminal rate	1/20 (5%)	1/29 (3%)	0/25 (0%)	0/28 (0%)
First incidence (days)	563	729 (T)	718	708
Poly-3 test	P = 0.241N	P = 0.284N	P = 0.302N	P = 0.285N
All Organs: Benign Neoplasms				
Overall rate	48/50 (96%)	49/50 (98%)	48/50 (96%)	49/50 (98%)
Adjusted rate	96.4%	99.4%	97.5%	99.7%
Terminal rate	19/20 (95%)	29/29 (100%)	24/25 (96%)	28/28 (100%)
First incidence (days)	372	513	480	483
Poly-3 test	P = 0.236	P = 0.357	P = 0.600	P = 0.304
All Organs: Malignant Neoplasms				
Overall rate	33/50 (66%)	29/50 (58%)	21/50 (42%)	20/50 (40%)
Adjusted rate	71.2%	61.0%	47.4%	42.7%
Terminal rate	12/20 (60%)	16/29 (55%)	10/25 (40%)	8/28 (29%)
First incidence (days)	557	500	438	381
Poly-3 test	P = 0.002N	P = 0.197N	P = 0.013N	P = 0.003N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	98.0%	100.0%
Terminal rate	20/20 (100%)	29/29 (100%)	24/25 (96%)	28/28 (100%)
First incidence (days)	372	500	438	381
Poly-3 test	P = 0.694N	—	P = 0.500N	—

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

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

Study (Study Start)	Incidence in Controls
Historical Incidence: Inhalation Studies	
1-Bromopropane (July, 2003)	1/50
Cumene (June, 2001)	1/50
Divinylbenzene (September, 1999)	0/50
Methyl isobutyl ketone (May, 2000)	0/50
α-Methylstyrene (August, 2001)	0/50
Propargyl alcohol (October, 2001)	0/49
Tetralin (June, 2003)	0/50
Total (%)	2/349 (0.6%)
Mean ± standard deviation	0.6% ± 1.0%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	8/1,394 (0.6%)
Mean ± standard deviation	0.6% ± 1.0%
Range	0%-2%

[a] Data as of November 17, 2008

TABLE A3b
Historical Incidence of Interstitial Cell Adenoma of the Testis in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Inhalation Studies	
1-Bromopropane (July, 2003)	34/50
Cumene (June, 2001)	36/50
Divinylbenzene (September, 1999)	38/50
Methyl isobutyl ketone (May, 2000)	42/50
α-Methylstyrene (August, 2001)	33/50
Propargyl alcohol (October, 2001)	38/49
Tetralin (June, 2003)	29/50
Total (%)	250/349 (71.6%)
Mean ± standard deviation	71.7% ± 8.5%
Range	58%-84%
Overall Historical Incidence: All Routes	
Total (%)	1,170/1,399 (83.6%)
Mean ± standard deviation	83.6% ± 11.5%
Range	58%-98%

[a] Data as of November 17, 2008

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	24	14	20	19
Natural deaths	6	7	5	3
Survivors				
Died last week of study		1		
Terminal sacrifice	20	28	25	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, duodenum	(49)	(47)	(48)	(50)
Intestine small, ileum	(44)	(46)	(45)	(48)
Muscularis, hyperplasia		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		1 (2%)
Basophilic focus	2 (4%)		5 (10%)	
Basophilic focus, multiple	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Clear cell focus	5 (10%)	3 (6%)	3 (6%)	6 (12%)
Clear cell focus, multiple	7 (14%)	11 (22%)	8 (16%)	7 (14%)
Degeneration, cystic	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule	4 (8%)	4 (8%)	4 (8%)	4 (8%)
Infarct		1 (2%)		
Mixed cell focus				1 (2%)
Necrosis	3 (6%)	3 (6%)	1 (2%)	
Vacuolization cytoplasmic	4 (8%)	5 (10%)	4 (8%)	8 (16%)
Bile duct, hyperplasia	15 (30%)	3 (6%)	4 (8%)	9 (18%)
Centrilobular, degeneration				2 (4%)
Hepatocyte, regeneration	2 (4%)			
Periportal, inflammation, chronic	3 (6%)	3 (6%)	2 (4%)	5 (10%)
Serosa, fibrosis			1 (2%)	
Mesentery	(15)	(13)	(12)	(11)
Hemorrhage	1 (7%)			1 (9%)
Necrosis	12 (80%)	13 (100%)	12 (100%)	11 (100%)
Fat, hemorrhage	2 (13%)			1 (9%)
Oral mucosa	(1)		(3)	
Hyperplasia, squamous	1 (100%)			
Pharyngeal, fibrosis			1 (33%)	
Pharyngeal, hyperplasia, squamous			2 (67%)	
Pancreas	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)		
Inflammation, chronic			1 (2%)	
Acinus, atrophy	9 (18%)	5 (10%)	3 (6%)	5 (10%)
Artery, inflammation				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)		
Erosion		1 (2%)		
Hyperplasia, squamous	2 (4%)		1 (2%)	
Inflammation, suppurative	2 (4%)	1 (2%)	1 (2%)	
Ulcer	5 (10%)	1 (2%)	3 (6%)	2 (4%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Alimentary System *(continued)*				
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion		2 (4%)		1 (2%)
Ulcer	1 (2%)			1 (2%)
Tongue	(1)	(3)	(3)	(1)
Epithelium, hyperplasia	1 (100%)	2 (67%)	3 (100%)	1 (100%)
Tooth	(3)	(2)	(1)	
Inflammation, suppurative		2 (100%)	1 (100%)	
Malformation	2 (67%)			
Epithelium alveolus, hyperplasia	1 (33%)			
Peridontal tissue, inflammation	1 (33%)			
Cardiovascular System				
Blood vessel				(1)
Inflammation, chronic active				1 (100%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	35 (70%)	35 (70%)	38 (76%)	32 (64%)
Atrium, thrombosis	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Ventricle, inflammation, suppurative		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Atrophy			1 (2%)	
Hemorrhage	1 (2%)			
Hyperplasia	13 (26%)	17 (34%)	18 (37%)	14 (28%)
Vacuolization cytoplasmic	17 (34%)	11 (22%)	19 (39%)	15 (30%)
Adrenal medulla	(50)	(50)	(49)	(50)
Hyperplasia	16 (32%)	15 (30%)	15 (31%)	13 (26%)
Infiltration cellular, lymphocyte			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia		2 (4%)	1 (2%)	
Pituitary gland	(50)	(49)	(49)	(49)
Cyst	2 (4%)	3 (6%)		
Hemorrhage		2 (4%)	4 (8%)	4 (8%)
Hyperplasia	7 (14%)	8 (16%)	4 (8%)	6 (12%)
Pars intermedia, cyst				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	23 (46%)	17 (34%)	16 (32%)	10 (20%)
Follicle, cyst	1 (2%)			
Follicular cell, hyperplasia		1 (2%)	1 (2%)	1 (2%)
General Body System				
Peritoneum		(2)		(3)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Necrosis, fatty				1 (2%)
Preputial gland	(50)	(50)	(50)	(49)
Cyst	1 (2%)	1 (2%)	3 (6%)	
Hyperplasia	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Inflammation, suppurative	1 (2%)			1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Genital System *(continued)*				
Prostate gland	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	1 (2%)	5 (10%)	5 (10%)
Inflammation, suppurative	40 (80%)	36 (72%)	38 (76%)	34 (68%)
Inflammation, chronic				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation			1 (2%)	
Inflammation, suppurative			1 (2%)	
Inflammation, chronic				1 (2%)
Testes	(50)	(50)	(50)	(50)
Mineralization		2 (4%)		1 (2%)
Artery, inflammation, chronic active	1 (2%)	1 (2%)	6 (12%)	2 (4%)
Germinal epithelium, atrophy	32 (64%)	42 (84%)	34 (68%)	45 (90%)
Interstitial cell, hyperplasia	4 (8%)	4 (8%)	4 (8%)	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia, reticulum cell	2 (4%)		1 (2%)	
Lymph node	(8)	(2)	(7)	(1)
Deep cervical, ectasia			1 (14%)	
Pancreatic, ectasia			1 (14%)	
Pancreatic, hyperplasia, lymphoid				1 (100%)
Pancreatic, pigmentation, hemosiderin		1 (50%)		
Lymph node, bronchial	(7)	(5)	(4)	(4)
Angiectasis	1 (14%)		2 (50%)	
Ectasia	1 (14%)	1 (20%)		
Hemorrhage		1 (20%)		1 (25%)
Hyperplasia, lymphoid				1 (25%)
Infiltration cellular, histiocyte				1 (25%)
Inflammation, chronic	1 (14%)			
Inflammation, chronic active	1 (14%)			
Pigmentation, hemosiderin		1 (20%)		
Lymph node, mandibular		(1)	(3)	
Inflammation, chronic active			1 (33%)	
Lymph node, mediastinal	(33)	(36)	(36)	(31)
Angiectasis		1 (3%)		
Ectasia	1 (3%)		1 (3%)	1 (3%)
Hemorrhage	1 (3%)	1 (3%)		2 (6%)
Hyperplasia, lymphoid		1 (3%)	1 (3%)	1 (3%)
Inflammation, suppurative			1 (3%)	
Inflammation, chronic	1 (3%)			
Inflammation, chronic active	1 (3%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	1 (2%)			
Fibrosis			1 (2%)	
Necrosis		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Accessory spleen	2 (4%)	1 (2%)		
Fibrosis		3 (6%)		
Hematopoietic cell proliferation	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Hemorrhage	2 (4%)	2 (4%)		1 (2%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
Necrosis	2 (4%)	2 (4%)	1 (2%)	
Pigmentation, hemosiderin		1 (2%)		
Thymus	(47)	(43)	(44)	(43)
Ectopic thyroid				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	4 (8%)	3 (6%)		3 (6%)
Hyperkeratosis		1 (2%)	2 (4%)	
Hyperplasia, squamous			2 (4%)	
Inflammation, chronic	1 (2%)	1 (2%)		
Ulcer	5 (10%)	1 (2%)		
Subcutaneous tissue, fibrosis		1 (2%)		
Subcutaneous tissue, hemorrhage			1 (2%)	
Subcutaneous tissue, metaplasia, osseous		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	13 (26%)	13 (26%)	17 (34%)	4 (8%)
Hemorrhage	6 (12%)	5 (10%)	4 (8%)	2 (4%)
Cerebrum, mineralization	1 (2%)			
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	6 (12%)	2 (4%)	4 (8%)	3 (6%)
Inflammation, suppurative	5 (10%)	2 (4%)	7 (14%)	7 (14%)
Inflammation, chronic	1 (2%)			
Epiglottis, metaplasia, squamous				1 (2%)
Respiratory epithelium, hyperplasia	1 (2%)			
Respiratory epithelium, metaplasia, squamous		1 (2%)		
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)		1 (2%)	
Hemorrhage	5 (10%)	3 (6%)	5 (10%)	3 (6%)
Inflammation, suppurative			2 (4%)	
Inflammation, chronic	8 (16%)	10 (20%)	7 (14%)	9 (18%)
Alveolar epithelium, hyperplasia	12 (24%)	7 (14%)	11 (22%)	15 (30%)
Alveolar epithelium, metaplasia, squamous	1 (2%)		1 (2%)	1 (2%)
Alveolar epithelium, metaplasia, mucous	1 (2%)		2 (4%)	2 (4%)
Alveolus, emphysema				1 (2%)
Alveolus, foreign body				2 (4%)
Alveolus, infiltration cellular, histiocyte	13 (26%)	16 (32%)	14 (28%)	15 (30%)
Alveolus, proteinosis	3 (6%)	1 (2%)	3 (6%)	4 (8%)
Artery, mineralization			1 (2%)	1 (2%)
Bronchiole, hyperplasia		1 (2%)		1 (2%)
Bronchiole, inflammation, suppurative				1 (2%)
Bronchiole, glands, degeneration, mucoid	5 (10%)		1 (2%)	4 (8%)
Bronchiole, goblet cell, hyperplasia	1 (2%)			
Interstitium, fibrosis	1 (2%)	1 (2%)	1 (2%)	
Mediastinum, fibrosis			2 (4%)	
Mediastinum, hyperplasia, lymphoid, focal			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control		30 ppm		60 ppm		120 ppm	
Respiratory System *(continued)*								
Nose	(50)		(50)		(49)		(50)	
Foreign body	11	(22%)	5	(10%)	5	(10%)	3	(6%)
Inflammation, suppurative	6	(12%)	6	(12%)	5	(10%)	4	(8%)
Inflammation, chronic	1	(2%)						
Inflammation, chronic active	1	(2%)						
Glands, dilatation			3	(6%)	3	(6%)	16	(32%)
Goblet cell, hyperplasia			1	(2%)				
Nasolacrimal duct, inflammation, suppurative	1	(2%)	3	(6%)				
Olfactory epithelium, atrophy							1	(2%)
Olfactory epithelium, degeneration	1	(2%)	40	(80%)	43	(88%)	42	(84%)
Olfactory epithelium, degeneration, hyaline	1	(2%)						
Olfactory epithelium, hyperplasia, basal cell			38	(76%)	48	(98%)	48	(96%)
Olfactory epithelium, inflammation, suppurative			12	(24%)	8	(16%)	10	(20%)
Olfactory epithelium, metaplasia			17	(34%)	31	(63%)	37	(74%)
Olfactory epithelium, mineralization			5	(10%)	12	(24%)	17	(34%)
Respiratory epithelium, degeneration, hyaline	1	(2%)						
Respiratory epithelium, hyperplasia	1	(2%)	2	(4%)	3	(6%)	3	(6%)
Respiratory epithelium, inflammation, chronic	4	(8%)	4	(8%)	18	(37%)	16	(32%)
Respiratory epithelium, metaplasia, squamous			1	(2%)				
Pleura	(2)		(1)		(3)		(10)	
Fibrosis	2	(100%)			1	(33%)	2	(20%)
Inflammation, chronic							1	(10%)
Special Senses System								
Eye	(50)		(49)		(48)		(50)	
Atrophy	1	(2%)						
Inflammation, chronic	1	(2%)						
Anterior chamber, edema					1	(2%)		
Anterior chamber, inflammation, suppurative	1	(2%)						
Cornea, degeneration			1	(2%)				
Cornea, inflammation, suppurative			1	(2%)			1	(2%)
Cornea, mineralization	1	(2%)	1	(2%)			1	(2%)
Lens, cataract	9	(18%)	10	(20%)	10	(21%)	10	(20%)
Retina, atrophy	2	(4%)	3	(6%)	5	(10%)	4	(8%)
Sclera, metaplasia, osseous	4	(8%)	11	(22%)	3	(6%)	7	(14%)
Harderian gland	(50)		(50)		(50)		(50)	
Infiltration cellular, lymphocyte					1	(2%)		
Inflammation, chronic			1	(2%)	2	(4%)	3	(6%)
Zymbal's gland					(1)		(1)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation, suppurative		1 (2%)		
Nephropathy, chronic	48 (96%)	50 (100%)	48 (96%)	50 (100%)
Artery, necrosis			1 (2%)	
Cortex, renal tubule, accumulation, hyaline droplet	1 (2%)	2 (4%)	1 (2%)	
Cortex, renal tubule, dilatation				1 (2%)
Cortex, renal tubule, hyperplasia	1 (2%)	2 (4%)		3 (6%)
Cortex, renal tubule, hyperplasia, oncocytic		1 (2%)		
Cortex, renal tubule, pigmentation	1 (2%)			
Medulla, casts			1 (2%)	
Papilla, mineralization	13 (26%)	11 (22%)	10 (20%)	18 (36%)
Pelvis, transitional epithelium, dilatation				1 (2%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)	1 (2%)		7 (14%)
Urinary bladder	(50)	(50)	(50)	(50)
Calculus microscopic observation only	1 (2%)		1 (2%)	
Inflammation, chronic	1 (2%)			1 (2%)
Transitional epithelium, hyperplasia			2 (4%)	1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR INHALATION STUDY OF TETRALIN

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

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	11	15	11
Natural deaths	3	3	4	1
Survivors				
Terminal sacrifice	31	36	31	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(48)	(49)
Intestine large, colon	(50)	(50)	(50)	(49)
Intestine large, rectum	(50)	(50)	(50)	(49)
Polyp adenomatous		1 (2%)		
Intestine small, ileum	(49)	(50)	(48)	(49)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Fibrous histiocytoma			1 (2%)	
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Hepatocellular adenoma				3 (6%)
Hepatocellular carcinoma			1 (2%)	1 (2%)
Mesentery	(10)	(18)	(18)	(14)
Oral mucosa	(1)		(1)	(1)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Epithelium, adenoma			1 (2%)	
Tongue	(1)	(1)		(3)
Squamous cell carcinoma	1 (100%)			
Cardiovascular System				
Blood vessel				(1)
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	5 (10%)	4 (8%)	13 (26%)
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Bilateral, adenoma	1 (2%)		1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Pheochromocytoma benign	2 (4%)	1 (2%)		1 (2%)
Pheochromocytoma malignant	1 (2%)		1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Carcinoma		1 (2%)		
Parathyroid gland	(49)	(50)	(48)	(47)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Endocrine System *(continued)*				
Pituitary gland	(49)	(50)	(50)	(50)
Adenoma	36 (73%)	29 (58%)	32 (64%)	37 (74%)
Carcinoma		1 (2%)	1 (2%)	1 (2%)
Thyroid gland	(50)	(50)	(50)	(49)
C-cell, adenoma	3 (6%)	3 (6%)	4 (8%)	5 (10%)
C-cell, carcinoma		2 (4%)		1 (2%)
Follicular cell, adenoma		1 (2%)		
Follicular cell, carcinoma			1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Carcinoma	4 (8%)	2 (4%)	3 (6%)	4 (8%)
Ovary	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Granulosa cell tumor benign				1 (2%)
Granulosa cell tumor malignant		1 (2%)		1 (2%)
Oviduct		(1)		
Uterus	(50)	(50)	(50)	(50)
Leiomyosarcoma		1 (2%)		
Polyp stromal	6 (12%)	8 (16%)	9 (18%)	17 (34%)
Sarcoma stromal		1 (2%)	1 (2%)	
Bilateral, polyp stromal		2 (4%)		
Endometrium, adenoma	1 (2%)			
Endometrium, carcinoma	1 (2%)	1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(3)	(3)	(3)
Renal, stromal nephroma, metastatic, kidney			1 (33%)	
Lymph node, bronchial	(3)	(5)	(3)	(2)
Carcinoma, metastatic, kidney				1 (50%)
Lymph node, mediastinal	(33)	(36)	(40)	(34)
Fibrous histiocytoma, metastatic, skin			1 (3%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Osteosarcoma		1 (2%)		
Thymus	(44)	(45)	(48)	(46)
Thymoma benign			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	3 (6%)	3 (6%)	5 (10%)	4 (8%)
Fibroadenoma	13 (26%)	14 (28%)	19 (38%)	11 (22%)
Fibroadenoma, multiple	1 (2%)	9 (18%)	4 (8%)	5 (10%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Integumentary System *(continued)*				
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma		2 (4%)		1 (2%)
Fibrous histiocytoma			1 (2%)	
Keratoacanthoma		2 (4%)		1 (2%)
Lip, squamous cell carcinoma	1 (2%)			
Subcutaneous tissue, fibroma		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, skin	1 (2%)			
Rib, osteosarcoma			1 (2%)	
Skeletal muscle	(1)	(1)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Spinal cord		(1)		
Oligodendroglioma malignant		1 (100%)		
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Osteosarcoma, metastatic, bone			1 (2%)	
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Squamous cell carcinoma, metastatic, tongue	1 (2%)			
Stromal nephroma, metastatic, kidney			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Respiratory epithelium, turbinate, adenoma		1 (2%)		
Pleura	(9)	(16)	(15)	(17)
Carcinoma, metastatic, kidney				1 (6%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(50)	(49)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(1)			
Carcinoma	1 (100%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Stromal nephroma			1 (2%)	
Cortex, renal tubule, adenoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Leukemia mononuclear	17 (34%)	2 (4%)		4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	47	47	49
Total primary neoplasms	101	100	95	117
Total animals with benign neoplasms	40	43	44	48
Total benign neoplasms	70	79	76	96
Total animals with malignant neoplasms	25	18	16	19
Total malignant neoplasms	31	21	19	21
Total animals with metastatic neoplasms	4	1	3	1
Total metastatic neoplasms	4	1	9	8
Total animals with malignant neoplasms of uncertain primary site		1		

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	8/50 (16%)	5/50 (10%)	5/50 (10%)	13/50 (26%)
Adjusted rate[b]	18.3%	11.2%	11.4%	28.1%
Terminal rate[c]	6/31 (19%)	4/36 (11%)	5/31 (16%)	12/38 (32%)
First incidence (days)	675	703	730 (T)	694
Poly-3 test[d]	P = 0.067	P = 0.261N	P = 0.268N	P = 0.198
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.9%	2.3%	2.3%	4.3%
Terminal rate	3/31 (10%)	1/36 (3%)	1/31 (3%)	2/38 (5%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test	P = 0.464N	P = 0.296N	P = 0.299N	P = 0.471N
Clitoral Gland: Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	4/50 (8%)
Adjusted rate	9.2%	4.5%	6.6%	8.7%
Terminal rate	2/31 (7%)	2/36 (6%)	1/31 (3%)	3/38 (8%)
First incidence (days)	675	730 (T)	425	703
Poly-3 test	P = 0.508	P = 0.329N	P = 0.482N	P = 0.612N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	5/50 (10%)
Adjusted rate	9.2%	4.5%	6.6%	10.8%
Terminal rate	2/31 (7%)	2/36 (6%)	1/31 (3%)	4/38 (11%)
First incidence (days)	675	730 (T)	425	703
Poly-3 test	P = 0.344	P = 0.329N	P = 0.482N	P = 0.535
Kidney (Renal Tubule): Adenoma Carcinoma or Hyperplasia (Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.3%	4.5%	2.3%	2.1%
Terminal rate	1/31 (3%)	1/36 (3%)	1/31 (3%)	0/38 (0%)
First incidence (days)	730 (T)	699	730 (T)	442
Poly-3 test	P = 0.507N	P = 0.510	P = 0.757N	P = 0.743N
Kidney (Renal Tubule): Adenoma Carcinoma or Hyperplasia (Single and Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.3%	4.5%	2.3%	4.3%
Terminal rate	1/31 (3%)	1/36 (3%)	1/31 (3%)	1/38 (3%)
First incidence (days)	730 (T)	699	730 (T)	442
Poly-3 test	P = 0.476	P = 0.510	P = 0.757N	P = 0.528
Liver: Hepatocellular Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.5%
Terminal rate	0/31 (0%)	0/36 (0%)	0/31 (0%)	2/38 (5%)
First incidence (days)	—[e]	—	—	685
Poly-3 test	P = 0.012	—[f]	—	P = 0.131
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	2.3%	8.6%
Terminal rate	0/31 (0%)	0/36 (0%)	1/31 (3%)	3/38 (8%)
First incidence (days)	—	—	730 (T)	685
Poly-3 test	P = 0.006	—	P = 0.503	P = 0.069

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Mammary Gland: Fibroadenoma				
Overall rate	14/50 (28%)	23/50 (46%)	23/50 (46%)	16/50 (32%)
Adjusted rate	31.7%	51.1%	50.1%	34.5%
Terminal rate	10/31 (32%)	18/36 (50%)	15/31 (48%)	14/38 (37%)
First incidence (days)	624	680	509	681
Poly-3 test	P = 0.442N	P = 0.046	P = 0.055	P = 0.478
Mammary Gland: Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	5/50 (10%)	4/50 (8%)
Adjusted rate	6.9%	6.7%	11.2%	8.5%
Terminal rate	1/31 (3%)	2/36 (6%)	3/31 (10%)	3/38 (8%)
First incidence (days)	682	680	591	488
Poly-3 test	P = 0.422	P = 0.651N	P = 0.369	P = 0.539
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	16/50 (32%)	24/50 (48%)	25/50 (50%)	17/50 (34%)
Adjusted rate	36.1%	53.4%	53.9%	36.1%
Terminal rate	11/31 (36%)	19/36 (53%)	16/31 (52%)	14/38 (37%)
First incidence (days)	624	680	509	488
Poly-3 test	P = 0.352N	P = 0.072	P = 0.063	P = 0.586N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	36/49 (73%)	29/50 (58%)	32/50 (64%)	37/50 (74%)
Adjusted rate	78.0%	62.2%	66.9%	75.4%
Terminal rate	22/31 (71%)	22/36 (61%)	19/31 (61%)	27/38 (71%)
First incidence (days)	522	438	515	488
Poly-3 test	P = 0.457	P = 0.071N	P = 0.162N	P = 0.481N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	36/49 (73%)	30/50 (60%)	33/50 (66%)	38/50 (76%)
Adjusted rate	78.0%	63.5%	69.0%	77.5%
Terminal rate	22/31 (71%)	22/36 (61%)	20/31 (65%)	28/38 (74%)
First incidence (days)	522	438	515	488
Poly-3 test	P = 0.369	P = 0.091N	P = 0.222N	P = 0.576N
Skin: Keratoacanthoma or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted rate	2.3%	8.8%	0.0%	4.3%
Terminal rate	0/31 (0%)	2/36 (6%)	0/31 (0%)	2/38 (5%)
First incidence (days)	717	516	—	730 (T)
Poly-3 test	P = 0.557N	P = 0.195	P = 0.497N	P = 0.521
Thyroid Gland (C-cell): Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	5/49 (10%)
Adjusted rate	6.9%	6.8%	9.1%	10.9%
Terminal rate	3/31 (10%)	3/36 (8%)	3/31 (10%)	2/38 (5%)
First incidence (days)	730 (T)	730 (T)	704	648
Poly-3 test	P = 0.277	P = 0.650N	P = 0.510	P = 0.391
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	6/49 (12%)
Adjusted rate	6.9%	11.2%	9.1%	13.0%
Terminal rate	3/31 (10%)	4/36 (11%)	3/31 (10%)	3/38 (8%)
First incidence (days)	730 (T)	644	704	648
Poly-3 test	P = 0.256	P = 0.375	P = 0.510	P = 0.273

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Uterus: Stromal Polyp				
Overall rate	6/50 (12%)	10/50 (20%)	9/50 (18%)	17/50 (34%)
Adjusted rate	13.8%	22.2%	19.8%	36.5%
Terminal rate	5/31 (16%)	7/36 (19%)	4/31 (13%)	15/38 (40%)
First incidence (days)	697	644	591	647
Poly-3 test	P = 0.008	P = 0.228	P = 0.318	P = 0.011
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	6/50 (12%)	11/50 (22%)	10/50 (20%)	17/50 (34%)
Adjusted rate	13.8%	24.4%	22.0%	36.5%
Terminal rate	5/31 (16%)	8/36 (22%)	4/31 (13%)	15/38 (40%)
First incidence (days)	697	644	591	647
Poly-3 test	P = 0.010	P = 0.160	P = 0.234	P = 0.011
All Organs: Mononuclear Cell Leukemia				
Overall rate	17/50 (34%)	2/50 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	37.0%	4.5%	0.0%	8.6%
Terminal rate	9/31 (29%)	0/36 (0%)	0/31 (0%)	2/38 (5%)
First incidence (days)	420	644	—	703
Poly-3 test	P < 0.001N	P < 0.001N	P < 0.001N	P < 0.001N
All Organs: Benign Neoplasms				
Overall rate	40/50 (80%)	43/50 (86%)	44/50 (88%)	48/50 (96%)
Adjusted rate	84.4%	89.9%	89.4%	97.5%
Terminal rate	25/31 (81%)	32/36 (89%)	26/31 (84%)	37/38 (97%)
First incidence (days)	522	438	509	488
Poly-3 test	P = 0.021	P = 0.307	P = 0.332	P = 0.022
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	19/50 (38%)	16/50 (32%)	19/50 (38%)
Adjusted rate	53.1%	39.5%	34.3%	39.7%
Terminal rate	13/31 (42%)	11/36 (31%)	7/31 (23%)	15/38 (40%)
First incidence (days)	420	196	425	442
Poly-3 test	P = 0.146N	P = 0.130N	P = 0.048N	P = 0.134N
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	47/50 (94%)	47/50 (94%)	49/50 (98%)
Adjusted rate	93.9%	94.0%	94.0%	98.0%
Terminal rate	28/31 (90%)	33/36 (92%)	28/31 (90%)	37/38 (97%)
First incidence (days)	420	196	425	442
Poly-3 test	P = 0.217	P = 0.652	P = 0.652	P = 0.299

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, liver, 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 chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed

TABLE B3a
Historical Incidence of Renal Tubule Neoplasms in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July, 2003)	0/49	1/49	1/49
Cumene (June, 2001)	0/50	0/50	0/50
Divinylbenzene (September, 1999)	0/50	0/50	0/50
Methyl isobutyl ketone (May, 2000)	0/50	0/50	0/50
α-Methylstyrene (August, 2001)	0/49	0/49	0/49
Propargyl alcohol (October, 2001)	0/50	0/50	0/50
Tetralin (June, 2003)	0/50	0/50	0/50
Total (%)	0/348	1/348 (0.3%)	1/348 (0.3%)
Mean ± standard deviation		0.3% ± 0.8%	0.3% ± 0.8%
Range		0%-2%	0%-2%
Overall Historical Incidence: All Routes			
Total (%)	1/1,340 (0.1%)	1/1,340 (0.1%)	2/1,340 (0.2%)
Mean ± standard deviation	0.1% ± 0.4%	0.1% ± 0.4%	0.2% ± 0.5%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of November 17, 2008

TABLE B3b
Historical Incidence of Hepatocellular Neoplasms in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July, 2003)	0/50	1/50	1/50
Cumene (June, 2001)	0/50	0/50	0/50
Divinylbenzene (September, 1999)	0/50	0/50	0/50
Methyl isobutyl ketone (May, 2000)	0/50	0/50	0/50
α-Methylstyrene (August, 2001)	0/50	0/50	0/50
Propargyl alcohol (October, 2001)	0/50	0/50	0/50
Tetralin (June, 2003)	0/50	0/50	0/50
Total (%)	0/350	1/350 (0.3%)	1/350 (0.3%)
Mean ± standard deviation		0.3% ± 0.8%	0.3% ± 0.8%
Range		0%-2%	0%-2%
Overall Historical Incidence: All Routes			
Total (%)	16/1,350 (1.2%)	1/1,350 (0.1%)	17/1,350 (1.3%)
Mean ± standard deviation	1.2% ± 2.6%	0.1% ± 0.4%	1.3% ± 2.6%
Range	0%-12%	0%-2%	0%-12%

[a] Data as of November 17, 2008

TABLE B3c
Historical Incidence of Uterine Neoplasms in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls		
	Stromal Polyp	Stromal Sarcoma	Stromal Polyp or Stromal Sarcoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July, 2003)	8/50	2/50	10/50
Cumene (June, 2001)	13/50	1/50	14/50
Divinylbenzene (September, 1999)	9/50	0/50	9/50
Methyl isobutyl ketone (May, 2000)	12/50	1/50	13/50
α-Methylstyrene (August, 2001)	6/50	0/50	6/50
Propargyl alcohol (October, 2001)	12/50	0/50	12/50
Tetralin (June, 2003)	6/50	0/50	6/50
Total (%)	66/350 (18.9%)	4/350 (1.1%)	70/350 (20.0%)
Mean ± standard deviation	18.9% ± 5.9%	1.1% ± 1.2%	20.0% ± 6.4%
Range	12%-26%	0%-4%	12%-28%
Overall Historical Incidence: All Routes			
Total (%)	241/1,350 (17.9%)	9/1,350 (0.7%)	250/1,350 (18.5%)
Mean ± standard deviation	17.9% ± 6.6%	0.7% ± 1.2%	18.5% ± 7.0%
Range	4%-32%	0%-4%	6%-34%

[a] Data as of November 17, 2008

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	11	15	11
Natural deaths	3	3	4	1
Survivors				
Terminal sacrifice	31	36	31	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(48)	(49)
Intestine large, colon	(50)	(50)	(50)	(49)
Inflammation, suppurative				1 (2%)
Ulcer				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, ileum	(49)	(50)	(48)	(49)
Inflammation, chronic			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	4 (8%)	1 (2%)	4 (8%)
Basophilic focus	7 (14%)	7 (14%)	7 (14%)	2 (4%)
Basophilic focus, multiple	18 (36%)	13 (26%)	16 (32%)	20 (40%)
Clear cell focus	6 (12%)	6 (12%)	7 (14%)	9 (18%)
Clear cell focus, multiple	9 (18%)	2 (4%)	5 (10%)	10 (20%)
Degeneration, cystic			1 (2%)	
Hematopoietic cell proliferation			2 (4%)	
Hepatodiaphragmatic nodule	5 (10%)	6 (12%)	9 (18%)	6 (12%)
Mixed cell focus				1 (2%)
Mixed cell focus, multiple		1 (2%)		
Necrosis	2 (4%)		2 (4%)	
Vacuolization cytoplasmic	6 (12%)	2 (4%)	2 (4%)	3 (6%)
Bile duct, hyperplasia	2 (4%)			1 (2%)
Periportal, inflammation, chronic		2 (4%)	3 (6%)	3 (6%)
Serosa, fibrosis			1 (2%)	
Mesentery	(10)	(18)	(18)	(14)
Necrosis	10 (100%)	18 (100%)	18 (100%)	14 (100%)
Fat, hemorrhage				1 (7%)
Oral mucosa	(1)		(1)	(1)
Pharyngeal, hyperplasia, squamous	1 (100%)		1 (100%)	1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Inflammation, chronic		1 (2%)		
Acinus, atrophy	2 (4%)	1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	1 (2%)		1 (2%)	
Inflammation, suppurative	2 (4%)		1 (2%)	
Ulcer				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Ulcer	1 (2%)			
Epithelium, hyperplasia		1 (2%)		
Tongue	(1)	(1)		(3)
Epithelium, hyperplasia		1 (100%)		3 (100%)

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control		30 ppm		60 ppm		120 ppm	
Cardiovascular System								
Blood vessel							(1)	
Media, inflammation, chronic							1	(100%)
Heart	(50)		(50)		(50)		(50)	
Cardiomyopathy	22	(44%)	24	(48%)	32	(64%)	34	(68%)
Atrium, thrombosis	1	(2%)						
Pericardium, fibrosis	1	(2%)						
Pericardium, infiltration cellular, mixed cell	1	(2%)						
Ventricle, thrombosis			1	(2%)				
Endocrine System								
Adrenal cortex	(50)		(50)		(50)		(50)	
Hematopoietic cell proliferation					2	(4%)		
Hemorrhage	3	(6%)	1	(2%)	1	(2%)		
Hyperplasia	14	(28%)	17	(34%)	14	(28%)	18	(36%)
Metaplasia, osseous					1	(2%)		
Necrosis			1	(2%)	1	(2%)		
Vacuolization cytoplasmic	4	(8%)	7	(14%)	8	(16%)	4	(8%)
Capsule, hyperplasia							1	(2%)
Adrenal medulla	(50)		(50)		(50)		(50)	
Hyperplasia	1	(2%)	2	(4%)	3	(6%)		
Islets, pancreatic	(50)		(50)		(50)		(50)	
Hyperplasia	1	(2%)						
Parathyroid gland	(49)		(50)		(48)		(47)	
Hyperplasia	1	(2%)			1	(2%)	1	(2%)
Pituitary gland	(49)		(50)		(50)		(50)	
Cyst	3	(6%)	2	(4%)	4	(8%)	4	(8%)
Hyperplasia	5	(10%)	11	(22%)	10	(20%)	5	(10%)
Thyroid gland	(50)		(50)		(50)		(49)	
C-cell, hyperplasia	27	(54%)	23	(46%)	17	(34%)	19	(39%)
Follicle, cyst							1	(2%)
Follicular cell, hyperplasia	1	(2%)			1	(2%)		
General Body System								
None								
Genital System								
Clitoral gland	(50)		(50)		(50)		(50)	
Cyst	2	(4%)					3	(6%)
Hyperplasia	4	(8%)	4	(8%)	4	(8%)	2	(4%)
Inflammation, suppurative							1	(2%)
Inflammation, chronic					1	(2%)		
Ovary	(50)		(50)		(50)		(50)	
Cyst	6	(12%)	4	(8%)	7	(14%)	9	(18%)
Hyperplasia, adenomatous							1	(2%)
Oviduct			(1)					
Cyst			1	(100%)				
Inflammation, suppurative			1	(100%)				
Uterus	(50)		(50)		(50)		(50)	
Decidual reaction	1	(2%)	1	(2%)				
Hemorrhage	1	(2%)	1	(2%)	2	(4%)	3	(6%)
Hydrometra			1	(2%)				
Necrosis			3	(6%)			2	(4%)
Thrombosis					1	(2%)		
Cervix, myometrium, hypertrophy			1	(2%)				
Endometrium, hyperplasia	2	(4%)	5	(10%)	7	(14%)	11	(22%)
Endometrium, inflammation, suppurative					3	(6%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	1 (2%)
Hyperplasia, reticulum cell		2 (4%)	3 (6%)	
Erythroid cell, hyperplasia			1 (2%)	
Lymph node	(4)	(3)	(3)	(3)
Pancreatic, ectasia			1 (33%)	
Pancreatic, erythrophagocytosis		1 (33%)		
Pancreatic, hemorrhage		1 (33%)		1 (33%)
Pancreatic, infiltration cellular, histiocyte	1 (25%)	1 (33%)		
Pancreatic, inflammation, chronic				1 (33%)
Pancreatic, pigmentation		1 (33%)	1 (33%)	1 (33%)
Lymph node, bronchial	(3)	(5)	(3)	(2)
Ectasia			1 (33%)	
Hyperplasia, lymphoid		1 (20%)		
Infiltration cellular, histiocyte		2 (40%)		
Pigmentation		1 (20%)		
Lymph node, mediastinal	(33)	(36)	(40)	(34)
Angiectasis	1 (3%)		1 (3%)	
Ectasia				1 (3%)
Hyperplasia, lymphoid	2 (6%)			
Inflammation, chronic	1 (3%)			
Pigmentation	2 (6%)		1 (3%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia				1 (2%)
Pigmentation				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	2 (4%)	4 (8%)	5 (10%)	1 (2%)
Hemorrhage		1 (2%)		
Necrosis	2 (4%)			
Thymus	(44)	(45)	(48)	(46)
Cyst		1 (2%)		
Ectopic parathyroid gland				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Hyperplasia				1 (2%)
Epithelium, hyperplasia		1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Hyperkeratosis				1 (2%)
Hyperplasia, squamous				1 (2%)
Inflammation, chronic			1 (2%)	1 (2%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Subcutaneous tissue, hemorrhage		1 (2%)		
Subcutaneous tissue, inflammation, chronic	1 (2%)			
Subcutaneous tissue, ulcer			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control		30 ppm		60 ppm		120 ppm	
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Compression	11	(22%)	6	(12%)	13	(26%)	12	(24%)
Hemorrhage	2	(4%)	3	(6%)	4	(8%)	5	(10%)
Hydrocephalus							1	(2%)
Necrosis							1	(2%)
Thrombosis	1	(2%)						
Choroid plexus, hyperplasia							1	(2%)
Medulla, gliosis					1	(2%)		
Spinal cord			(1)					
Respiratory System								
Larynx	(50)		(50)		(50)		(50)	
Foreign body	3	(6%)	4	(8%)	4	(8%)	3	(6%)
Inflammation, suppurative	6	(12%)	2	(4%)	6	(12%)	9	(18%)
Inflammation, chronic			1	(2%)	1	(2%)	1	(2%)
Epiglottis, metaplasia, squamous							1	(2%)
Respiratory epithelium, hyperplasia	3	(6%)	1	(2%)			1	(2%)
Lung	(50)		(50)		(50)		(50)	
Hemorrhage	2	(4%)	1	(2%)				
Inflammation, suppurative			1	(2%)				
Inflammation, chronic	12	(24%)	16	(32%)	14	(28%)	21	(42%)
Alveolar epithelium, hyperplasia	6	(12%)	5	(10%)	4	(8%)	3	(6%)
Alveolar epithelium, metaplasia, squamous	1	(2%)						
Alveolus, infiltration cellular, histiocyte	21	(42%)	30	(60%)	24	(48%)	34	(68%)
Alveolus, pigmentation	1	(2%)						
Alveolus, proteinosis			5	(10%)	3	(6%)	2	(4%)
Bronchiole, hyperplasia	1	(2%)	3	(6%)	2	(4%)	3	(6%)
Bronchiole, glands, degeneration, mucoid	2	(4%)	2	(4%)	2	(4%)		
Interstitium, fibrosis							1	(2%)
Mediastinum, infiltration cellular, histiocyte			1	(2%)				
Nose	(50)		(50)		(50)		(50)	
Foreign body	1	(2%)	1	(2%)	2	(4%)	5	(10%)
Inflammation, suppurative	7	(14%)	1	(2%)	4	(8%)	4	(8%)
Glands, dilatation			6	(12%)	10	(20%)	16	(32%)
Nasolacrimal duct, inflammation, suppurative	3	(6%)	2	(4%)	1	(2%)	1	(2%)
Olfactory epithelium, degeneration			47	(94%)	50	(100%)	46	(92%)
Olfactory epithelium, degeneration, hyaline	3	(6%)						
Olfactory epithelium, hyperplasia, basal cell			48	(96%)	50	(100%)	49	(98%)
Olfactory epithelium, inflammation, suppurative			16	(32%)	15	(30%)	19	(38%)
Olfactory epithelium, metaplasia			41	(82%)	43	(86%)	49	(98%)
Olfactory epithelium, mineralization			2	(4%)	8	(16%)	13	(26%)
Respiratory epithelium, degeneration, hyaline	1	(2%)						
Respiratory epithelium, hyperplasia	7	(14%)	1	(2%)	2	(4%)	5	(10%)
Respiratory epithelium, inflammation, chronic	1	(2%)	7	(14%)	11	(22%)	12	(24%)
Pleura	(9)		(16)		(15)		(17)	
Fibrosis					2	(13%)		
Infiltration cellular, histiocyte	1	(11%)						
Mesothelium, hyperplasia					1	(7%)		
Trachea	(50)		(50)		(50)		(50)	
Inflammation, chronic					1	(2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Special Senses System				
Eye	(49)	(50)	(49)	(50)
Cornea, inflammation	1 (2%)			
Cornea, inflammation, suppurative		1 (2%)		
Cornea, mineralization				1 (2%)
Lens, cataract	2 (4%)	6 (12%)	7 (14%)	11 (22%)
Retina, atrophy	4 (8%)	7 (14%)	7 (14%)	7 (14%)
Sclera, metaplasia, osseous		1 (2%)	1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Inflammation, chronic	2 (4%)	1 (2%)	2 (4%)	
Zymbal's gland	(1)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Nephropathy, chronic	40 (80%)	41 (82%)	44 (88%)	49 (98%)
Cortex, infarct, multiple	1 (2%)			
Cortex, renal tubule, accumulation, hyaline droplet	1 (2%)		2 (4%)	
Papilla, mineralization	28 (56%)	16 (32%)	23 (46%)	20 (40%)
Pelvis, inflammation, suppurative				1 (2%)
Pelvis, inflammation, chronic			1 (2%)	
Pelvis, mineralization	1 (2%)			
Pelvis, transitional epithelium, hyperplasia		4 (8%)	1 (2%)	2 (4%)
Pelvis, transitional epithelium, mineralization	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Renal tubule, dilatation		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, hyperplasia		2 (4%)		1 (2%)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF TETRALIN

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	7	11
Natural deaths	5	7	5	3
Survivors				
Terminal sacrifice	36	35	38	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(43)	(41)	(43)	(41)
Intestine large, cecum	(47)	(45)	(45)	(47)
Intestine small, duodenum	(45)	(45)	(45)	(47)
Polyp adenomatous		1 (2%)		1 (2%)
Intestine small, ileum	(47)	(44)	(45)	(47)
Intestine small, jejunum	(45)	(44)	(45)	(47)
Carcinoma		1 (2%)		1 (2%)
Liver	(49)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	3 (6%)		1 (2%)
Hepatocellular adenoma	18 (37%)	13 (26%)	16 (32%)	14 (28%)
Hepatocellular adenoma, multiple	15 (31%)	18 (36%)	19 (38%)	13 (26%)
Hepatocellular carcinoma	11 (22%)	14 (28%)	11 (22%)	13 (26%)
Hepatocellular carcinoma, multiple		3 (6%)	3 (6%)	1 (2%)
Hepatocholangiocarcinoma, multiple		1 (2%)		
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (2%)		
Mesentery	(13)	(4)	(10)	(2)
Pancreas	(49)	(49)	(50)	(50)
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (2%)		
Stomach, forestomach	(49)	(48)	(50)	(50)
Squamous cell papilloma				1 (2%)
Stomach, glandular	(49)	(47)	(48)	(48)
Tooth	(7)	(6)	(7)	(1)
Odontoma	1 (14%)			
Cardiovascular System				
Blood vessel				(1)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(49)	(49)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	
Capsule, adenoma	1 (2%)			2 (4%)
Capsule, carcinoma		1 (2%)		
Adrenal medulla	(47)	(49)	(49)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)		
Islets, pancreatic	(49)	(48)	(50)	(50)
Adenoma	2 (4%)			
Carcinoma			1 (2%)	
Pituitary gland	(50)	(49)	(48)	(49)
Pars distalis, adenoma		1 (2%)		
Thyroid gland	(49)	(49)	(49)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
General Body System				
Peritoneum		(1)		
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (100%)		
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(49)	(50)
Prostate gland	(49)	(48)	(49)	(49)
Seminal vesicle	(49)	(47)	(48)	(48)
Testes	(50)	(50)	(50)	(50)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(49)
Hemangiosarcoma		1 (2%)		2 (4%)
Lymph node	(1)			
Lymph node, bronchial	(35)	(30)	(22)	(27)
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (3%)		
Lymph node, mandibular	(24)	(26)	(28)	(25)
Sarcoma, metastatic, uncertain primary site				1 (4%)
Lymph node, mediastinal	(34)	(36)	(34)	(33)
Hepatocholangiocarcinoma, metastatic, liver		1 (3%)		
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (3%)		
Lymph node, mesenteric	(47)	(48)	(48)	(48)
Plasma cell tumor malignant			1 (2%)	
Spleen	(49)	(49)	(49)	(49)
Hemangiosarcoma	1 (2%)			2 (4%)
Thymus	(47)	(40)	(45)	(43)
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (3%)		
Integumentary System				
Skin	(50)	(49)	(50)	(50)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)		
Subcutaneous tissue, hemangioma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Skeletal muscle		(1)		
Hepatocholangiocarcinoma, metastatic, liver		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Spinal cord	(1)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	5 (10%)	6 (12%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		
Alveolar/bronchiolar carcinoma	11 (22%)	5 (10%)	11 (22%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple		2 (4%)	1 (2%)	2 (4%)
Carcinoma, metastatic, Harderian gland	1 (2%)			
Carcinoma, metastatic, kidney		1 (2%)		
Hemangiosarcoma			1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	9 (18%)	5 (10%)	6 (12%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Plasma cell tumor malignant, metastatic, uncertain primary site		1 (2%)		
Sarcoma, metastatic, uncertain primary site				1 (2%)
Nose	(49)	(49)	(50)	(50)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Trachea	(49)	(48)	(50)	(50)
Special Senses System				
Eye	(48)	(46)	(47)	(47)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Harderian gland	(49)	(47)	(50)	(50)
Adenoma	2 (4%)	5 (11%)	4 (8%)	1 (2%)
Carcinoma	3 (6%)	3 (6%)		1 (2%)
Sarcoma		1 (2%)		
Sarcoma, metastatic, uncertain primary site				1 (2%)
Urinary System				
Kidney	(49)	(49)	(50)	(49)
Carcinoma, metastatic, intestine small, jejunum		1 (2%)		
Renal tubule, adenoma		1 (2%)		
Transitional epithelium, carcinoma		1 (2%)		
Urinary bladder	(49)	(47)	(50)	(48)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	45	46	42
Total primary neoplasms	85	85	80	73
Total animals with benign neoplasms	42	36	38	31
Total benign neoplasms	52	46	46	40
Total animals with malignant neoplasms	26	26	24	25
Total malignant neoplasms	33	39	34	33
Total animals with metastatic neoplasms	5	12	5	7
Total metastatic neoplasms	5	21	5	11
Total animals with malignant neoplasms of uncertain primary site		1		1

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Harderian Gland: Adenoma				
Overall rate[a]	2/50 (4%)	5/50 (10%)	4/50 (8%)	1/50 (2%)
Adjusted rate[b]	4.4%	11.0%	8.7%	2.3%
Terminal rate[c]	1/36 (3%)	4/35 (11%)	3/38 (8%)	1/36 (3%)
First incidence (days)	593	602	656	729 (T)
Poly-3 test[d]	P = 0.281N	P = 0.219	P = 0.346	P = 0.506N
Harderian Gland: Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.7%	6.7%	0.0%	2.3%
Terminal rate	3/36 (8%)	3/35 (9%)	0/38 (0%)	1/36 (3%)
First incidence (days)	729 (T)	729 (T)	—[e]	729 (T)
Poly-3 test	P = 0.133N	P = 0.658N	P = 0.114N	P = 0.307N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	8/50 (16%)	4/50 (8%)	2/50 (4%)
Adjusted rate	11.1%	17.6%	8.7%	4.5%
Terminal rate	4/36 (11%)	7/35 (20%)	3/38 (8%)	2/36 (6%)
First incidence (days)	593	602	656	729 (T)
Poly-3 test	P = 0.092N	P = 0.280	P = 0.488N	P = 0.223N
Liver: Hemangiosarcoma				
Overall rate	1/49 (2%)	3/50 (6%)	0/50 (0%)	1/50(2%)
Adjusted rate	2.3%	6.6%	0.0%	2.2%
Terminal rate	0/36 (0%)	1/35 (3%)	0/38 (0%)	0/36 (0%)
First incidence (days)	592	705	—	591
Poly-3 test	P = 0.403N	P = 0.314	P = 0.493N	P = 0.757N
Liver: Hepatocellular Adenoma				
Overall rate	33/49 (67%)	31/50 (62%)	35/50 (70%)	27/50 (54%)
Adjusted rate	73.4%	64.6%	71.5%	59.0%
Terminal rate	29/36 (81%)	24/35 (69%)	26/38 (68%)	21/36 (58%)
First incidence (days)	516	454	493	591
Poly-3 test	P = 0.124N	P = 0.239N	P = 0.510N	P = 0.102N
Liver: Hepatocellular Carcinoma				
Overall rate	11/49 (22%)	17/50 (34%)	14/50 (28%)	14/50 (28%)
Adjusted rate	23.5%	35.5%	29.3%	29.2%
Terminal rate	5/36 (14%)	9/35 (26%)	8/38 (21%)	6/36 (17%)
First incidence (days)	422	454	593	478
Poly-3 test	P = 0.443	P = 0.146	P = 0.344	P = 0.349
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	37/49 (76%)	40/50 (80%)	40/50 (80%)	35/50 (70%)
Adjusted rate	78.8%	81.4%	80.2%	72.7%
Terminal rate	29/36 (81%)	28/35 (80%)	29/38 (76%)	25/36 (69%)
First incidence (days)	422	454	493	478
Poly-3 test	P = 0.222N	P = 0.476	P = 0.531	P = 0.321N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	10/50 (20%)	6/50 (12%)	6/50 (12%)	8/50 (16%)
Adjusted rate	21.8%	13.3%	13.1%	17.7%
Terminal rate	7/36 (19%)	5/35 (14%)	5/38 (13%)	7/36 (19%)
First incidence (days)	422	705	694	444
Poly-3 test	P = 0.426N	P = 0.215N	P = 0.205N	P = 0.412N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	11/50 (22%)	7/50 (14%)	12/50 (24%)	8/50 (16%)
Adjusted rate	24.3%	15.5%	26.2%	18.0%
Terminal rate	8/36 (22%)	7/35 (20%)	11/38 (29%)	8/36 (22%)
First incidence (days)	618	729 (T)	716	729 (T)
Poly-3 test	P = 0.397N	P = 0.218N	P = 0.511	P = 0.321N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	20/50 (40%)	13/50 (26%)	18/50 (36%)	14/50 (28%)
Adjusted rate	42.9%	28.8%	39.2%	31.0%
Terminal rate	14/36 (39%)	12/35 (34%)	16/38 (42%)	13/36 (36%)
First incidence (days)	422	705	694	444
Poly-3 test	P = 0.230N	P = 0.113N	P = 0.440N	P = 0.165N
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	5/50 (10%)	1/50 (2%)	2/50(4%)
Adjusted rate	8.8%	11.1%	2.2%	4.5%
Terminal rate	2/36 (6%)	3/35 (9%)	0/38 (0%)	0/36 (0%)
First incidence (days)	592	705	700	591
Poly-3 test	P = 0.157N	P = 0.498	P = 0.175N	P = 0.341N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/50 (10%)	5/50 (10%)	1/50 (2%)	2/50 (4%)
Adjusted rate	11.0%	11.1%	2.2%	4.5%
Terminal rate	3/36 (8%)	3/35 (9%)	0/38 (0%)	0/36 (0%)
First incidence (days)	592	705	700	591
Poly-3 test	P = 0.095N	P = 0.628	P = 0.099N	P = 0.220N
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	4.5%	2.2%	8.7%	4.5%
Terminal rate	2/36 (6%)	1/35 (3%)	3/38 (8%)	2/36 (6%)
First incidence (days)	729 (T)	729 (T)	716	729 (T)
Poly-3 test	P = 0.471	P = 0.497N	P = 0.349	P = 0.692
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	36/50 (72%)	38/50 (76%)	31/50 (62%)
Adjusted rate	86.7%	74.9%	77.4%	66.7%
Terminal rate	32/36 (89%)	28/35 (80%)	28/38 (74%)	24/36 (67%)
First incidence (days)	422	454	493	444
Poly-3 test	P = 0.019N	P = 0.101N	P = 0.168N	P = 0.014N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	27/50 (54%)	24/50 (48%)	26/50 (52%)
Adjusted rate	54.0%	55.3%	50.2%	53.3%
Terminal rate	18/36 (50%)	15/35 (43%)	17/38 (45%)	16/36 (44%)
First incidence (days)	422	454	593	444
Poly-3 test	P = 0.471N	P = 0.531	P = 0.432N	P = 0.551N
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	46/50 (92%)	46/50 (92%)	42/50 (84%)
Adjusted rate	95.6%	92.0%	92.0%	85.7%
Terminal rate	35/36 (97%)	31/35 (89%)	34/38 (90%)	30/36 (83%)
First incidence (days)	422	454	493	444
Poly-3 test	P = 0.057N	P = 0.367N	P = 0.367N	P = 0.080N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	7	11
Natural deaths	5	7	5	3
Survivors				
Terminal sacrifice	36	35	38	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(43)	(41)	(43)	(41)
Degeneration, hyaline				1 (2%)
Infiltration cellular, polymorphonuclear		1 (2%)		
Intestine large, cecum	(47)	(45)	(45)	(47)
Hemorrhage		1 (2%)		
Intestine small, duodenum	(45)	(45)	(45)	(47)
Necrosis	1 (2%)			
Intestine small, ileum	(47)	(44)	(45)	(47)
Infiltration cellular, mixed cell	2 (4%)			
Peyer's patch, inflammation, granulomatous		1 (2%)		
Intestine small, jejunum	(45)	(44)	(45)	(47)
Inflammation, suppurative	1 (2%)			
Liver	(49)	(50)	(50)	(50)
Angiectasis	2 (4%)	1 (2%)		
Basophilic focus	4 (8%)	4 (8%)	4 (8%)	1 (2%)
Clear cell focus	16 (33%)	16 (32%)	14 (28%)	18 (36%)
Eosinophilic focus	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Hepatodiaphragmatic nodule				1 (2%)
Infarct		1 (2%)		
Mixed cell focus	1 (2%)	1 (2%)	1 (2%)	
Necrosis	2 (4%)	2 (4%)		3 (6%)
Tension lipidosis	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Vacuolization cytoplasmic				1 (2%)
Mesentery	(13)	(4)	(10)	(2)
Artery, inflammation				1 (50%)
Fat, necrosis	13 (100%)	4 (100%)	10 (100%)	1 (50%)
Pancreas	(49)	(49)	(50)	(50)
Basophilic focus	1 (2%)		1 (2%)	
Hemorrhage			1 (2%)	
Inflammation, granulomatous		1 (2%)		
Inflammation, acute			1 (2%)	
Stomach, forestomach	(49)	(48)	(50)	(50)
Hyperplasia, squamous	1 (2%)	1 (2%)	4 (8%)	4 (8%)
Infiltration cellular, mast cell	1 (2%)			
Inflammation		1 (2%)	4 (8%)	3 (6%)
Ulcer	1 (2%)		3 (6%)	3 (6%)
Stomach, glandular	(49)	(47)	(48)	(48)
Necrosis	3 (6%)	2 (4%)		
Artery, inflammation, chronic active	1 (2%)		1 (2%)	
Tooth	(7)	(6)	(7)	(1)
Malformation	6 (86%)	6 (100%)	7 (100%)	1 (100%)

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

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Cardiovascular System				
Blood vessel				(1)
Aorta, mineralization				1 (100%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	6 (12%)	2 (4%)	5 (10%)	5 (10%)
Inflammation, suppurative				1 (2%)
Mineralization		1 (2%)		2 (4%)
Thrombosis		1 (2%)		
Artery, inflammation, chronic active			3 (6%)	
Endocrine System				
Adrenal cortex	(49)	(49)	(50)	(50)
Atrophy	2 (4%)			
Hyperplasia	17 (35%)	14 (29%)	10 (20%)	9 (18%)
Hypertrophy	24 (49%)	21 (43%)	16 (32%)	18 (36%)
Necrosis				1 (2%)
Capsule, hyperplasia	1 (2%)			
Adrenal medulla	(47)	(49)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)		3 (6%)
Islets, pancreatic	(49)	(48)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	3 (6%)	
Pituitary gland	(50)	(49)	(48)	(49)
Pars distalis, hyperplasia		1 (2%)		
Thyroid gland	(49)	(49)	(49)	(50)
Follicle, cyst				1 (2%)
Follicular cell, hyperplasia			1 (2%)	1 (2%)
General Body System				
Peritoneum		(1)		
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			2 (4%)
Preputial gland	(50)	(50)	(49)	(50)
Ectasia	1 (2%)			
Prostate	(49)	(48)	(49)	(49)
Inflammation, chronic active			1 (2%)	
Seminal vesicle	(49)	(47)	(48)	(48)
Inflammation, chronic active	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Atrophy			2 (4%)	
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(49)
Angiectasis	1 (2%)			
Lymph node	(1)			
Lymph node, bronchial	(35)	(30)	(22)	(27)
Lymph node, mandibular	(24)	(26)	(28)	(25)
Lymph node, mediastinal	(34)	(36)	(34)	(33)
Lymph node, mesenteric	(47)	(48)	(48)	(48)
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, mixed cell	2 (4%)			
Infiltration cellular, plasma cell	2 (4%)			
Spleen	(49)	(49)	(49)	(49)
Depletion cellular	1 (2%)			
Hematopoietic cell proliferation	5 (10%)	5 (10%)	2 (4%)	5 (10%)
Hyperplasia, lymphoid		1 (2%)		
Thymus	(47)	(40)	(45)	(43)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Integumentary System				
Skin	(50)	(49)	(50)	(50)
Hyperplasia, squamous				1 (2%)
Inflammation, chronic active	3 (6%)	4 (8%)	7 (14%)	6 (12%)
Metaplasia, osseous		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Hyperostosis		1 (2%)		
Skeletal muscle		(1)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Inflammation, acute			1 (2%)	
Meninges, infiltration cellular, mononuclear cell	1 (2%)			
Spinal cord	(1)			
Demyelination, focal	1 (100%)			
Gliosis	1 (100%)			
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)			
Squamous epithelium, polyp, inflammatory				1 (2%)
Lung	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Inflammation, chronic active				1 (2%)
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	2 (4%)	6 (12%)	8 (16%)	8 (16%)
Alveolar epithelium, metaplasia, squamous		1 (2%)		
Alveolus, infiltration cellular, histiocyte	1 (2%)		2 (4%)	1 (2%)
Arteriole, mineralization				1 (2%)
Bronchiole, hyperplasia	4 (8%)	2 (4%)	1 (2%)	3 (6%)
Nose	(49)	(49)	(50)	(50)
Inflammation				1 (2%)
Inflammation, suppurative	2 (4%)	26 (53%)	45 (90%)	45 (90%)
Glands, olfactory epithelium, hyperplasia	14 (29%)	49 (100%)	50 (100%)	49 (98%)
Glands, olfactory epithelium, inflammation				1 (2%)
Olfactory epithelium, atrophy	2 (4%)	49 (100%)	50 (100%)	50 (100%)
Olfactory epithelium, metaplasia, respiratory	2 (4%)	47 (96%)	50 (100%)	49 (98%)
Trachea	(49)	(48)	(50)	(50)
Inflammation, suppurative				1 (2%)
Mineralization			1 (2%)	
Special Senses System				
Eye	(48)	(46)	(47)	(47)
Cataract	1 (2%)	1 (2%)	1 (2%)	
Degeneration				1 (2%)
Inflammation, suppurative				1 (2%)
Cornea, inflammation, chronic active	1 (2%)	3 (7%)		
Cornea, mineralization			1 (2%)	2 (4%)
Harderian gland	(49)	(47)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)	2 (4%)	2 (4%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Urinary System				
Kidney	(49)	(49)	(50)	(49)
Cyst		1 (2%)		1 (2%)
Hemorrhage		1 (2%)		
Infarct		2 (4%)	1 (2%)	
Metaplasia, osseous		1 (2%)	2 (4%)	1 (2%)
Mineralization				1 (2%)
Nephropathy	46 (94%)	47 (96%)	48 (96%)	47 (96%)
Thrombosis		1 (2%)		
Artery, inflammation, chronic active		1 (2%)		
Pelvis, inflammation, chronic active		1 (2%)		
Renal tubule, hyperplasia	1 (2%)	4 (8%)	1 (2%)	4 (8%)
Urinary bladder	(49)	(47)	(50)	(48)
Transitional epithelium, eosinophilic granules, cytoplasmic		47 (100%)	50 (100%)	48 (100%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF TETRALIN

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	9	6	6
Natural deaths	2	3	2	1
Survivors				
Terminal sacrifice	31	38	42	43
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(42)	(43)	(44)	(47)
Intestine large, colon	(49)	(49)	(49)	(48)
Leiomyosarcoma	1 (2%)			
Intestine small, duodenum	(48)	(48)	(48)	(49)
Leiomyosarcoma	1 (2%)			
Polyp adenomatous				1 (2%)
Intestine small, ileum	(48)	(48)	(49)	(49)
Polyp adenomatous	1 (2%)		1 (2%)	
Intestine small, jejunum	(48)	(48)	(48)	(49)
Carcinoma			1 (2%)	1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)			1 (2%)
Hepatocellular adenoma	10 (20%)	13 (26%)	14 (28%)	14 (28%)
Hepatocellular adenoma, multiple	4 (8%)	3 (6%)	3 (6%)	8 (16%)
Hepatocellular carcinoma	5 (10%)	4 (8%)	5 (10%)	2 (4%)
Hepatocellular carcinoma, multiple	2 (4%)	1 (2%)	4 (8%)	
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Mesentery	(16)	(17)	(15)	(10)
Hemangiosarcoma	1 (6%)			
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Stomach, glandular	(49)	(49)	(49)	(49)
Tooth	(1)			
Cardiovascular System				
Blood vessel	(2)	(1)	(1)	
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Capsule, adenoma		1 (2%)		1 (2%)
Capsule, carcinoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant		1 (2%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, adenoma	6 (12%)	4 (8%)	8 (16%)	7 (14%)
Pars intermedia, adenoma	1 (2%)			1 (2%)
Thyroid gland	(50)	(50)	(49)	(49)
Follicular cell, carcinoma	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
General Body System				
Peritoneum		(1)		
Genital System				
Ovary	(50)	(50)	(50)	(49)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Cystadenoma	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Hemangioma				1 (2%)
Hemangiosarcoma			1 (2%)	
Luteoma	1 (2%)		1 (2%)	1 (2%)
Teratoma benign	1 (2%)			
Teratoma malignant	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma	1 (2%)			1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Hemangiosarcoma	1 (2%)	1 (2%)		1 (2%)
Leiomyosarcoma	1 (2%)			
Polyp stromal	1 (2%)	3 (6%)	4 (8%)	
Sarcoma stromal		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)			2 (4%)
Lymph node	(7)	(7)	(3)	(2)
Lymph node, bronchial	(33)	(32)	(28)	(37)
Lymph node, mandibular	(33)	(34)	(30)	(31)
Hemangiosarcoma	1 (3%)			
Lymph node, mediastinal	(35)	(42)	(38)	(35)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (3%)			
Osteosarcoma, metastatic, uncertain primary site	1 (3%)			
Lymph node, mesenteric	(49)	(48)	(49)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Teratoma malignant, metastatic, ovary	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)	4 (8%)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (2%)			
Thymus	(48)	(49)	(49)	(47)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Carcinoma	1 (2%)	2 (4%)		
Skin	(50)	(50)	(50)	(50)
Sebaceous gland, adenoma	1 (2%)			1 (2%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			1 (2%)
Skeletal muscle	(1)		(1)	(1)
Hemangiosarcoma	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(49)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	6 (12%)	1 (2%)	2 (4%)	4 (8%)
Alveolar/bronchiolar carcinoma		4 (8%)	2 (4%)	2 (4%)
Carcinoma, metastatic, Harderian gland	1 (2%)			
Carcinoma, metastatic, uncertain primary site				1 (2%)
Hepatocellular carcinoma, metastatic, liver	3 (6%)	1 (2%)	4 (8%)	1 (2%)
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Mediastinum, hemangiosarcoma	1 (2%)			
Nose	(50)	(50)	(50)	(49)
Pleura	(1)			
Osteosarcoma, metastatic, uncertain primary site	1 (100%)			
Trachea	(50)	(50)	(50)	(49)
Special Senses System				
Eye	(49)	(49)	(49)	(49)
Harderian gland	(50)	(50)	(49)	(49)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)		1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Renal tubule, adenoma		1 (2%)		
Urinary bladder	(49)	(50)	(49)	(49)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant	12 (24%)	12 (24%)	10 (20%)	12 (24%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	39	38	38	43
Total primary neoplasms	77	63	64	74
Total animals with benign neoplasms	24	27	26	34
Total benign neoplasms	37	32	37	43
Total animals with malignant neoplasms	25	25	23	23
Total malignant neoplasms	40	31	27	31
Total animals with metastatic neoplasms	7	1	5	1
Total metastatic neoplasms	12	1	5	9
Total animals with malignant neoplasms of uncertain primary site	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 D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Liver: Hepatocellular Adenoma				
Overall rate[a]	14/50 (28%)	16/50 (32%)	17/50 (34%)	22/50 (44%)
Adjusted rate[b]	33.8%	34.7%	34.6%	45.2%
Terminal rate[c]	10/31 (32%)	12/38 (32%)	15/42 (36%)	21/43 (49%)
First incidence (days)	666	639	656	721
Poly-3 test[d]	P = 0.129	P = 0.553	P = 0.556	P = 0.187
Liver: Hepatocellular Carcinoma				
Overall rate	7/50 (14%)	5/50 (10%)	9/50 (18%)	2/50 (4%)
Adjusted rate	17.2%	10.9%	18.2%	4.1%
Terminal rate	7/31 (23%)	3/38 (8%)	5/42 (12%)	0/43 (0%)
First incidence (days)	731 (T)	645	653	721
Poly-3 test	P = 0.057N	P = 0.300N	P = 0.558	P = 0.044N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	20/50 (40%)	20/50 (40%)	23/50 (46%)	23/50 (46%)
Adjusted rate	48.3%	43.0%	46.3%	47.2%
Terminal rate	16/31 (52%)	14/38 (37%)	18/42 (43%)	21/43 (49%)
First incidence (days)	666	639	653	721
Poly-3 test	P = 0.502	P = 0.389N	P = 0.511N	P = 0.546N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	14.7%	2.2%	4.1%	8.2%
Terminal rate	5/31 (16%)	1/38 (3%)	1/42 (2%)	4/43 (9%)
First incidence (days)	674	731 (T)	704	731 (T)
Poly-3 test	P = 0.411N	P = 0.041N	P = 0.084N	P = 0.268N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	0.0%	8.8%	4.1%	4.1%
Terminal rate	0/31 (0%)	4/38 (11%)	1/42 (2%)	2/43 (5%)
First incidence (days)	—[e]	731 (T)	704	731 (T)
Poly-3 test	P = 0.515	P = 0.075	P = 0.280	P = 0.279
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	5/50 (10%)	3/50 (6%)	6/50 (12%)
Adjusted rate	14.7%	11.0%	6.1%	12.3%
Terminal rate	5/31 (16%)	5/38 (13%)	2/42 (5%)	6/43 (14%)
First incidence (days)	674	731 (T)	704	731 (T)
Poly-3 test	P = 0.481N	P = 0.429N	P = 0.163N	P = 0.496N
Ovary: Cystadenoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	1/49 (2%)
Adjusted rate	4.9%	6.6%	2.1%	2.1%
Terminal rate	2/31 (7%)	2/38 (5%)	1/42 (2%)	1/43 (2%)
First incidence (days)	731 (T)	704	731 (T)	731 (T)
Poly-3 test	P = 0.236N	P = 0.550	P = 0.437N	P = 0.444N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	6/49 (12%)	4/50 (8%)	8/50 (16%)	7/50 (14%)
Adjusted rate	15.0%	8.8%	16.4%	14.4%
Terminal rate	4/30 (13%)	3/38 (8%)	8/42 (19%)	6/43 (14%)
First incidence (days)	674	690	731 (T)	705
Poly-3 test	P = 0.447	P = 0.291N	P = 0.544	P = 0.585N
Spleen: Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate	2.5%	0.0%	2.1%	8.2%
Terminal rate	1/31 (3%)	0/38 (0%)	1/42 (2%)	3/43 (7%)
First incidence (days)	731 (T)	—	731 (T)	705
Poly-3 test	P = 0.041	P = 0.479N	P = 0.718N	P = 0.239
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.5%	6.6%	8.2%	0.0%
Terminal rate	1/31 (3%)	3/38 (8%)	3/42 (7%)	0/43(0%)
First incidence (days)	731 (T)	731 (T)	656	—
Poly-3 test	P = 0.224N	P = 0.344	P = 0.241	P = 0.465N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.5%	8.8%	8.2%	0.0%
Terminal rate	1/31 (3%)	4/38 (11%)	3/42 (7%)	0/43 (0%)
First incidence (days)	731 (T)	731 (T)	656	—
Poly-3 test	P = 0.175N	P = 0.213	P = 0.241	P = 0.465N
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.9%	4.4%	4.1%	8.2%
Terminal rate	2/31 (7%)	1/38 (3%)	1/42 (2%)	3/43 (7%)
First incidence (days)	731 (T)	704	704	705
Poly-3 test	P = 0.279	P = 0.654N	P = 0.626N	P = 0.423
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted rate	4.9%	4.4%	4.1%	10.3%
Terminal rate	2/31 (7%)	1/38 (3%)	1/42 (2%)	4/43 (9%)
First incidence (days)	731 (T)	704	704	705
Poly-3 test	P = 0.153	P = 0.654N	P = 0.626N	P = 0.296
All Organs: Malignant Lymphoma				
Overall rate	12/50 (24%)	12/50 (24%)	10/50 (20%)	12/50 (24%)
Adjusted rate	29.2%	25.8%	20.3%	24.4%
Terminal rate	10/31 (32%)	8/38 (21%)	7/42 (17%)	9/43 (21%)
First incidence (days)	673	514	656	648
Poly-3 test	P = 0.362N	P = 0.455N	P = 0.234N	P = 0.393N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
All Organs: Benign Neoplasms				
Overall rate	24/50 (48%)	27/50 (54%)	26/50 (52%)	34/50 (68%)
Adjusted rate	56.6%	58.5%	52.8%	69.7%
Terminal rate	18/31 (58%)	22/38 (58%)	23/42 (55%)	31/43 (72%)
First incidence (days)	432	639	656	705
Poly-3 test	P = 0.106	P = 0.511	P = 0.439N	P = 0.134
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	25/50 (50%)	23/50 (46%)	23/50 (46%)
Adjusted rate	61.1%	51.0%	46.0%	46.6%
Terminal rate	18/31 (58%)	15/38 (40%)	15/42 (36%)	17/43 (40%)
First incidence (days)	432	298	653	648
Poly-3 test	P = 0.122N	P = 0.220N	P = 0.103N	P = 0.114N
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	38/50 (76%)	38/50 (76%)	43/50 (86%)
Adjusted rate	89.5%	77.3%	76.0%	87.1%
Terminal rate	28/31 (90%)	27/38 (71%)	30/42 (71%)	37/43 (86%)
First incidence (days)	432	298	653	648
Poly-3 test	P = 0.484	P = 0.089N	P = 0.067N	P = 0.483N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, pituitary gland, and spleen; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE D3
Historical Incidence of Hemangiosarcoma of the Spleen in Control Female B6C3F1 Mice[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Inhalation Studies	
1-Bromopropane (July, 2003)	1/50
Cumene (June, 2001)	0/49
Diethylamine (August, 2003)	2/50
Divinylbenzene (September, 1999)	0/49
Methyl isobutyl ketone (June, 2000)	1/50
α-Methylstyrene (July, 2001)	0/50
Propargyl alcohol (September, 2001)	1/50
Tetralin (June, 2003)	1/50
Total (%)	6/398 (1.5%)
Mean ± standard deviation	1.5% ± 1.4%
Range	0%-4%
Overall Historical Incidence: All Routes	
Total (%)	27/1,478 (1.8%)
Mean ± standard deviation	1.8% ± 2.4%
Range	0%-10%

[a] Data as of November 19, 2008

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	9	6	6
Natural deaths	2	3	2	1
Survivors				
Terminal sacrifice	31	38	42	43
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Gallbladder	(42)	(43)	(44)	(47)
Infiltration cellular, polymorphonuclear				1 (2%)
Intestine large, colon	(49)	(49)	(49)	(48)
Intestine small, duodenum	(48)	(48)	(48)	(49)
Necrosis	1 (2%)			
Intestine small, ileum	(48)	(48)	(49)	(49)
Infiltration cellular, polymorphonuclear	1 (2%)			
Intestine small, jejunum	(48)	(48)	(48)	(49)
Inflammation, acute		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis				2 (4%)
Basophilic focus	3 (6%)			1 (2%)
Clear cell focus	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Eosinophilic focus	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule				1 (2%)
Infarct	1 (2%)			
Inflammation, chronic			1 (2%)	1 (2%)
Mitotic alteration			1 (2%)	1 (2%)
Mixed cell focus				1 (2%)
Necrosis	2 (4%)			2 (4%)
Tension lipidosis	3 (6%)	8 (16%)	3 (6%)	3 (6%)
Mesentery	(16)	(17)	(15)	(10)
Fat, hemorrhage		3 (18%)	1 (7%)	
Fat, necrosis	14 (88%)	14 (82%)	13 (87%)	10 (100%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	1 (2%)
Inflammation, acute				1 (2%)
Inflammation, chronic active	1 (2%)			
Necrosis			1 (2%)	
Acinus, hypertrophy	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	2 (4%)	3 (6%)		2 (4%)
Inflammation, suppurative	1 (2%)			
Ulcer	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Artery, inflammation, chronic active	1 (2%)			
Stomach, glandular	(49)	(49)	(49)	(49)
Mineralization	1 (2%)			
Tooth	(1)			

[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 Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Cardiovascular System				
Blood vessel	(2)	(1)	(1)	
Inflammation, chronic active	1 (50%)			
Adventitia, metaplasia, respiratory		1 (100%)		
Aorta, mineralization	1 (50%)		1 (100%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	6 (12%)	5 (10%)	1 (2%)	4 (8%)
Inflammation, suppurative	1 (2%)			
Mineralization	1 (2%)		1 (2%)	1 (2%)
Thrombosis			1 (2%)	
Artery, inflammation, chronic active	1 (2%)		1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	3 (6%)	12 (24%)	5 (10%)
Hypertrophy	5 (10%)	1 (2%)	2 (4%)	3 (6%)
Necrosis	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	1 (2%)	5 (10%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)			
Pituitary gland	(49)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Pars distalis, hyperplasia	9 (18%)	11 (22%)	10 (20%)	7 (14%)
Thyroid gland	(50)	(50)	(49)	(49)
Inflammation, chronic active	1 (2%)			
Follicular cell, hyperplasia	1 (2%)			
General Body System				
Peritoneum		(1)		
Genital System				
Ovary	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)		2 (4%)	1 (2%)
Cyst	10 (20%)	11 (22%)	6 (12%)	11 (22%)
Hemorrhage		1 (2%)		
Thrombosis			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation, suppurative		1 (2%)		
Endometrium, hyperplasia, cystic	46 (92%)	50 (100%)	47 (94%)	48 (96%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Atrophy		1 (2%)		
Lymph node	(7)	(7)	(3)	(2)
Angiectasis				1 (50%)
Hemorrhage		1 (14%)		
Iliac, angiectasis			1 (33%)	
Lumbar, angiectasis			1 (33%)	
Lumbar, ectasia	1 (14%)	2 (29%)		
Lumbar, hyperplasia, lymphoid	2 (29%)			
Renal, ectasia				1 (50%)
Renal, hyperplasia, lymphoid	2 (29%)			
Lymph node, bronchial	(33)	(32)	(28)	(37)
Infiltration cellular, mixed cell	1 (3%)			
Lymph node, mandibular	(33)	(34)	(30)	(31)
Hyperplasia, lymphoid	1 (3%)			
Necrosis, lymphoid	1 (3%)			
Lymph node, mediastinal	(35)	(42)	(38)	(35)
Infiltration cellular, mixed cell	1 (3%)			
Lymph node, mesenteric	(49)	(48)	(49)	(50)
Angiectasis			1 (2%)	
Ectasia	1 (2%)		1 (2%)	2 (4%)
Infiltration cellular, mixed cell	1 (2%)			
Necrosis, lymphoid	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	5 (10%)	3 (6%)	6 (12%)	4 (8%)
Hyperplasia, lymphoid	3 (6%)		2 (4%)	2 (4%)
Necrosis, lymphoid	1 (2%)			
Thymus	(48)	(49)	(49)	(47)
Inflammation, granulomatous			1 (2%)	
Necrosis, lymphoid	1 (2%)			
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Inflammation, chronic	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Infiltration cellular, mixed cell			1 (2%)	
Inflammation, chronic active	3 (6%)	3 (6%)	1 (2%)	1 (2%)
Hair follicle, inflammation, chronic	1 (2%)			
Subcutaneous tissue, inflammation, granulomatous				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)		(1)	(1)
Inflammation, chronic active			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis	1 (2%)			
Inflammation, chronic	1 (2%)			
Necrosis	1 (2%)		1 (2%)	
Meninges, infiltration cellular, mononuclear cell	1 (2%)			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Tetralin

	Chamber Control	30 ppm	60 ppm	120 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(49)
Mineralization			1 (2%)	
Lung	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia	4 (8%)	6 (12%)	3 (6%)	6 (12%)
Alveolar epithelium, metaplasia, squamous			1 (2%)	
Alveolus, infiltration cellular, histiocyte				2 (4%)
Bronchiole, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Mediastinum, hemorrhage		1 (2%)		
Nose	(50)	(50)	(50)	(49)
Inflammation, suppurative	3 (6%)	28 (56%)	48 (96%)	46 (94%)
Glands, olfactory epithelium, hyperplasia	17 (34%)	50 (100%)	50 (100%)	49 (100%)
Olfactory epithelium, atrophy	1 (2%)	50 (100%)	50 (100%)	49 (100%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	49 (98%)	50 (100%)	49 (100%)
Pleura	(1)			
Trachea	(50)	(50)	(50)	(49)
Inflammation, suppurative			1 (2%)	
Mineralization			1 (2%)	
Special Senses System				
Eye	(49)	(49)	(49)	(49)
Cataract	2 (4%)	1 (2%)	1 (2%)	
Cornea, hyperplasia, squamous		1 (2%)		1 (2%)
Cornea, inflammation, chronic active				1 (2%)
Cornea, mineralization		3 (6%)	3 (6%)	12 (24%)
Harderian gland	(50)	(50)	(49)	(49)
Hyperplasia	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Infarct	1 (2%)	1 (2%)		
Metaplasia, osseous		3 (6%)	4 (8%)	2 (4%)
Mineralization			1 (2%)	
Nephropathy	40 (80%)	40 (80%)	43 (86%)	38 (76%)
Artery, inflammation, chronic active	1 (2%)			
Urinary bladder	(49)	(50)	(49)	(49)
Infiltration cellular, mixed cell		1 (2%)		
Transitional epithelium, eosinophilic granules, cytoplasmic		50 (100%)	49 (100%)	49 (100%)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Two independent bacterial mutagenicity assays were conducted with tetralin. The first test was performed as reported by Zeiger *et al.* (1992). Tetralin was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. The second test was conducted with the same lot of tetralin used in the 2-year study. Tetralin was incubated for 20 minutes with *S. typhimurium* strains TA98 and TA100 and with *Escherichia coli* strain WP2 *uvrA*/pKM101, either in buffer or rat liver S9 mix. In both tests following incubation, 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 at least five doses of tetralin. The high dose was limited by toxicity. All trials were repeated, and those conducted with S9 were repeated using the same or a higher concentration of S9.

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

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic (mature) erythrocytes (NCEs) in each of 10 animals per exposure group. In addition, the percentage of polychromatic (immature) erythrocytes (PCEs) in a population of 1,000 erythrocytes per animal 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 exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

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

Tetralin (0.3 to 333 μg/plate) was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 when testing was conducted with or without induced rat or hamster liver metabolic activation enzymes (Table E1). A second bacterial mutagenicity assay conducted with the same lot of tetralin (2 to 500 μg/plate) used in the 2-year study showed no mutagenicity in *S. typhimurium* strains TA98 or TA100 or in *Escherichia coli* strain WP2 *uvrA*, with or without rat liver activation enzymes (Table E2). At the end of the 3-month study, no increase in the frequency of micronucleated NCEs was seen in peripheral blood samples of male or female B6C3F1 mice (Table E3). In both male and female mice, the percentages of PCEs generally increased with increasing tetralin concentration, suggesting possible stimulation of erythropoiesis as a response to exposure.

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

Strain	Dose (µg/plate)	Revertants/Plate[b] – S9 Trial 1	– S9 Trial 2	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
TA100	0	125 ± 3	112 ± 4	115 ± 4	117 ± 5	111 ± 6	135 ± 3
	0.3	107 ± 8	107 ± 6				
	1	93 ± 8	112 ± 2				
	3	119 ± 3	110 ± 5	104 ± 7	112 ± 5	115 ± 3	146 ± 6
	10	111 ± 2	101 ± 11	103 ± 5	130 ± 18	117 ± 6	142 ± 9
	33	91 ± 4	75 ± 4	137 ± 29	127 ± 6	118 ± 7	135 ± 4
	100			101 ± 12	136 ± 6	96 ± 6	137 ± 4
	333			50 ± 3[c]	120 ± 5	56 ± 7[c]	126 ± 3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		870 ± 15	833 ± 25	453 ± 13	415 ± 11	386 ± 7	366 ± 8
TA1535	0	7 ± 1	9 ± 2	9 ± 1	10 ± 2	12 ± 2	12 ± 2
	0.3	11 ± 2	8 ± 1				
	1	9 ± 1	9 ± 1				
	3	7 ± 0	8 ± 1	10 ± 1	13 ± 1	9 ± 2	10 ± 2
	10	7 ± 1	8 ± 0	10 ± 1	11 ± 1	9 ± 1	11 ± 3
	33	7 ± 1	7 ± 1	7 ± 1	12 ± 1	8 ± 0	13 ± 1
	100			9 ± 1	10 ± 1	7 ± 1	13 ± 2
	333			2[c]	12 ± 0	3[c]	11 ± 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		851 ± 16	732 ± 8	105 ± 5	198 ± 14	97 ± 10	138 ± 8
TA97	0	108 ± 3	123 ± 6	113 ± 7	121 ± 3	148 ± 10	154 ± 6
	0.3	109 ± 5	116 ± 10				
	1	116 ± 4	133 ± 7				
	3	104 ± 3	139 ± 16	123 ± 4	135 ± 7	138 ± 6	165 ± 6
	10	112 ± 3	120 ± 6	104 ± 4	134 ± 7	121 ± 8	158 ± 6
	33	119 ± 4	101 ± 9	135 ± 12	131 ± 7	136 ± 7	163 ± 6
	100			108 ± 11[e]	118 ± 6	124 ± 8	160 ± 6
	333			52 ± 8[c]	141 ± 5	50 ± 4[c]	130 ± 12
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		392 ± 12	387 ± 6	393 ± 40	409 ± 9	330 ± 15	374 ± 11
TA98	0	16 ± 2	10 ± 1	18 ± 2	17 ± 1	11 ± 1	19 ± 2
	0.3	14 ± 1	11 ± 3				
	1	18 ± 2	8 ± 1				
	3	18 ± 1	10 ± 1	13 ± 1	14 ± 2	11 ± 1	21 ± 2
	10	18 ± 3	12 ± 2	22 ± 8	18 ± 3	11 ± 1	20 ± 2
	33	8 ± 1[c]	8 ± 1	14 ± 2	23 ± 3	12 ± 0	18 ± 1
	100			10 ± 2	18 ± 1	13 ± 1	21 ± 2
	333			3 ± 1[c]	18 ± 3	2 ± 0[c]	21 ± 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		374 ± 13	272 ± 4	276 ± 21	328 ± 21	168 ± 3	187 ± 15

[a] Study performed at SRI International. The detailed protocol is presented by Zeiger *et al.* (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] Contamination

TABLE E2
Mutagenicity of Tetralin (Lot 139699) in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	89 ± 6.0	85 ± 11.0	88 ± 6.0	90 ± 4.0
	2	126 ± 11.0	94 ± 4.0		
	5	115 ± 10.0	90 ± 7.0	112 ± 3.0	108 ± 4.0
	10	119 ± 3.0	86 ± 7.0	92 ± 6.0	95 ± 5.0
	20	108 ± 5.0	97 ± 6.0	91 ± 4.0	111 ± 4.0
	50	96 ± 2.0	78 ± 8.0	94 ± 6.0	82 ± 1.0
	200	Toxic	Toxic	67 ± 14.0	88 ± 2.0
	500			2.3 ± 12.0	Toxic
Trial summary		Negative	Negative	Negative	Negative
Positive control[c]		1,014 ± 26.0	625 ± 18.0	3,125 ± 90.0	3,823 ± 47.0
TA98	0	26 ± 2.0	23 ± 4.0	23 ± 3.0	25 ± 2.0
	2	23 ± 1.0	14 ± 1.0		
	5	24 ± 2.0	19 ± 5.0	33 ± 3.0	30 ± 4.0
	10	22 ± 1.0	21 ± 3.0	35 ± 4.0	32 ± 1.0
	20	24 ± 3.0	23 ± 0.0	31 ± 2.0	26 ± 5.0
	50	19 ± 2.0	22 ± 4.0	26 ± 3.0	28 ± 6.0
	200	31 ± 1.5	Toxic	25 ± 2.0	28 ± 4.0
	500			Toxic	Toxic
Trial summary		Negative	Negative	Negative	Negative
Positive control		951 ± 18.0	697 ± 15.0	1,474 ± 64.0	1,622 ± 139.0
***Escherichia coli* WP2 *uvrA*/pKM101 (Analogous to TA102)**					
	0	130 ± 2.0	133 ± 11.0	152 ± 15.0	157 ± 9.0
	2	131 ± 9.0	155 ± 11.0		
	5	127 ± 5.0	123 ± 5.0	159 ± 7.0	149 ± 11.0
	10	120 ± 8.0	141 ± 1.0	150 ± 4.0	157 ± 9.0
	20	122 ± 5.0	138 ± 2.0	140 ± 9.0	142 ± 4.0
	50	98 ± 9.0	106 ± 2.0	149 ± 4.0	150 ± 6.0
	200	Toxic	16 ± 7	142 ± 5.0	128 ± 3.0
	500			86 ± 18.0	Toxic
Trial summary		Negative	Negative	Negative	Negative
Positive control		998 ± 32.0	1,008 ± 107.0	1,215 ± 54.0	1,108 ± 123.0

[a] Study performed at ILS, Inc. 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (WP2 *uvrA*/pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.

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

Compound	Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Air[d]	0	10	1.05 ± 0.24		1.75 ± 0.10
Tetralin	7.5	10	1.60 ± 0.23	0.0653	1.94 ± 0.13
	15	10	1.25 ± 0.26	0.2776	1.89 ± 0.31
	30	10	1.00 ± 0.18	0.5621	1.94 ± 0.12
	60	10	1.15 ± 0.18	0.3814	2.51 ± 0.23
	120	10	0.90 ± 0.18	0.6846	2.70 ± 0.26
			P = 0.903[e]		
Female					
Air	0	10	0.95 ± 0.12		1.14 ± 0.10
Tetralin	7.5	10	0.70 ± 0.11	0.8081	1.80 ± 0.15
	15	10	0.80 ± 0.15	0.6940	2.19 ± 0.13
	30	10	0.75 ± 0.15	0.7537	2.33 ± 0.11
	60	10	1.05 ± 0.14	0.3759	2.19 ± 0.11
	120	10	0.50 ± 0.13	0.9527	2.59 ± 0.11
			P = 0.856		

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

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 3	43.9 ± 0.5	45.0 ± 0.9	45.2 ± 0.5	44.1 ± 0.5	45.0 ± 0.7	44.4 ± 0.3
Day 23	47.6 ± 0.3	47.5 ± 0.4	47.8 ± 0.3	48.3 ± 0.6	48.5 ± 0.4	47.6 ± 0.3
Week 14	46.9 ± 0.1	46.9 ± 0.3	46.5 ± 0.3	46.4 ± 0.4	46.0 ± 0.3	46.2 ± 0.3
Packed cell volume (mL/dL)						
Day 3	43.2 ± 0.6	43.6 ± 0.8	44.3 ± 0.5	42.7 ± 0.5	43.9 ± 0.7	43.7 ± 0.5
Day 23	46.3 ± 0.5	45.9 ± 0.4	46.2 ± 0.3	47.3 ± 0.7	46.9 ± 0.4	46.4 ± 0.2
Week 14	46.2 ± 0.3	46.4 ± 0.2	45.8 ± 0.3	45.9 ± 0.5	45.9 ± 0.3	45.7 ± 0.3
Hemoglobin (g/dL)						
Day 3	13.3 ± 0.2	13.7 ± 0.3	14.0 ± 0.2	13.3 ± 0.1	13.6 ± 0.3	13.6 ± 0.2
Day 23	15.1 ± 0.1	15.1 ± 0.2	15.2 ± 0.1	15.4 ± 0.1	15.4 ± 0.2	15.2 ± 0.1
Week 14	15.3 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	15.1 ± 0.1	15.0 ± 0.1*
Erythrocytes (10^6/μL)						
Day 3	6.74 ± 0.11	6.83 ± 0.14	6.98 ± 0.07	6.74 ± 0.09	7.02 ± 0.13	6.95 ± 0.09
Day 23	7.50 ± 0.11	7.49 ± 0.09	7.49 ± 0.07	7.77 ± 0.11	7.66 ± 0.11	7.49 ± 0.06
Week 14	8.40 ± 0.06	8.40 ± 0.06	8.27 ± 0.08	8.29 ± 0.10	8.21 ± 0.04*	8.07 ± 0.06**
Reticulocytes (10^6/μL)						
Day 3	0.28 ± 0.02	0.24 ± 0.03	0.26 ± 0.04	0.37 ± 0.05	0.36 ± 0.05	0.28 ± 0.03
Day 23	0.29 ± 0.02	0.32 ± 0.02	0.34 ± 0.02	0.31 ± 0.02	0.32 ± 0.03	0.38 ± 0.03*
Week 14	0.09 ± 0.01	0.09 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.16 ± 0.02**
Reticulocytes/1,000 erythrocytes						
Day 3	42.10 ± 2.49	35.40 ± 4.46	38.00 ± 6.10	55.00 ± 8.22	51.60 ± 7.06	40.30 ± 4.62
Day 23	38.20 ± 1.91	43.30 ± 2.94	45.20 ± 2.41	39.80 ± 2.49	41.80 ± 4.53	50.60 ± 4.32
Week 14	10.10 ± 1.10	10.40 ± 1.80	14.90 ± 1.62	15.20 ± 2.00	15.70 ± 2.11	19.60 ± 1.78**
Nucleated erythrocytes/100 leukocytes						
Day 3	0.50 ± 0.30	0.50 ± 0.30	1.20 ± 0.50	0.40 ± 0.20	0.30 ± 0.20	0.30 ± 0.20
Mean cell volume (fL)						
Day 3	64.2 ± 0.4	63.7 ± 0.4	63.4 ± 0.3	63.4 ± 0.3	62.6 ± 0.3**	62.9 ± 0.2**
Day 23	61.8 ± 0.4	61.2 ± 0.9	61.6 ± 0.3	60.9 ± 0.4	61.2 ± 0.6	61.9 ± 0.5
Week 14	55.1 ± 0.2	55.1 ± 0.2	55.5 ± 0.2	55.3 ± 0.2	55.8 ± 0.2*	56.6 ± 0.2**
Mean cell hemoglobin (pg)						
Day 3	19.8 ± 0.1	20.1 ± 0.2	20.0 ± 0.1	19.8 ± 0.2	19.4 ± 0.1	19.6 ± 0.1
Day 23	20.2 ± 0.2	20.2 ± 0.3	20.3 ± 0.2	19.9 ± 0.2	20.2 ± 0.2	20.3 ± 0.2
Week 14	18.3 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	30.8 ± 0.1	31.4 ± 0.1*	31.5 ± 0.1**	31.3 ± 0.2	31.0 ± 0.2	31.1 ± 0.1
Day 23	32.7 ± 0.1	33.0 ± 0.2	32.8 ± 0.1	32.6 ± 0.2	32.9 ± 0.1	32.9 ± 0.1
Week 14	33.2 ± 0.2	33.1 ± 0.1	33.1 ± 0.1	33.0 ± 0.1	32.9 ± 0.2	32.9 ± 0.2
Platelets (10^3/μL)						
Day 3	876.1 ± 22.9	935.9 ± 13.3	880.3 ± 20.6	885.4 ± 25.3	883.7 ± 15.2	894.0 ± 16.2
Day 23	796.2 ± 13.9	773.2 ± 15.8	779.0 ± 11.4	786.3 ± 12.7	757.5 ± 11.5	763.8 ± 12.8
Week 14	560.7 ± 12.5	557.0 ± 10.5	582.8 ± 9.9	556.7 ± 20.5	608.5 ± 7.7**	631.3 ± 4.4**
Leukocytes (10^3/μL)						
Day 3	8.85 ± 0.66	9.28 ± 0.57	8.84 ± 0.43	9.24 ± 0.52	8.13 ± 0.42	7.86 ± 0.41
Day 23	11.65 ± 0.32	11.54 ± 0.34	11.86 ± 0.42	11.60 ± 0.51	11.55 ± 0.56	10.95 ± 0.26
Week 14	7.43 ± 0.22	7.48 ± 0.46	7.93 ± 0.40	7.69 ± 0.43	8.23 ± 0.47	8.43 ± 0.55
Segmented neutrophils (10^3/μL)						
Day 3	0.94 ± 0.09	1.04 ± 0.14	0.97 ± 0.13	1.28 ± 0.14	0.99 ± 0.08	1.03 ± 0.08
Day 23	0.91 ± 0.08	0.84 ± 0.05	0.94 ± 0.06	1.12 ± 0.10	1.05 ± 0.08	1.14 ± 0.08*
Week 14	0.89 ± 0.06	0.84 ± 0.03	0.88 ± 0.05	0.85 ± 0.07	1.08 ± 0.09	1.03 ± 0.05

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male *(continued)*						
n	10	10	10	10	10	10
Hematology *(continued)*						
Bands (10^3/μL)						
Day 3	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 3	7.58 ± 0.60	7.97 ± 0.61	7.56 ± 0.41	7.64 ± 0.43	6.74 ± 0.35	6.43 ± 0.33
Day 23	10.39 ± 0.23	10.33 ± 0.37	10.54 ± 0.38	10.10 ± 0.44	10.05 ± 0.52	9.38 ± 0.24
Week 14	6.19 ± 0.16	6.29 ± 0.44	6.69 ± 0.33	6.54 ± 0.37	6.74 ± 0.42	7.06 ± 0.51
Monocytes (10^3/μL)						
Day 3	0.26 ± 0.07	0.24 ± 0.05	0.23 ± 0.05	0.24 ± 0.05	0.34 ± 0.06	0.37 ± 0.05
Day 23	0.27 ± 0.05	0.30 ± 0.02	0.31 ± 0.02	0.31 ± 0.04	0.35 ± 0.04	0.35 ± 0.03
Week 14	0.30 ± 0.02	0.28 ± 0.02	0.30 ± 0.04	0.24 ± 0.03	0.32 ± 0.04	0.28 ± 0.02
Basophils (10^3/μL)						
Day 3	0.010 ± 0.010	0.016 ± 0.010	0.018 ± 0.012	0.021 ± 0.014	0.008 ± 0.008	0.007 ± 0.007
Day 23	0.060 ± 0.015	0.032 ± 0.003	0.031 ± 0.005	0.045 ± 0.005	0.064 ± 0.013	0.045 ± 0.006
Week 14	0.020 ± 0.004	0.031 ± 0.008	0.021 ± 0.003	0.025 ± 0.006	0.039 ± 0.014	0.026 ± 0.005
Eosinophils (10^3/μL)						
Day 3	0.06 ± 0.03	0.02 ± 0.01	0.06 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	0.02 ± 0.01
Day 23	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
Week 14	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.00**	0.03 ± 0.00
Hemolysis						
Day 3	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
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	7.3 ± 0.5	6.4 ± 0.5	7.1 ± 0.5	7.2 ± 0.4	9.6 ± 0.5**	10.2 ± 0.6**
Day 23	8.1 ± 0.3	8.2 ± 0.2	7.8 ± 0.4	8.3 ± 0.3	7.7 ± 0.4	8.5 ± 0.4
Week 14	16.5 ± 0.7	15.8 ± 0.5	15.6 ± 0.5	15.9 ± 0.7	14.9 ± 0.3	14.3 ± 0.7
Creatinine (mg/dL)						
Day 3	0.61 ± 0.01	0.64 ± 0.02	0.63 ± 0.02	0.64 ± 0.02	0.63 ± 0.02	0.69 ± 0.01**
Day 23	0.71 ± 0.02	0.69 ± 0.02	0.69 ± 0.01	0.71 ± 0.01	0.71 ± 0.01	0.73 ± 0.02
Week 14	0.87 ± 0.02	0.83 ± 0.04	0.83 ± 0.03	0.88 ± 0.02	0.80 ± 0.03	0.76 ± 0.03
Total protein (g/dL)						
Day 3	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.0	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1
Day 23	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.0	6.3 ± 0.0	6.1 ± 0.1	6.1 ± 0.1
Week 14	6.8 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1
Albumin (g/dL)						
Day 3	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	3.8 ± 0.0	3.8 ± 0.1
Day 23	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.0	3.8 ± 0.1	3.9 ± 0.1
Week 14	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.2 ± 0.0	4.1 ± 0.0	4.2 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	58 ± 2	52 ± 1*	48 ± 2**	51 ± 1**	44 ± 2**	51 ± 2**
Day 23	44 ± 1	40 ± 1**	42 ± 1*	39 ± 1**	37 ± 1**	35 ± 1**
Week 14	120 ± 11	128 ± 13	100 ± 7	95 ± 7	76 ± 6**	63 ± 3**
Alkaline phosphatase (IU/L)						
Day 3	784 ± 9	762 ± 17	723 ± 15**	747 ± 13*	692 ± 17**	716 ± 22**
Day 23	555 ± 9	556 ± 11	568 ± 7	541 ± 10	521 ± 10*	528 ± 10*
Week 14	302 ± 11	282 ± 11	306 ± 9	300 ± 10	268 ± 7	285 ± 9

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Male *(continued)*						
n	10	10	10	10	10	10
Clinical Chemistry *(continued)*						
Creatine kinase (IU/L)						
Day 3	393 ± 38	439 ± 38	420 ± 65	428 ± 62	315 ± 19	378 ± 22
Day 23	378 ± 39	410 ± 61	367 ± 34	319 ± 48	389 ± 38	403 ± 75
Week 14	192 ± 24	174 ± 22	168 ± 22	166 ± 21	167 ± 29	164 ± 21
Sorbitol dehydrogenase (IU/L)						
Day 3	12 ± 1	13 ± 1	13 ± 1	12 ± 1	12 ± 0	12 ± 1
Day 23	12 ± 1	13 ± 1	12 ± 1	13 ± 1	11 ± 1	11 ± 1
Week 14	24 ± 2	29 ± 2	25 ± 2	23 ± 2	16 ± 1*	17 ± 2*
Bile acids (μmol/L)						
Day 3	31.7 ± 1.1	26.8 ± 1.0	29.8 ± 5.2**	23.1 ± 0.6**	27.9 ± 1.4	27.0 ± 1.9
Day 23	28.7 ± 1.4	29.6 ± 3.3	27.3 ± 1.0	28.5 ± 2.2	32.3 ± 2.6	32.4 ± 2.4
Week 14	32.7 ± 4.5	45.3 ± 6.8	29.3 ± 2.4	29.6 ± 1.7	30.3 ± 1.6	29.8 ± 1.1
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	9	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 3	46.1 ± 0.4	46.1 ± 0.6	46.1 ± 0.4	45.7 ± 0.5	46.3 ± 0.4	45.5 ± 0.4
Day 23	48.4 ± 0.2	48.1 ± 0.3	47.7 ± 0.2	48.2 ± 0.2	48.0 ± 0.4	46.9 ± 0.2**
Week 14	47.1 ± 0.4	47.1 ± 0.4	46.5 ± 0.5	46.0 ± 0.2	46.8 ± 0.3	46.2 ± 0.3
Packed cell volume (mL/dL)						
Day 3	44.5 ± 0.4	45.0 ± 0.8	45.3 ± 0.5	44.6 ± 0.6	45.1 ± 0.6	44.9 ± 0.6
Day 23	47.1 ± 0.3	47.2 ± 0.4	46.6 ± 0.3	46.9 ± 0.2	46.6 ± 0.4	46.3 ± 0.4
Week 14	47.2 ± 0.5	47.1 ± 0.4	46.0 ± 0.6	45.7 ± 0.3	46.4 ± 0.3	45.8 ± 0.3
Hemoglobin (g/dL)						
Day 3	14.1 ± 0.2	14.3 ± 0.2	14.3 ± 0.2	14.1 ± 0.2	14.3 ± 0.2	14.0 ± 0.2
Day 23	15.6 ± 0.1	15.8 ± 0.2	15.5 ± 0.1	15.6 ± 0.1	15.6 ± 0.1	15.3 ± 0.1
Week 14	15.4 ± 0.2	15.3 ± 0.1	15.0 ± 0.2	14.8 ± 0.1**	14.9 ± 0.1**	14.8 ± 0.1**
Erythrocytes (10^6/μL)						
Day 3	6.99 ± 0.08	7.08 ± 0.14	7.12 ± 0.11	7.01 ± 0.12	7.09 ± 0.12	7.09 ± 0.11
Day 23	7.62 ± 0.06	7.73 ± 0.07	7.55 ± 0.07	7.53 ± 0.05	7.57 ± 0.10	7.63 ± 0.07
Week 14	8.01 ± 0.10	7.94 ± 0.08	7.69 ± 0.11*	7.51 ± 0.05**	7.50 ± 0.04**	7.40 ± 0.07**
Reticulocytes (10^6/μL)						
Day 3	0.49 ± 0.03	0.47 ± 0.04	0.49 ± 0.04	0.45 ± 0.04	0.47 ± 0.04	0.47 ± 0.05
Day 23	0.22 ± 0.02	0.26 ± 0.01	0.27 ± 0.02	0.27 ± 0.01	0.22 ± 0.02	0.24 ± 0.02
Week 14	0.10 ± 0.01	0.12 ± 0.02	0.13 ± 0.03	0.15 ± 0.01*	0.19 ± 0.02**	0.16 ± 0.02*
Reticulocytes/1,000 erythrocytes						
Day 3	69.80 ± 4.23	66.50 ± 5.66	69.30 ± 5.65	64.00 ± 4.74	67.10 ± 6.00	67.40 ± 7.49
Day 23	28.80 ± 2.15	33.78 ± 1.84	36.00 ± 2.13	36.00 ± 1.11	29.30 ± 2.41	31.40 ± 2.30
Week 14	12.60 ± 1.61	15.00 ± 2.15	16.80 ± 3.75	20.50 ± 1.70*	25.90 ± 2.79**	21.60 ± 2.59**
Nucleated erythrocytes/100 leukocytes						
Day 3	0.40 ± 0.30	0.10 ± 0.10	0.20 ± 0.10	0.40 ± 0.20	0.10 ± 0.10	0.40 ± 0.20

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Female *(continued)*						
Hematology *(continued)*						
n						
Day 3	10	10	10	10	10	10
Day 23	10	9	10	10	10	10
Week 14	10	10	10	10	10	10
Mean cell volume (fL)						
Day 3	63.6 ± 0.3	63.6 ± 0.5	63.7 ± 0.3	63.5 ± 0.3	63.9 ± 0.6	63.3 ± 0.2
Day 23	62.0 ± 0.5	61.0 ± 0.6	61.8 ± 0.4	62.3 ± 0.5	61.7 ± 0.5	60.5 ± 0.5
Week 14	59.0 ± 0.3	59.3 ± 0.4	59.8 ± 0.3	61.0 ± 0.3**	61.8 ± 0.3**	61.8 ± 0.3**
Mean cell hemoglobin (pg)						
Day 3	20.2 ± 0.1	20.2 ± 0.2	20.1 ± 0.1	20.1 ± 0.2	20.2 ± 0.1	19.8 ± 0.2
Day 23	20.4 ± 0.2	20.5 ± 0.2	20.5 ± 0.1	20.7 ± 0.2	20.7 ± 0.2	20.1 ± 0.2
Week 14	19.3 ± 0.1	19.3 ± 0.1	19.5 ± 0.1	19.7 ± 0.1**	19.9 ± 0.1**	20.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.7 ± 0.2	31.7 ± 0.2	31.6 ± 0.2	31.6 ± 0.2	31.7 ± 0.2	31.2 ± 0.2
Day 23	33.0 ± 0.2	33.5 ± 0.2	33.1 ± 0.1	33.2 ± 0.2	33.5 ± 0.1	33.1 ± 0.1
Week 14	32.7 ± 0.2	32.6 ± 0.2	32.7 ± 0.2	32.3 ± 0.1	32.2 ± 0.2	32.3 ± 0.1
Platelets (10^3/μL)						
Day 3	828.0 ± 17.1	822.5 ± 16.4	804.5 ± 12.0	825.4 ± 19.8	842.7 ± 23.3	833.1 ± 17.3
Day 23	755.2 ± 19.0	734.4 ± 9.5	742.0 ± 20.5	736.8 ± 10.3	734.0 ± 17.6	764.6 ± 11.1
Week 14	504.8 ± 7.1	536.7 ± 10.8*	562.6 ± 9.0**	596.6 ± 9.8**	579.7 ± 10.2**	609.6 ± 16.6**
Leukocytes (10^3/μL)						
Day 3	9.81 ± 0.58	10.56 ± 0.43	9.23 ± 0.68	10.08 ± 0.40	8.68 ± 0.65	7.97 ± 0.69
Day 23	12.82 ± 0.29	13.71 ± 0.55	12.55 ± 0.63	11.80 ± 0.33	11.49 ± 0.68	11.15 ± 0.43*
Week 14	9.31 ± 0.78	8.10 ± 0.53	9.36 ± 0.60	8.48 ± 0.52	8.43 ± 0.75	8.84 ± 0.26
Segmented neutrophils (10^3/μL)						
Day 3	0.89 ± 0.08	0.89 ± 0.13	0.84 ± 0.09	0.74 ± 0.08	0.69 ± 0.10	0.86 ± 0.13
Day 23	0.94 ± 0.06	1.26 ± 0.08	1.10 ± 0.07	1.12 ± 0.15	0.95 ± 0.12	1.03 ± 0.09
Week 14	1.16 ± 0.17	0.93 ± 0.08	1.12 ± 0.13	0.85 ± 0.08	0.91 ± 0.11	1.04 ± 0.10
Bands (10^3/μL)						
Day 3	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 3	8.83 ± 0.54	9.61 ± 0.40	8.35 ± 0.65	9.24 ± 0.34	7.89 ± 0.67	6.95 ± 0.68
Day 23	11.50 ± 0.26	12.00 ± 0.48	11.04 ± 0.56	10.26 ± 0.29*	10.10 ± 0.54*	9.75 ± 0.37**
Week 14	7.63 ± 0.58	6.81 ± 0.47	7.76 ± 0.48	7.30 ± 0.48	7.17 ± 0.62	7.40 ± 0.20
Monocytes (10^3/μL)						
Day 3	0.05 ± 0.03	0.02 ± 0.01	0.03 ± 0.02	0.07 ± 0.03	0.07 ± 0.03	0.14 ± 0.06
Day 23	0.30 ± 0.04	0.34 ± 0.03	0.33 ± 0.04	0.34 ± 0.04	0.37 ± 0.07	0.29 ± 0.02
Week 14	0.46 ± 0.08	0.31 ± 0.03	0.49 ± 0.09	0.27 ± 0.04	0.29 ± 0.04	0.34 ± 0.04
Basophils (10^3/μL)						
Day 3	0.011 ± 0.011	0.000 ± 0.000	0.009 ± 0.009	0.000 ± 0.000	0.007 ± 0.007	0.000 ± 0.000
Day 23	0.041 ± 0.004	0.057 ± 0.007	0.045 ± 0.005	0.042 ± 0.005	0.031 ± 0.002	0.044 ± 0.006
Week 14	0.023 ± 0.005	0.021 ± 0.003	0.020 ± 0.003	0.023 ± 0.004	0.021 ± 0.003	0.027 ± 0.003
Eosinophils (10^3/μL)						
Day 3	0.02 ± 0.02	0.04 ± 0.03	0.01 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01
Day 23	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.01
Week 14	0.03 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Hemolysis						
Day 3	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

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Female *(continued)*						
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	8.3 ± 0.5	8.3 ± 0.8	9.2 ± 0.6	8.7 ± 0.6	10.2 ± 0.6*	10.6 ± 0.5*
Day 23	9.9 ± 0.3	9.5 ± 0.2	9.6 ± 0.4	11.3 ± 0.8	11.1 ± 0.5	11.0 ± 0.5
Week 14	15.4 ± 0.4	14.6 ± 0.4	14.6 ± 0.6	14.7 ± 0.5	14.6 ± 0.4	14.2 ± 0.6
Creatinine (mg/dL)						
Day 3	0.63 ± 0.02	0.63 ± 0.02	0.62 ± 0.01	0.62 ± 0.01	0.67 ± 0.02	0.70 ± 0.00**
Day 23	0.68 ± 0.01	0.68 ± 0.01	0.66 ± 0.02	0.69 ± 0.01	0.75 ± 0.02**	0.77 ± 0.02**
Week 14	0.74 ± 0.02	0.75 ± 0.02	0.73 ± 0.02	0.78 ± 0.02	0.75 ± 0.02	0.72 ± 0.02
Total protein (g/dL)						
Day 3	5.9 ± 0.0	5.7 ± 0.1	5.9 ± 0.1	5.7 ± 0.1	5.9 ± 0.1	5.8 ± 0.1
Day 23	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.1
Week 14	6.4 ± 0.1	6.5 ± 0.0	6.5 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.5 ± 0.1
Albumin (g/dL)						
Day 3	3.9 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
Day 23	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.1
Week 14	4.3 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	47 ± 1	39 ± 1**	41 ± 1**	42 ± 1**	41 ± 2**	39 ± 1**
Day 23	38 ± 1	37 ± 1	35 ± 1	35 ± 2	33 ± 1**	33 ± 1**
Week 14	87 ± 8	100 ± 15	95 ± 7	75 ± 5	77 ± 7	59 ± 3**
Alkaline phosphatase (IU/L)						
Day 3	636 ± 17	591 ± 16	603 ± 19	606 ± 17	603 ± 21	581 ± 15
Day 23	408 ± 14	397 ± 12	390 ± 12	409 ± 14	390 ± 10	389 ± 13
Week 14	332 ± 11	285 ± 10	284 ± 11	289 ± 17	286 ± 10	302 ± 8
Creatine kinase (IU/L)						
Day 3	391 ± 88	296 ± 27	375 ± 104	319 ± 17	340 ± 25	328 ± 29
Day 23	265 ± 22	254 ± 47	271 ± 33	211 ± 14	286 ± 28	308 ± 31
Week 14	138 ± 26	132 ± 20	117 ± 22	147 ± 19	147 ± 24	121 ± 20
Sorbitol dehydrogenase (IU/L)						
Day 3	12 ± 0	12 ± 0	12 ± 1	12 ± 0	13 ± 0	12 ± 0
Day 23	14 ± 1	14 ± 0	14 ± 1	14 ± 0	15 ± 1	12 ± 1
Week 14	19 ± 1	21 ± 2	21 ± 1	17 ± 1	18 ± 1	14 ± 1**
Bile acids (μmol/L)						
Day 3	26.5 ± 1.8	24.7 ± 1.4	22.2 ± 1.2	19.8 ± 1.0**	22.2 ± 1.5	23.8 ± 0.5
Day 23	20.6 ± 0.7	22.0 ± 1.8	19.1 ± 0.5	19.5 ± 1.0	25.6 ± 4.6	24.0 ± 1.4
Week 14	31.8 ± 3.6	40.8 ± 6.7	31.4 ± 3.4	41.8 ± 5.9	34.4 ± 4.5	27.7 ± 2.9

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

TABLE F2
Hematology Data for Mice in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	10	10	10	10	10	10
Male						
Hematocrit (%)	49.5 ± 0.4	48.2 ± 0.4	48.6 ± 0.7	48.2 ± 0.3*	48.2 ± 0.4*	47.3 ± 0.2**
Packed cell volume (mL/dL)	48.4 ± 0.3	47.2 ± 0.3	47.5 ± 0.7	47.5 ± 0.3	46.8 ± 0.4**	45.6 ± 0.4**
Hemoglobin (g/dL)	15.7 ± 0.1	15.3 ± 0.1	15.5 ± 0.2	15.5 ± 0.1	15.7 ± 0.1	16.1 ± 0.1
Erythrocytes (10^6/µL)	9.86 ± 0.07	9.76 ± 0.07	9.68 ± 0.13	9.58 ± 0.05**	9.33 ± 0.10**	9.05 ± 0.08**
Reticulocytes (10^6/µL)	0.15 ± 0.02	0.17 ± 0.02	0.23 ± 0.02	0.16 ± 0.02	0.23 ± 0.02*	0.24 ± 0.03**
Reticulocytes/ 1,000 erythrocytes	15.20 ± 1.68	17.70 ± 1.75	23.50 ± 2.81	16.80 ± 2.29	24.20 ± 2.13**	27.10 ± 3.05**
Nucleated erythrocytes/ 100 leukocytes	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)	49.0 ± 0.3	48.4 ± 0.2	49.0 ± 0.2	49.6 ± 0.3	50.3 ± 0.2**	50.4 ± 0.3**
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.6 ± 0.1	16.0 ± 0.1	16.2 ± 0.1	16.9 ± 0.1**	17.8 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.5 ± 0.1	32.3 ± 0.2	32.7 ± 0.2	32.6 ± 0.1	33.6 ± 0.2**	35.3 ± 0.2**
Platelets (10^3/µL)	731.6 ± 16.8	730.9 ± 10.9	759.6 ± 10.2	760.0 ± 13.3	785.5 ± 11.7*	817.7 ± 9.6**
Leukocytes (10^3/µL)	2.40 ± 0.28	2.23 ± 0.20	2.68 ± 0.25	2.74 ± 0.23	2.75 ± 0.18	3.74 ± 0.41*
Segmented neutrophils (10^3/µL)	0.31 ± 0.05	0.30 ± 0.04	0.33 ± 0.05	0.40 ± 0.05	0.29 ± 0.04	0.53 ± 0.06*
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	1.98 ± 0.21	1.89 ± 0.19	2.26 ± 0.20	2.23 ± 0.20	2.38 ± 0.16	3.08 ± 0.40*
Monocytes (10^3/µL)	0.06 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.08 ± 0.02	0.05 ± 0.01	0.08 ± 0.02
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	0.02 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
Female						
Hematocrit (%)	49.3 ± 0.4	49.9 ± 0.4	49.4 ± 0.4	49.2 ± 0.5	48.3 ± 0.5	47.6 ± 0.5*
Packed cell volume (mL/dL)	48.8 ± 0.3	48.8 ± 0.5	47.7 ± 0.4	47.6 ± 0.6	46.9 ± 0.5**	45.8 ± 0.4**
Hemoglobin (g/dL)	16.0 ± 0.1	16.0 ± 0.1	15.8 ± 0.2	15.7 ± 0.2	15.7 ± 0.2	16.0 ± 0.2
Erythrocytes (10^6/µL)	9.88 ± 0.06	9.89 ± 0.09	9.58 ± 0.07*	9.54 ± 0.09*	9.36 ± 0.11**	8.93 ± 0.07**
Reticulocytes (10^6/µL)	0.27 ± 0.02	0.27 ± 0.01	0.27 ± 0.02	0.32 ± 0.02*	0.36 ± 0.02**	0.37 ± 0.02**
Reticulocytes/ 1,000 erythrocytes	26.80 ± 1.51	27.10 ± 1.45	28.40 ± 2.11	34.00 ± 1.59*	38.10 ± 1.48**	41.60 ± 2.24**
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10	0.10 ± 0.10	0.20 ± 0.10	0.00 ± 0.00
Mean cell volume (fL)	49.3 ± 0.2	49.3 ± 0.3	49.6 ± 0.2	49.9 ± 0.2*	50.1 ± 0.2**	51.3 ± 0.2**
Mean cell hemoglobin (pg)	16.2 ± 0.1	16.2 ± 0.1	16.5 ± 0.1	16.5 ± 0.1*	16.7 ± 0.1**	18.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.9 ± 0.2	33.1 ± 0.2	33.1 ± 0.2	33.4 ± 0.1*	35.0 ± 0.2**
Platelets (10^3/µL)	723.3 ± 16.5	761.7 ± 19.9	752.1 ± 15.9	740.7 ± 14.1	827.7 ± 12.8**	827.2 ± 22.6**
Leukocytes (10^3/µL)	2.72 ± 0.20	2.63 ± 0.16	2.51 ± 0.10	2.58 ± 0.24	3.26 ± 0.36	3.03 ± 0.25
Segmented neutrophils (10^3/µL)	0.22 ± 0.01	0.24 ± 0.03	0.19 ± 0.03	0.18 ± 0.02	0.35 ± 0.09	0.33 ± 0.05
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)	2.47 ± 0.19	2.35 ± 0.15	2.29 ± 0.08	2.38 ± 0.23	2.83 ± 0.25	2.66 ± 0.23
Monocytes (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.03	0.02 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.02 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.01

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

APPENDIX G
RENAL TOXICITY, URINALYSIS, AND URINARY METABOLITE RESULTS

TABLE G1
Renal Toxicity Data for Male Rats in the 2-Week Inhalation Studies of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	5	5	5	5	5	5
F344/N						
Cells labeled	89 ± 6	81 ± 11	83 ± 5	84 ± 2	59 ± 9	69 ± 5
Cells counted	2,133 ± 26	2,075 ± 18	2,105 ± 20	2,063 ± 24	2,065 ± 20	2,065 ± 24
Labeling index (%)[b]	4.19 ± 0.24	3.90 ± 0.50	3.96 ± 0.22	4.09 ± 0.11	2.83 ± 0.43	3.31 ± 0.22
α2u-Globulin (ng/μg soluble protein)	55.7 ± 6.4	104.3 ± 25.0*	119.7 ± 25.3*	99.3 ± 9.2*	144.3 ± 32.2*	164.2 ± 20.1**
NBR						
Cells labeled	57 ± 6	42 ± 12	40 ± 4	32 ± 4*	48 ± 8	38 ± 3
Cells counted	2,083 ± 31	2,079 ± 15	2,079 ± 21	2,054 ± 22	2,083 ± 29	2,130 ± 29
Labeling index (%)	2.76 ± 0.30	2.01 ± 0.59	1.94 ± 0.17	1.56 ± 0.17*	2.31 ± 0.39	1.80 ± 0.17

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test
** $P \leq 0.01$ by Shirley's test
[a] Data are presented as mean ± standard error.
[b] Labeling index was calculated as the number of labeled cells divided by the total number of cells counted times 100. A minimum of 2,000 cells were counted.

TABLE G2
Renal Toxicity Data for Male F344/N Rats in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	5	5	5	5	5	5
Cells labeled						
Week 2	95 ± 9	79 ± 3	80 ± 3	75 ± 5	72 ± 4	79 ± 9
Week 6	69 ± 4	72 ± 2	81 ± 4	80 ± 4	85 ± 5*	99 ± 3**
Week 14	52 ± 8	80 ± 6*	81 ± 7*	79 ± 7*	88 ± 3**	113 ± 5**
Cells counted						
Week 2	2,296 ± 49	2,101 ± 34*	2,175 ± 59	2,225 ± 40	2,206 ± 57	2,195 ± 47
Week 6	2,212 ± 51	2,095 ± 30	2,168 ± 16	2,293 ± 35	2,100 ± 25	2,147 ± 47
Week 14	2,282 ± 41	2,172 ± 76	2,189 ± 63	2,116 ± 35	2,187 ± 28	2,199 ± 55
Labeling index (%)[b]						
Week 2	4.1083 ± 0.3236	3.7593 ± 0.1936	3.6989 ± 0.1198	3.3606 ± 0.2077	3.2465 ± 0.1532	3.6038 ± 0.4045
Week 6	3.1456 ± 0.2447	3.4190 ± 0.0986	3.7545 ± 0.1714	3.5015 ± 0.1799	4.0573 ± 0.2442**	4.6233 ± 0.1406**
Week 14	2.2679 ± 0.3738	3.7086 ± 0.2979*	3.6883 ± 0.3331*	3.7147 ± 0.3182*	4.0077 ± 0.1403**	5.1597 ± 0.3387**
Soluble protein (g/dL)						
Week 2	2.788 ± 0.142	2.668 ± 0.066	2.742 ± 0.089	2.800 ± 0.064	2.940 ± 0.044	2.898 ± 0.090
Week 6	2.958 ± 0.074	3.100 ± 0.038	2.820 ± 0.143	3.146 ± 0.086	3.020 ± 0.064	2.980 ± 0.085
Week 14	2.532 ± 0.205	2.258 ± 0.038	2.360 ± 0.078	2.386 ± 0.041	2.486 ± 0.077	2.652 ± 0.047
α2u-Globulin (nmol/g kidney)						
Week 2	19.60 ± 5.13	24.86 ± 6.44	18.28 ± 6.65	71.31 ± 28.96	69.16 ± 22.59*	66.74 ± 29.14*
Week 6	274.20 ± 85.72	435.80 ± 128.82	622.80 ± 161.96	623.20 ± 102.08	425.40 ± 123.13	498.80 ± 112.86
Week 14	80.46 ± 9.73	252.14 ± 85.56*	157.68 ± 26.86*	314.60 ± 49.60**	297.18 ± 125.69**	564.60 ± 178.90**
α2u-Globulin (ng/μg soluble protein)						
Week 2	6.46 ± 1.64	8.66 ± 2.11	6.10 ± 2.17	23.80 ± 9.82	22.01 ± 7.06*	21.37 ± 9.42*
Week 6	86.58 ± 27.57	131.94 ± 39.65	200.66 ± 48.10	183.60 ± 26.12	132.78 ± 39.71	155.32 ± 32.84
Week 14	30.22 ± 4.04	103.50 ± 34.62*	62.60 ± 10.46*	123.14 ± 18.64**	110.68 ± 44.32**	197.98 ± 61.48**

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test
** $P \leq 0.01$ by Shirley's test
[a] Data are presented as mean ± standard error.
[b] Labeling index was calculated as the number of labeled cells divided by the total number of cells counted times 100. A minimum of 2,000 cells were counted.

TABLE G3
Urinalysis Data for F344/N Rats at 12 Weeks in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	10	10	10	10	10	10
Male						
Creatinine (mg/dL)	59.10 ± 9.00	46.60 ± 7.70	73.30 ± 18.50	54.60 ± 12.70	44.90 ± 6.10	41.90 ± 7.30
Glucose (mg/dL)	7 ± 1	6 ± 1	8 ± 2	7 ± 2	6 ± 1	6 ± 1
Glucose/creatinine ratio	0.11 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01*
Protein (mg/dL)	34 ± 7	29 ± 7	41 ± 5	37 ± 9	28 ± 4	26 ± 5
Protein/creatinine ratio	0.57 ± 0.05	0.58 ± 0.06	0.70 ± 0.07	0.70 ± 0.05	0.67 ± 0.09	0.62 ± 0.07
Alkaline phosphatase (IU/L)	187 ± 41	135 ± 27	165 ± 20	124 ± 19	104 ± 12*	91 ± 12**
Alkaline phosphatase/ creatinine ratio	3.06 ± 0.28	2.78 ± 0.24	2.75 ± 0.26	2.61 ± 0.18	2.45 ± 0.20	2.27 ± 0.16
Aspartate aminotransferase (IU/L)	6 ± 1	6 ± 1	9 ± 1	10 ± 2	9 ± 1*	15 ± 3**
Aspartate aminotransferase/ creatinine ratio	0.102 ± 0.011	0.13 ± 0.01	0.13 ± 0.01	0.18 ± 0.01**	0.22 ± 0.03**	0.35 ± 0.03**
Lactate dehydrogenase (IU/L)	28 ± 5	23 ± 5	31 ± 5	31 ± 8	26 ± 2	31 ± 4
Lactate dehydrogenase/ creatinine ratio	0.48 ± 0.03	0.48 ± 0.03	0.48 ± 0.03	0.57 ± 0.05	0.63 ± 0.04*	0.78 ± 0.04**
γ-Glutamyltransferase (IU/L)	1,256 ± 177	997 ± 190	1,482 ± 345	1,064 ± 289	811 ± 92*	618 ± 112**
γ-Glutamyltransferase/ creatinine ratio	21.63 ± 1.09	20.87 ± 0.84	20.87 ± 0.61	18.92 ± 0.80*	18.59 ± 0.85*	14.86 ± 0.92**
N-acetyl-β-D-glucosaminidase (IU/L)	12 ± 2	8 ± 1	12 ± 2	16 ± 6	8 ± 1	8 ± 1
N-acetyl-β-D-glucosaminidase/ creatinine ratio	0.21 ± 0.04	0.16 ± 0.01	0.17 ± 0.01	0.36 ± 0.18	0.19 ± 0.01	0.19 ± 0.01
Volume (mL/16 hours)	13.0 ± 1.6	17.7 ± 2.4	13.3 ± 2.5	18.1 ± 3.6	17.6 ± 2.4	19.8 ± 2.2
Specific gravity	1.015 ± 0.002	1.013 ± 0.002	1.019 ± 0.004	1.015 ± 0.003	1.012 ± 0.002	1.011 ± 0.002

TABLE G3
Urinalysis Data for F344/N Rats at 12 Weeks in the 3-Month Inhalation Study of Tetralin

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	10	10	10	10	10	10
Female						
Creatinine (mg/dL)	50.10 ± 7.70	41.20 ± 4.20	31.50 ± 2.90	36.40 ± 3.10	31.30 ± 3.50	68.70 ± 9.20
Glucose (mg/dL)	6 ± 1	5 ± 1	4 ± 0	4 ± 0	4 ± 0	8 ± 1
Glucose/creatinine ratio	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.00
Protein (mg/dL)	5 ± 1	4 ± 0	3 ± 0	4 ± 0	3 ± 0*	6 ± 1
Protein/creatinine ratio	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.00
Alkaline phosphatase (IU/L)	86 ± 10	83 ± 12	62 ± 9	77 ± 10	56 ± 7	89 ± 10
Alkaline phosphatase/ creatinine ratio	1.82 ± 0.12	2.01 ± 0.16	1.94 ± 0.17	2.07 ± 0.16	1.79 ± 0.11	1.34 ± 0.08*
Aspartate aminotransferase (IU/L)	1 ± 0	1 ± 0	1 ± 0	2 ± 0*	3 ± 1**	14 ± 2**
Aspartate aminotransferase/ creatinine ratio	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01**	0.09 ± 0.01**	0.20 ± 0.01**
Lactate dehydrogenase (IU/L)	16 ± 3	15 ± 2	11 ± 1	13 ± 2	11 ± 1	29 ± 4
Lactate dehydrogenase/ creatinine ratio	0.32 ± 0.02	0.38 ± 0.03	0.37 ± 0.04	0.36 ± 0.03	0.38 ± 0.03	0.45 ± 0.05
γ-Glutamyltransferase (IU/L)	513 ± 53	513 ± 100	349 ± 59	421 ± 61	370 ± 60	505 ± 68
γ-Glutamyltransferase/ creatinine ratio	11.21 ± 1.15	12.45 ± 1.75	10.66 ± 0.95	11.20 ± 0.85	11.91 ± 1.34	7.47 ± 0.52
N-acetyl-β-D-glucosaminidase (IU/L)	7 ± 1	6 ± 1	4 ± 0	5 ± 1	4 ± 1	8 ± 1
N-acetyl-β-D-glucosaminidase/ creatinine ratio	0.13 ± 0.00	0.14 ± 0.01	0.13 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.01
Volume (mL/16 hours)	9.3 ± 1.4	10.3 ± 1.3	14.1 ± 1.6	11.9 ± 1.3	14.8 ± 1.7	6.6 ± 1.0
Specific gravity	1.015 ± 0.002	1.014 ± 0.001	1.011 ± 0.001	1.013 ± 0.001	1.010 ± 0.001	1.022 ± 0.003

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

TABLE G4
Urinalysis and Urinary Metabolite Data for F344/N Rats at 12 Months in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
n	5	5	5	5
Male				
Creatinine (mg/dL)	92.30 ± 12.70	71.60 ± 5.20	70.20 ± 5.70	63.40 ± 12.90
Glucose	Negative	Negative	Negative	Negative
Protein (mg/dL)	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Volume (mL/16 hours)	10.3 ± 2.1	13.2 ± 1.7	13.3 ± 1.4	18.6 ± 3.6
Specific gravity	1.022 ± 0.003	1.018 ± 0.001	1.018 ± 0.001	1.016 ± 0.003
pH	6.50 ± 0.00	6.50 ± 0.00	6.50 ± 0.00	6.60 ± 0.10
Urobilinogen	Negative	Negative	Negative	Negative
Bilirubin (ordinal 1-3)	2.0 ± 0.0	1.8 ± 0.2	2.0 ± 0.0	1.4 ± 0.2
Blood (ordinal 1-3)	0.2 ± 0.2	Negative	Negative	0.6 ± 0.6
Ketones (mg/dL)	1.0 ± 0.3	1.0 ± 0.3	1.2 ± 0.4	0.6 ± 0.6
Leukocytes (ordinal 1-3)	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Nitrites	Negative	Negative	Negative	Negative
1-Tetralol concentration (µg/mL)	0.2 ± 0.0	37.0 ± 2.8**	75.0 ± 8.8**	154.7 ± 30.7**
1-Tetralol normalized to creatinine (ng/µg)	0.2 ± 0.0	51.7 ± 2.7**	107.2 ± 9.0**	246.0 ± 9.9**
2-Tetralol concentration (µg/mL)	0.2 ± 0.0	10.4 ± 1.1**	18.0 ± 2.5**	33.0 ± 8.8**
2-Tetralol normalized to creatinine (ng/µg)	0.2 ± 0.1	14.6 ± 1.7**	25.7 ± 3.0**	50.2 ± 3.2**
2-Hydroxy-1-tetralone concentration (µg/mL)	0.0 ± 0.0	62.2 ± 3.5**	109.4 ± 13.9**	213.2 ± 41.2**
2-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.0 ± 0.0	87.6 ± 4.6**	155.8 ± 13.7**	339.8 ± 10.8**
4-Hydroxy-1-tetralone concentration (µg/mL)	0.3 ± 0.0	37.8 ± 3.2**	73.9 ± 8.1**	168.6 ± 33.1**
4-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.3 ± 0.1	52.8 ± 2.1**	106.0 ± 10.6**	267.6 ± 10.0**
Female				
Creatinine (mg/dL)	57.90 ± 6.00	51.50 ± 10.20	39.70 ± 5.60	50.50 ± 11.10
Glucose	Negative	Negative	Negative	Negative
Protein (mg/dL)	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Volume (mL/16 hours)	9.0 ± 1.1	11.6 ± 2.2	15.0 ± 3.3	12.6 ± 3.2
Specific gravity	1.017 ± 0.002	1.015 ± 0.003	1.011 ± 0.002	1.015 ± 0.003
pH	6.30 ± 0.10	6.50 ± 0.00	6.40 ± 0.20	6.40 ± 0.10
Urobilinogen	Negative	Negative	Negative	Negative
Bilirubin (ordinal 1-3)	2.0 ± 0.0	1.8 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
Blood (ordinal 1-3)	Negative	Negative	Negative	Negative
Ketones (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Leukocytes (ordinal 1-3)	0.2 ± 0.2	1.0 ± 0.0*	1.0 ± 0.0**	1.0 ± 0.0**
Nitrites	Negative	Negative	Negative	Negative
1-Tetralol concentration (µg/mL)	0.1 ± 0.0	17.2 ± 3.5**	28.7 ± 4.5**	89.3 ± 18.5**
1-Tetralol normalized to creatinine (ng/µg)	0.3 ± 0.0	33.7 ± 5.3**	72.5 ± 7.0**	177.2 ± 10.7**
2-Tetralol concentration (µg/mL)	0.1 ± 0.0	9.1 ± 1.9**	14.8 ± 2.5**	43.9 ± 9.6**
2-Tetralol normalized to creatinine (ng/µg)	0.3 ± 0.0	17.7 ± 3.0**	37.0 ± 4.1**	86.3 ± 5.8**
2-Hydroxy-1-tetralone concentration (µg/mL)	0.0 ± 0.0	29.6 ± 6.2**	56.3 ± 8.2**	163.1 ± 31.9**
2-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.0 ± 0.0	56.5 ± 4.7**	143.0 ± 11.8**	328.0 ± 13.1**
4-Hydroxy-1-tetralone concentration (µg/mL)	0.0 ± 0.0	14.5 ± 3.0**	27.0 ± 3.8**	99.4 ± 21.1**
4-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.1 ± 0.1	28.1 ± 4.0**	68.5 ± 5.1**	196.6 ± 12.1**

* Significantly different (P≤0.05) from the chamber control group by Shirley's test
** P≤0.01
[a] Data are given as mean ± standard error.

TABLE G5
Urinalysis and Urinary Metabolite Data for Mice at 12 Months in the 2-Year Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
n	5	5	5	5
Male				
Creatinine (mg/dL)	20.50 ± 1.30	19.40 ± 2.20	21.10 ± 1.90	17.30 ± 3.60
Glucose	Negative	Negative	Negative	Negative
Protein (mg/dL)	2 ± 0	2 ± 0	2 ± 0	1 ± 0
Volume (mL/16 hours)	2.0 ± 0.2	2.3 ± 0.4	2.8 ± 0.3	4.3 ± 1.7
Specific gravity	1.023 ± 0.001	1.022 ± 0.002	1.020 ± 0.002	1.016 ± 0.003
pH	6.50 ± 0.20	6.70 ± 0.10	6.70 ± 0.10	6.50 ± 0.00
Urobilinogen	Negative	Negative	Negative	Negative
Bilirubin (ordinal 1-3)	0.0 ± 0.0	0.6 ± 0.4	1.4 ± 0.2*	1.0 ± 0.4
Blood (ordinal 1-3)	Negative	Negative	Negative	Negative
Ketones (mg/dL)	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Leukocytes (ordinal 1-3)	0.4 ± 0.4	0.8 ± 0.2	1.0 ± 0.0	1.0 ± 0.0
Nitrites	Negative	Negative	Negative	Negative
1-Tetralol concentration (µg/mL)	0.0 ± 0.0	49.7 ± 8.2**	103.8 ± 10.0**	201.0 ± 27.0**
1-Tetralol normalized to creatinine (ng/µg)	0.0 ± 0.0	252.4 ± 24.5**	493.2 ± 21.7**	1,318.8 ± 278.5**
2-Tetralol concentration (µg/mL)	0.0 ± 0.0	13.5 ± 2.4**	39.5 ± 5.3**	93.3 ± 13.7**
2-Tetralol normalized to creatinine (ng/µg)	0.0 ± 0.0	68.3 ± 6.8**	186.0 ± 14.5**	608.4 ± 129.0**
2-Hydroxy-1-tetralone concentration (µg/mL)	0.0 ± 0.0	20.1 ± 3.6**	40.5 ± 4.9**	83.4 ± 12.8**
2-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.0 ± 0.0	101.4 ± 6.8**	191.6 ± 12.5**	547.2 ± 122.0**
4-Hydroxy-1-tetralone concentration (µg/mL)	0.0 ± 0.0	122.4 ± 17.2**	267.4 ± 29.8**	459.8 ± 55.1**
4-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.0 ± 0.0	627.0 ± 41.8**	1,262.0 ± 55.8**	3,014.0 ± 608.7**
Female				
Creatinine (mg/dL)	20.70 ± 2.00	15.00 ± 3.20	16.00 ± 3.20	15.10 ± 1.80
Glucose	Negative	Negative	Negative	Negative
Protein (mg/dL)	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Volume (mL/16 hours)	1.6 ± 0.1	3.8 ± 0.9*	2.2 ± 0.4	2.9 ± 0.5
Specific gravity	1.020 ± 0.002	1.015 ± 0.003	1.015 ± 0.002	1.015 ± 0.002
pH	6.60 ± 0.10	6.60 ± 0.10	6.80 ± 0.10	6.60 ± 0.10
Urobilinogen	Negative	Negative	Negative	Negative
Bilirubin (ordinal 1-3)	0.0 ± 0.0	1.0 ± 0.4	1.2 ± 0.5	0.8 ± 0.4
Blood (ordinal 1-3)	Negative	Negative	Negative	Negative
Ketones (mg/dL)	3.6 ± 0.2	4.4 ± 0.2	3.8 ± 0.2	3.4 ± 0.2
Leukocytes (ordinal 1-3)	0.0 ± 0.0	0.4 ± 0.2	0.8 ± 0.2*	1.0 ± 0.0**
Nitrites	Negative	Negative	Negative	Negative
1-Tetralol concentration (µg/mL)	0.1 ± 0.1	21.8 ± 6.7**	51.8 ± 24.7**	133.0 ± 26.5**
1-Tetralol normalized to creatinine (ng/µg)	0.7 ± 0.3	134.5 ± 25.3**	262.0 ± 97.9**	844.4 ± 85.1**
2-Tetralol concentration (µg/mL)	0.2 ± 0.0	4.2 ± 1.3**	12.2 ± 6.4**	31.7 ± 7.3**
2-Tetralol normalized to creatinine (ng/µg)	0.9 ± 0.2	25.9 ± 5.1**	59.1 ± 26.3**	197.8 ± 28.0**
2-Hydroxy-1-tetralone concentration (µg/mL)	0.0 ± 0.0	11.3 ± 3.4**	35.4 ± 17.3**	77.4 ± 17.0**
2-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.0 ± 0.0	71.3 ± 13.6**	176.7 ± 69.4**	484.6 ± 63.5**
4-Hydroxy-1-tetralone concentration (µg/mL)	0.2 ± 0.1	37.1 ± 10.8**	108.3 ± 48.9**	269.2 ± 55.3**
4-Hydroxy-1-tetralone normalized to creatinine (ng/µg)	0.7 ± 0.4	231.6 ± 36.2**	558.0 ± 187.0**	1,706.0 ± 185.7**

* Significantly different (P≤0.05) from the chamber control group by Dunn's or Shirley's test
** P≤0.01 by Shirley's test
[a] Data are given as mean ± standard error.

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

TABLE H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	242 ± 6	241 ± 4	236 ± 4	241 ± 5	232 ± 4	233 ± 6
R. Kidney						
Absolute	0.77 ± 0.03	0.80 ± 0.02	0.79 ± 0.02	0.81 ± 0.02	0.83 ± 0.02	0.82 ± 0.02
Relative	3.180 ± 0.035	3.320 ± 0.059	3.340 ± 0.052	3.355 ± 0.034*	3.565 ± 0.066**	3.508 ± 0.081**
Liver						
Absolute	8.42 ± 0.27	8.65 ± 0.22	8.46 ± 0.24	9.22 ± 0.47	9.10 ± 0.61	9.24 ± 0.40
Relative	34.831 ± 0.855	35.909 ± 0.990	35.827 ± 0.741	38.225 ± 1.318	39.118 ± 2.345*	39.552 ± 1.265*
Lung						
Absolute	1.33 ± 0.03	1.41 ± 0.05	1.39 ± 0.06	1.48 ± 0.03	1.35 ± 0.05	1.28 ± 0.04
Relative	5.481 ± 0.107	5.832 ± 0.137	5.881 ± 0.240	6.138 ± 0.064*	5.828 ± 0.208	5.500 ± 0.066
Female						
Necropsy body wt	161 ± 1	157 ± 3	155 ± 4	153 ± 1	155 ± 4	144 ± 2**
R. Kidney						
Absolute	0.54 ± 0.01	0.55 ± 0.01	0.57 ± 0.01	0.57 ± 0.01	0.60 ± 0.01**	0.56 ± 0.01
Relative	3.372 ± 0.051	3.491 ± 0.063	3.651 ± 0.043**	3.733 ± 0.049**	3.868 ± 0.086**	3.897 ± 0.023**
Liver						
Absolute	5.19 ± 0.15	5.20 ± 0.21	5.29 ± 0.29	5.29 ± 0.12	5.52 ± 0.18	5.07 ± 0.10
Relative	32.262 ± 0.757	33.099 ± 0.893	34.009 ± 1.062	34.535 ± 0.620	35.630 ± 0.755**	35.242 ± 0.268**
Lung						
Absolute	1.00 ± 0.03	0.99 ± 0.01	1.05 ± 0.06	1.03 ± 0.04	1.02 ± 0.02	1.04 ± 0.05
Relative	6.243 ± 0.188	6.310 ± 0.146	6.781 ± 0.300	6.741 ± 0.204	6.617 ± 0.161	7.222 ± 0.392*

* Significantly different ($P \leq 0.05$) from the chamber 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 H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male NBR Rats in the 2-Week Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	5	5	5	5	5	5
Necropsy body wt	281 ± 5	265 ± 4*	262 ± 5**	258 ± 4**	258 ± 5**	261 ± 4**
R. Kidney						
Absolute	0.94 ± 0.02	0.96 ± 0.01	0.93 ± 0.03	0.90 ± 0.02	0.89 ± 0.02	0.90 ± 0.02
Relative	3.341 ± 0.050	3.612 ± 0.039*	3.547 ± 0.105	3.494 ± 0.028	3.444 ± 0.034	3.444 ± 0.052
Liver						
Absolute	8.88 ± 0.15	8.33 ± 0.14	8.65 ± 0.06	8.56 ± 0.33	9.45 ± 0.44	10.95 ± 0.46**
Relative	31.574 ± 0.518	31.470 ± 0.390	32.986 ± 0.525	33.115 ± 0.741	36.698 ± 1.907**	41.980 ± 1.445**
Lung						
Absolute	1.52 ± 0.03	1.43 ± 0.04	1.44 ± 0.04	1.42 ± 0.04	1.40 ± 0.02	1.66 ± 0.23
Relative	5.418 ± 0.099	5.393 ± 0.097	5.499 ± 0.142	5.502 ± 0.121	5.460 ± 0.188	6.348 ± 0.797

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

TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	294 ± 8	301 ± 7	299 ± 6	301 ± 8	289 ± 8	276 ± 5
Heart						
Absolute	0.82 ± 0.03	0.84 ± 0.03	0.83 ± 0.01	0.84 ± 0.02	0.84 ± 0.03	0.77 ± 0.02
Relative	2.791 ± 0.034	2.776 ± 0.037	2.775 ± 0.025	2.795 ± 0.052	2.891 ± 0.041	2.800 ± 0.027
R. Kidney						
Absolute	0.86 ± 0.03	0.93 ± 0.03	0.94 ± 0.02	0.92 ± 0.03	0.92 ± 0.03	0.90 ± 0.02
Relative	2.930 ± 0.037	3.082 ± 0.070*	3.143 ± 0.043*	3.051 ± 0.032*	3.181 ± 0.044**	3.270 ± 0.032**
Liver						
Absolute	8.48 ± 0.31	8.88 ± 0.25	9.59 ± 0.31*	9.00 ± 0.32	8.61 ± 0.34	8.65 ± 0.25
Relative	28.795 ± 0.559	29.527 ± 0.356	31.992 ± 0.623**	29.860 ± 0.442	29.757 ± 0.494	31.264 ± 0.518**
Lung						
Absolute	1.51 ± 0.05	1.50 ± 0.06	1.48 ± 0.02	1.46 ± 0.04	1.42 ± 0.03	1.38 ± 0.05
Relative	5.136 ± 0.146	4.998 ± 0.238	4.958 ± 0.080	4.848 ± 0.145	4.929 ± 0.097	5.016 ± 0.182
R. Testis						
Absolute	1.276 ± 0.039	1.256 ± 0.037	1.323 ± 0.025	1.310 ± 0.034	1.220 ± 0.080	1.276 ± 0.043
Relative	4.335 ± 0.058	4.187 ± 0.120	4.423 ± 0.057	4.356 ± 0.083	4.240 ± 0.268	4.616 ± 0.128
Thymus						
Absolute	0.338 ± 0.024	0.341 ± 0.016	0.360 ± 0.017	0.332 ± 0.016	0.325 ± 0.015	0.319 ± 0.013
Relative	1.140 ± 0.060	1.142 ± 0.068	1.207 ± 0.061	1.104 ± 0.051	1.132 ± 0.063	1.162 ± 0.060
Female						
Necropsy body wt	183 ± 4	190 ± 4	184 ± 3	180 ± 4	178 ± 4	173 ± 3
Heart						
Absolute	0.59 ± 0.01	0.60 ± 0.01	0.59 ± 0.01	0.58 ± 0.01	0.57 ± 0.01	0.59 ± 0.01
Relative	3.229 ± 0.048	3.163 ± 0.036	3.203 ± 0.033	3.220 ± 0.040	3.237 ± 0.046	3.390 ± 0.095
R. Kidney						
Absolute	0.60 ± 0.01	0.63 ± 0.02	0.64 ± 0.01	0.64 ± 0.01	0.64 ± 0.02*	0.66 ± 0.01**
Relative	3.275 ± 0.044	3.314 ± 0.060	3.492 ± 0.041**	3.543 ± 0.059**	3.622 ± 0.056**	3.827 ± 0.048**
Liver						
Absolute	5.11 ± 0.11	5.23 ± 0.17	5.30 ± 0.15	5.27 ± 0.10	5.32 ± 0.13	5.25 ± 0.10
Relative	27.915 ± 0.615	27.533 ± 0.389	28.830 ± 0.624	29.294 ± 0.414	29.959 ± 0.479**	30.393 ± 0.475**
Lung						
Absolute	1.02 ± 0.03	1.09 ± 0.03	1.11 ± 0.04	1.06 ± 0.02	1.03 ± 0.02	1.03 ± 0.01
Relative	5.592 ± 0.141	5.751 ± 0.153	6.051 ± 0.142	5.910 ± 0.079	5.818 ± 0.151	5.963 ± 0.062
Thymus						
Absolute	0.267 ± 0.010	0.260 ± 0.008	0.252 ± 0.008	0.248 ± 0.010	0.262 ± 0.013	0.241 ± 0.011
Relative	1.457 ± 0.056	1.377 ± 0.053	1.371 ± 0.043	1.384 ± 0.063	1.474 ± 0.064	1.392 ± 0.060

* Significantly different ($P \leq 0.05$) from the chamber 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 H4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.2 ± 0.2	25.3 ± 0.8	25.4 ± 0.3	24.3 ± 0.6	25.2 ± 0.6	24.2 ± 0.5
R. Kidney						
Absolute	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.00	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
Relative	8.568 ± 0.220	9.136 ± 0.260	8.837 ± 0.238	8.237 ± 0.224	8.151 ± 0.139	8.840 ± 0.334
Liver						
Absolute	1.18 ± 0.01	1.26 ± 0.06	1.24 ± 0.02	1.20 ± 0.04	1.37 ± 0.03**	1.32 ± 0.05**
Relative	46.745 ± 0.232	49.616 ± 0.592	48.726 ± 0.895	49.687 ± 1.817	54.136 ± 0.567**	54.364 ± 0.927**
Lung						
Absolute	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.00	0.17 ± 0.01	0.18 ± 0.00
Relative	7.377 ± 0.143	7.275 ± 0.206	7.329 ± 0.183	7.349 ± 0.143	6.906 ± 0.227	7.375 ± 0.176
Female						
Necropsy body wt	21.3 ± 0.4	22.0 ± 0.4	22.0 ± 0.3	22.3 ± 0.2	21.4 ± 0.2	21.2 ± 0.4
R. Kidney						
Absolute	0.15 ± 0.01	0.16 ± 0.00	0.16 ± 0.00*	0.16 ± 0.01*	0.16 ± 0.00	0.16 ± 0.00
Relative	6.852 ± 0.186	7.270 ± 0.240	7.448 ± 0.160	7.275 ± 0.187	7.494 ± 0.176	7.358 ± 0.144
Liver						
Absolute	1.04 ± 0.02	1.16 ± 0.03	1.15 ± 0.03	1.26 ± 0.04**	1.14 ± 0.04	1.22 ± 0.06**
Relative	48.873 ± 1.142	52.520 ± 0.383	52.153 ± 1.414	56.583 ± 1.523**	53.340 ± 1.478**	57.524 ± 1.988**
Lung						
Absolute	0.17 ± 0.00	0.19 ± 0.01	0.18 ± 0.01	0.17 ± 0.00	0.18 ± 0.01	0.16 ± 0.01
Relative	8.086 ± 0.180	8.619 ± 0.370	8.264 ± 0.184	7.814 ± 0.129	8.530 ± 0.294	7.732 ± 0.372

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

TABLE H5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	7.5 ppm	15 ppm	30 ppm	60 ppm	120 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.0 ± 1.2	39.5 ± 0.8	37.7 ± 0.8	38.2 ± 1.2	35.9 ± 0.8	34.6 ± 0.8*
Heart						
Absolute	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.00*	0.14 ± 0.00**
Relative	4.571 ± 0.192	4.286 ± 0.109	4.310 ± 0.099	4.337 ± 0.095	4.382 ± 0.091	4.114 ± 0.089*
R. Kidney						
Absolute	0.33 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.32 ± 0.01	0.29 ± 0.01**	0.28 ± 0.01**
Relative	8.656 ± 0.257	8.095 ± 0.155	7.973 ± 0.082*	8.350 ± 0.162	7.953 ± 0.191*	7.990 ± 0.125*
Liver						
Absolute	1.55 ± 0.06	1.66 ± 0.03	1.52 ± 0.04	1.60 ± 0.04	1.47 ± 0.05	1.51 ± 0.05
Relative	40.769 ± 0.777	41.976 ± 0.539	40.387 ± 0.719	41.973 ± 0.821	40.951 ± 0.849	43.634 ± 0.640*
Lung						
Absolute	0.21 ± 0.00	0.22 ± 0.00	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.01**
Relative	5.688 ± 0.206	5.616 ± 0.120	5.924 ± 0.122	5.594 ± 0.151	5.653 ± 0.085	5.583 ± 0.095
R. Testis						
Absolute	0.120 ± 0.005	0.112 ± 0.004	0.115 ± 0.003	0.112 ± 0.003	0.111 ± 0.005	0.109 ± 0.004
Relative	3.192 ± 0.197	2.843 ± 0.073	3.067 ± 0.081	2.936 ± 0.100	3.083 ± 0.121	3.161 ± 0.113
Thymus						
Absolute	0.038 ± 0.005	0.041 ± 0.003	0.036 ± 0.002	0.041 ± 0.003	0.039 ± 0.002	0.038 ± 0.001
Relative	1.033 ± 0.157	1.021 ± 0.075	0.957 ± 0.054	1.070 ± 0.081	1.086 ± 0.047	1.097 ± 0.055
Female						
Necropsy body wt	31.4 ± 1.0	30.8 ± 0.9	31.8 ± 0.9	30.9 ± 1.1	30.8 ± 0.7	29.2 ± 0.9
Heart						
Absolute	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.13 ± 0.00
Relative	4.466 ± 0.182	4.592 ± 0.097	4.577 ± 0.136	4.862 ± 0.245	4.555 ± 0.122	4.387 ± 0.073
R. Kidney						
Absolute	0.20 ± 0.00	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Relative	6.408 ± 0.199	6.726 ± 0.139	6.593 ± 0.180	6.730 ± 0.210	6.821 ± 0.156	6.920 ± 0.103
Liver						
Absolute	1.37 ± 0.02	1.43 ± 0.06	1.43 ± 0.04	1.43 ± 0.05	1.50 ± 0.05	1.43 ± 0.04
Relative	43.767 ± 1.119	46.135 ± 0.586	45.078 ± 0.989	46.382 ± 0.708*	48.673 ± 0.539**	49.119 ± 0.836**
Lung						
Absolute	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.21 ± 0.01
Relative	6.958 ± 0.241	7.126 ± 0.140	7.028 ± 0.218	7.048 ± 0.165	7.480 ± 0.211	7.089 ± 0.239
Thymus						
Absolute	0.052 ± 0.002	0.049 ± 0.002	0.049 ± 0.003	0.053 ± 0.003	0.052 ± 0.002	0.047 ± 0.002
Relative	1.675 ± 0.089	1.590 ± 0.044	1.552 ± 0.088	1.727 ± 0.098	1.695 ± 0.058	1.626 ± 0.077

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

APPENDIX I
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE I1
Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	294 ± 8	301 ± 8	289 ± 8	275 ± 5
L. Cauda epididymis	0.1359 ± 0.0097	0.1538 ± 0.0087	0.1490 ± 0.0091	0.1371 ± 0.0070
L. Epididymis	0.3916 ± 0.0170	0.4194 ± 0.0129	0.4121 ± 0.0210	0.3930 ± 0.0122
L. Testis	1.3520 ± 0.0403	1.3979 ± 0.0331	1.3633 ± 0.0569	1.3475 ± 0.0427
Spermatid measurements				
Spermatid heads (10^3/mg testis)	154.60 ± 4.99	150.50 ± 4.08	148.07 ± 5.49	170.35 ± 25.24
Spermatid heads (10^6/testis)	191.38 ± 4.36	196.63 ± 6.95	188.13 ± 9.65	185.25 ± 5.78
Epididymal spermatozoal measurements				
Sperm motility (%)	94.0 ± 1.7	93.4 ± 2.6	92.0 ± 3.1	89.9 ± 3.4
Sperm count (10^6/cauda epididymis)	101.6 ± 10.1	106.0 ± 8.2	114.3 ± 11.4	94.9 ± 10.7
Sperm concentration (10^3/mg cauda epididymal tissue)	764.9 ± 78.2	691.4 ± 42.2	761.7 ± 52.6	679.6 ± 52.9

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

TABLE I2
Estrous Cycle Characterization for Female F344/N Rats in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	183 ± 4	180 ± 4	178 ± 4	173 ± 3
Proportion of regular cycling females[b,c]	6/10	10/10[*]	7/10	9/10
Estrous cycle length (days)	5.3 ± 0.3[d]	5.1 ± 0.1	6.3 ± 0.3[e]	5.4 ± 0.2[e]
Estrous stages (% of cycle)				
Diestrus	40.0	41.7	52.5	50.0
Proestrus	11.7	14.2	10.0	15.0
Estrus	18.3	20.0	19.2	17.5
Metestrus	17.5	20.0	16.7	17.5
Uncertain diagnoses	12.5	4.2	1.7	0.0

[*] $P \leq 0.05$ by Fisher's exact test.
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body weights) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females did not differ significantly from the chamber control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Regularity of cycling in the control group was difficult to determine because insufficient cells were collected on 13% of samples evaluated.
[d] Estrous cycle was longer than 12 days or unclear in two animals.
[e] Estrous cycle was longer than 12 days or unclear in one animal.

TABLE I3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.0 ± 1.2	38.2 ± 1.2	35.9 ± 0.8	34.6 ± 0.8
L. Cauda epididymis	0.0205 ± 0.0015	0.0195 ± 0.0009	0.0218 ± 0.0025	0.0212 ± 0.0017
L. Epididymis	0.0598 ± 0.0023	0.0558 ± 0.0014	0.0576 ± 0.0022	0.0635 ± 0.0042
L. Testis	0.1182 ± 0.0029	0.1153 ± 0.0039	0.1143 ± 0.0042	0.1128 ± 0.0035
Spermatid measurements				
Spermatid heads (10^3/mg testis)	214.92 ± 12.46	234.74 ± 8.83	237.39 ± 13.22	237.82 ± 14.81
Spermatid heads (10^6/testis)	19.97 ± 1.59	23.13 ± 0.90	21.19 ± 1.39	21.16 ± 1.99
Epididymal spermatozoal measurements				
Sperm motility (%)	85.3 ± 1.8	80.2 ± 1.7	80.6 ± 1.7	79.0 ± 1.8
Sperm count (10^6/cauda epididymis)	23.6 ± 1.5	22.2 ± 2.1	19.7 ± 1.0	21.5 ± 2.1
Sperm concentration (10^3/mg cauda epididymal tissue)	1,216.1 ± 116.5	1,153.9 ± 106.5	993.8 ± 99.8	1,072.7 ± 131.2

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

TABLE I4
Estrous Cycle Characterization for Female Mice in the 3-Month Inhalation Study of Tetralin[a]

	Chamber Control	30 ppm	60 ppm	120 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	31.4 ± 1.0	30.9 ± 1.1	30.8 ± 0.7	29.2 ± 0.9
Proportion of regular cycling females[b,c]	10/10	9/10	9/10	9/10
Estrous cycle length (days)	4.0 ± 0.0	4.5 ± 0.5	4.0 ± 0.1	4.6 ± 0.1**
Estrous stages (% of cycle)[d]				
Diestrus	25.0	27.5	25.8	30.8
Proestrus	21.7	20.0	19.2	17.5
Estrus	11.7	19.2	25.8	30.0
Metestrus	23.3	24.2	25.0	21.7
Uncertain diagnoses	18.3	9.2	4.2	0.0

** $P \leq 0.01$ by Dunn's test.
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group for body weights are not significant by Dunnett's test.
[b] Number of females with a regular cycle/number of females cycling
[c] No differences in regularity of cycling by Fisher's exact test
[d] By multivariate analysis of variance, exposed females differed significantly (Wilk's Criterion, $P \leq 0.05$) in the relative length of time spent in the estrous stages. The 60 and 120 ppm females spent more time in estrus than the chamber control females. However, the relative length of time spent in estrus by the chamber control animals was artificially low because insufficient cells were collected in most or all of the chamber control animals on the expected days of estrus.

APPENDIX J
CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

Procurement and Characterization of Tetralin

Tetralin was obtained from Sigma Aldrich Fluka Bulk Chemicals (St. Louis, MO) in two lots (00822JG and 07808LG) and from Advanced Aromatics, L.P. (Baytown, TX), in one lot (139699). Lots 00822JG and 07808LG were used in the 2-week and 3-month studies as a mixture combined by Research Triangle Institute (Research Triangle Park, NC) and assigned lot number 8359-80-01; lot 139699 was used during the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute, and the study laboratory, Battelle Toxicology Northwest (Richland, WA). The study laboratory also performed stability testing; additional testing was performed by Chemir/Polytech Laboratories, Inc. (St. Louis, MO), Chemir Analytical Services (Maryland Heights, MO), and Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the tetralin studies are on file at the National Institute of Environmental Health Sciences.

All lots of the chemical, a clear, colorless liquid, were identified as tetralin by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy and by gas chromatography (GC) coupled with mass spectroscopy (MS). Identity was confirmed using IR and proton NMR spectroscopy by Chemir/Polytech (combined lot 8359-80-01) and Chemir Analytical Services (lot 139699). All spectra were consistent with the structure and literature spectra of tetralin (*Aldrich*, 1981, 1983, 1993, 1997; NIST, 1995, 2005; NIAIST, 2003; *Sadtler*; *Wiley*, 2003). Representative IR, proton NMR, and MS spectra are presented in Figures J1, J2, and J3.

The analytical chemistry laboratory determined the purity of all lots using GC/MS by system A; for lot 139699, GC/MS by a system similar to system A and GC with flame ionization detection (FID) by system B were also used (Table J1). Purity was confirmed by the study laboratory using GC/FID by system C (lots 8359-80-01 and 139699) or a system similar to system C (lot 139699). For lot 8359-80-01, elemental analyses were performed by Chemir/Polytech Laboratories, Inc.; for lot 139699, Karl Fischer titration was performed by Chemir Analytical Services and elemental analyses were performed by Galbraith Laboratories, Inc.

For combined lot 8359-80-01, elemental analyses showed good agreement between found and theoretical values for carbon and hydrogen; oxygen and nitrogen were present at less than 0.5%, and sulfur content was less than 0.05%. For lots 00822JG and 07808LG, GC/MS by system A indicated one major peak and no impurities greater than 0.1% of the major peak area; the purity of each lot was determined to be greater than 97%. For combined lot 8359-80-01, GC/FID by system C indicated a major peak and three impurities 0.1% or greater of the total peak area. The overall purity for lots 00822JG, 07808LG, and/or combined lot 8359-80-01 was determined to be greater than 97%.

For lot 139699, Karl Fischer titration indicated a water content of 52 ppm. Elemental analyses showed good agreement between theoretical and found percentages for carbon and hydrogen; oxygen, nitrogen, and sulfur content were determined to be less than 0.5%. GC/MS by a system similar to system A indicated one major peak and six impurities greater than 0.1% of the total peak area. GC/FID by system B indicated a major peak and four impurities greater than 0.1% of the total peak area. GC/FID by system C or a system similar to system C indicated one major peak and three impurities greater than 0.1% of the total peak area. The overall purity for lot 139699 was determined to be greater than 98%.

The study laboratory identified the three impurities in lot 8359-80-01 using GC/MS by a system similar to system A as *trans*-decalin (0.40%), *cis*-decalin (0.98%), and octahydronapthalene (0.33%) by comparison to the spectra of reference standards obtained from Aldrich Chemical Company, Inc. The analytical chemistry laboratory identified the impurities in lot 139699 using GC/MS by a system similar to system A as *trans*-decahydronapthalene,

cis-decahydronapthalene, 1,2,3,4,5,6,7-hexahydro-1H-indene-1-one, 2,3-dihydro-1-methyl-1H-indene, 2,3-dihydro-5-methyl-1H-indene, and naphthalene. The spectra of all identified impurities were consistent with their structure and literature spectra (NIST, 2002; NIAIST, 2003; *Wiley*, 2003). In an attempt to identify the impurities in lot 139699, the study laboratory used GC/MS by a system similar to system A; naphthalene (0.15%) was positively identified by comparison to a spectrum of a reference standard obtained from Aldrich Chemical Company; the other two impurities (0.10% and 0.20%) were tentatively identified as dihydromethylindenes, but the identities could not be confirmed as no reference standards for these compounds were available.

Potentiometric titration was used to determine the peroxide content of each lot: 7.02 mEq/kg (00822JG), 8.79 mEq/kg (lot 7808LG), and 2.62 mEq/kg (lot 139699). To prevent the formation of hydroperoxides, 4-*tert*-butylcatechol was added to lot 139699 at a concentration of 50 ppm. The concentration was monitored every 6 months during the 2-year studies using high-performance liquid chromatography (HPLC). When the concentration of 4-*tert*-butylcatechol fell below 30 ppm, it was refortified to approximately 50 ppm. The HPLC system included a Hewlett-Packard HPLC (Hewlett-Packard, Palo Alto, CA) instrument with a Waters Nova-Pak C18 column (3.9 mm × 300 mm, 4-µm particle size) (Waters Corporation, Milford, MA), a mobile phase of 1% acetic acid in methanol (A) and 1% acetic acid in water (B), beginning with 0%A:100%B for 2 minutes, changed to 100%A:0%B over 11 minutes, held for 8 minutes, then rapidly reversed to 0%A:100%B in 0.1 minute, a flow rate of 0.75 mL/minute, and fluorescent detection at 274 and 298 nm.

To ensure stability, the bulk chemical was stored in the original shipping containers (55-gallon metal drums) under a nitrogen headspace at controlled room temperature (approximately 18° to 23° C). The bulk chemical was reanalyzed by the study laboratory 30 days prior to each study, at the beginning and end of the 2-week study, at the beginning, midpoint, and end of the 3-month studies, and at the beginning, end, and at least every 24 weeks during the 2-year studies using GC/FID by system C or a system similar to system C. No degradation of the chemical occurred.

Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the studies is shown in Figure J4. Preheated tetralin was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor out of the generator. Generator output was controlled by the delivery rate of the chemical metering pump.

Because the vapor leaving the generator was above room temperature, it was transported to the exposure room at an elevated temperature to prevent condensation. In the exposure room, the vapor was mixed with additional heated compressed air before entering a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, generator nitrogen flow rate, and dilution air flow rate. The pressure in the distribution manifold was kept fixed to ensure constant flows through the manifold and into the chambers.

An electronically actuated metering valve controlled the flow to each chamber; a pneumatically operated chamber exposure shutoff valve in line with the metering valve stopped flow to the chamber. Until the generation system was stable and exposures were ready to proceed, all chamber exposure valves were closed, and vapor was directed to the exposure chamber exhaust. When exposures started, the chambers' exposure valves were opened to allow the vapor to flow through the metering valves and then through temperature-controlled delivery lines to each exposure chamber. The vapor was then injected into the chamber inlet duct where it was further diluted with conditioned chamber air to achieve the desired concentrations.

The study laboratory designed the inhalation exposure chamber (Lab Products, Inc., Seaford, DE) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A condensation particle counter [Type CN, Gardner Associates,

Schenectady, NY (2-week and 3-month studies) or Model 3022A, TSI Inc., St. Paul, MN (2-year studies)] was used to count the particles in all chambers before and during generation to determine whether tetralin vapor, and not aerosol, was produced. No particle counts greater than 200 particles/cm^3 were detected.

Vapor Concentration Monitoring

The tetralin concentrations in the exposure chambers were monitored by an online GC by system D (2-week and 3-month studies) or E (2-year studies) (Table J1). Samples were drawn from each exposure chamber approximately every 24 (2-week and 3-month studies) or 26 (2-year studies) minutes during each 6-hour exposure period. Summaries of the chamber vapor concentrations are given in Tables J2, J3, and J4.

A 12- (2-week and 3-month studies) or a 16-port (2-year studies) stream select valve (VALCO Instruments Company, Houston, TX) directed a continuous stream of sampled atmosphere to a 6-port sampling valve (VALCO Instruments Company) with a 1.0 mL sample loop housed in a dedicated valve oven at 280° (2-week and 3-month studies) or 150° C (2-year studies). A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow. The online GC was checked throughout the day for instrument drift against an online standard of tetralin in nitrogen supplied by a diffusion standard generator (Kin-Tek Model 491, Precision Calibration Systems, La Marque, TX).

The online GC was calibrated monthly by a comparison of chamber concentration data to data from grab samples, which were collected with charcoal sampling tubes (ORBO^{TM}-101, Supelco, Bellefonte, PA). The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. These samples were extracted with toluene containing 1-phenylhexane as an internal standard and analyzed using an offline GC by system F. The offline GC was calibrated with gravimetrically prepared standard solutions of tetralin containing 1-phenylhexane as an internal standard in toluene.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined without and with animals present in the chambers. At a chamber airflow rate of 15 cfm, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) was approximately 12.5 minutes. For rats and mice in the 2-week and 3-month studies, T_{90} values ranged from 12 to 17 minutes without animals present and 10 to 13 minutes with animals present; T_{10} values ranged from 11 to 17 minutes without animals present and 14 to 20 minutes with animals present. For rats and mice in the 2-year studies, T_{90} values ranged from 8 to 10 minutes without animals present and from 12 to 16 minutes with animals present; T_{10} values ranged from 10 to 11 minutes without animals present and from 15 to 25 minutes with animals present. A T_{90} value of 12 minutes was selected for all studies.

The uniformity of tetralin vapor concentration in the inhalation exposure chambers without and with animals present in the chambers was measured once during the 2-week and 3-month studies and every 3 months during the 2-year studies. The vapor concentration was measured using the online GC (system D, Table J1) with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. Each exposure chamber has 12 sample ports; chamber uniformity measurements were taken at all 12 positions. Chamber concentration uniformity was maintained throughout the studies.

The persistence of tetralin in the chamber after vapor delivery ended was determined by monitoring the concentration without and with animals present in the 120 ppm chambers. In the 2-week and 3-month studies, the concentration decreased to 1% of the target concentration in approximately 118 minutes without animals present

and in 106 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 48 minutes (rats) and 97 minutes (mice) without animals present and in approximately 164 minutes (rats) and approximately 113 minutes (mice) with animals present.

Stability studies of tetralin in the generation and delivery system were performed. Samples of the test atmosphere from the distribution manifold and the high and low exposure chambers (7.5 and 120 ppm chambers for the 2-week and 3-month studies; 30 and 120 ppm for the 2-year studies) were collected with ORBO^{TM}-101 charcoal sampling tubes during the first and last hours of generation with animals present in the chambers. Additional samples were collected for the 2-year studies with tubes containing silica gel (ORBO^{TM}-52; Supelco), which provide good trapping efficiency for polar compounds. The samples were extracted with methylene chloride and analyzed with GC/FID by a system similar to system C. Resolved peaks corresponded to those in the initial bulk purity assays. No evidence of degradation was detected, and no impurities were found that were not present in the bulk material. The stability of tetralin in the generator reservoir was monitored during prestart testing prior to the subchronic studies at days 5, 17, and 24 after the initial filling of the reservoir and prior to and during the 2-year studies at approximately 2 weeks and 2 and 6 months after the initial filling of the reservoir. Samples were analyzed with GC/FID by system G and/or a system similar to system C. When compared to a spectrum of a frozen reference standard of the same lot, results indicated that tetralin was stable in the generator reservoir for at least 6 months. Further stability testing was performed every 6 months during the 2-year studies; no evidence of degradation of the test chemical was found. All measurements of 4-*tert*-butylcatechol concentration in exposure chambers and generator reservoir samples using GC/MS by system H were within the required specifications.

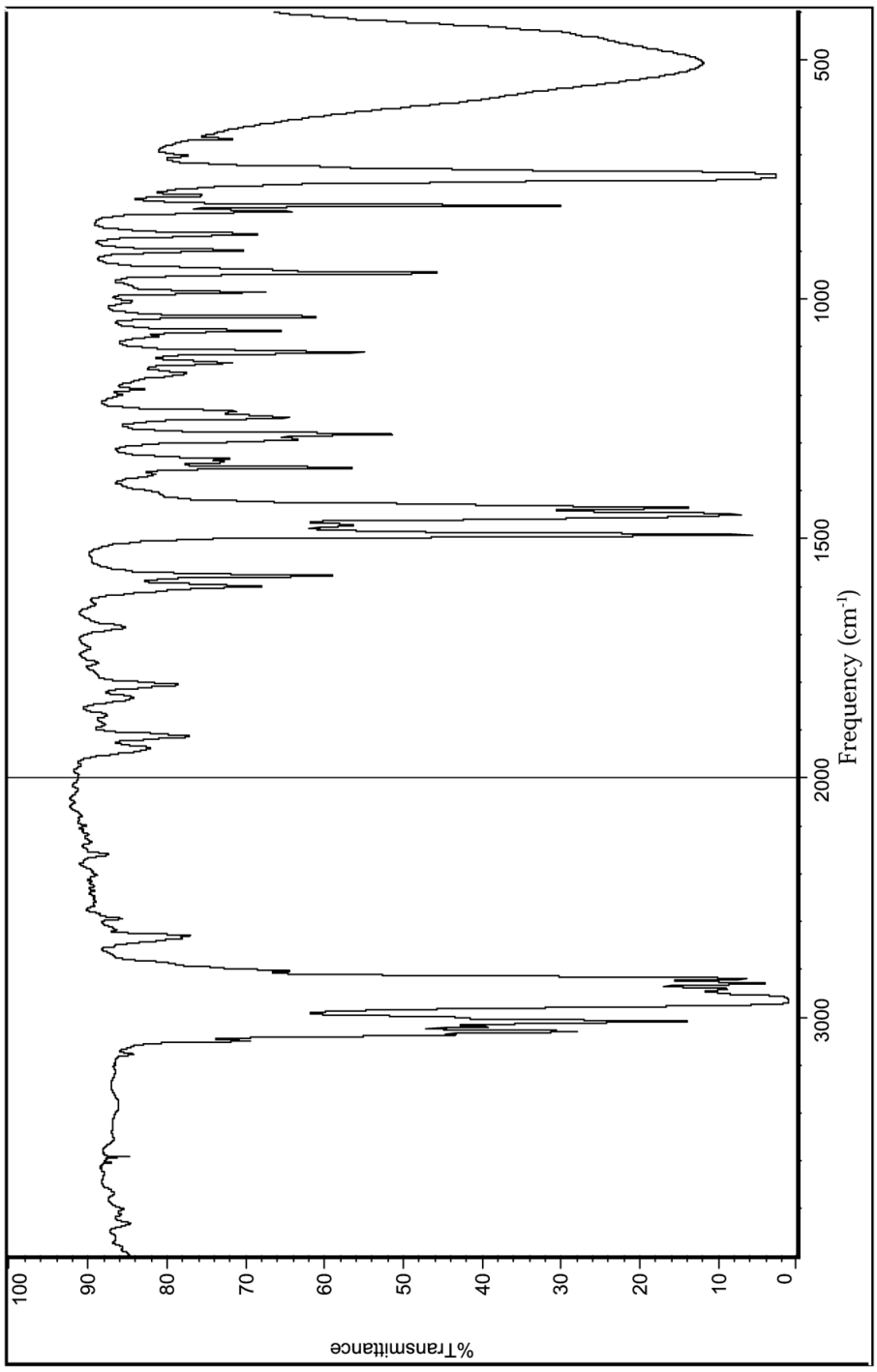

FIGURE J1
Infrared Absorption Spectrum of Tetralin

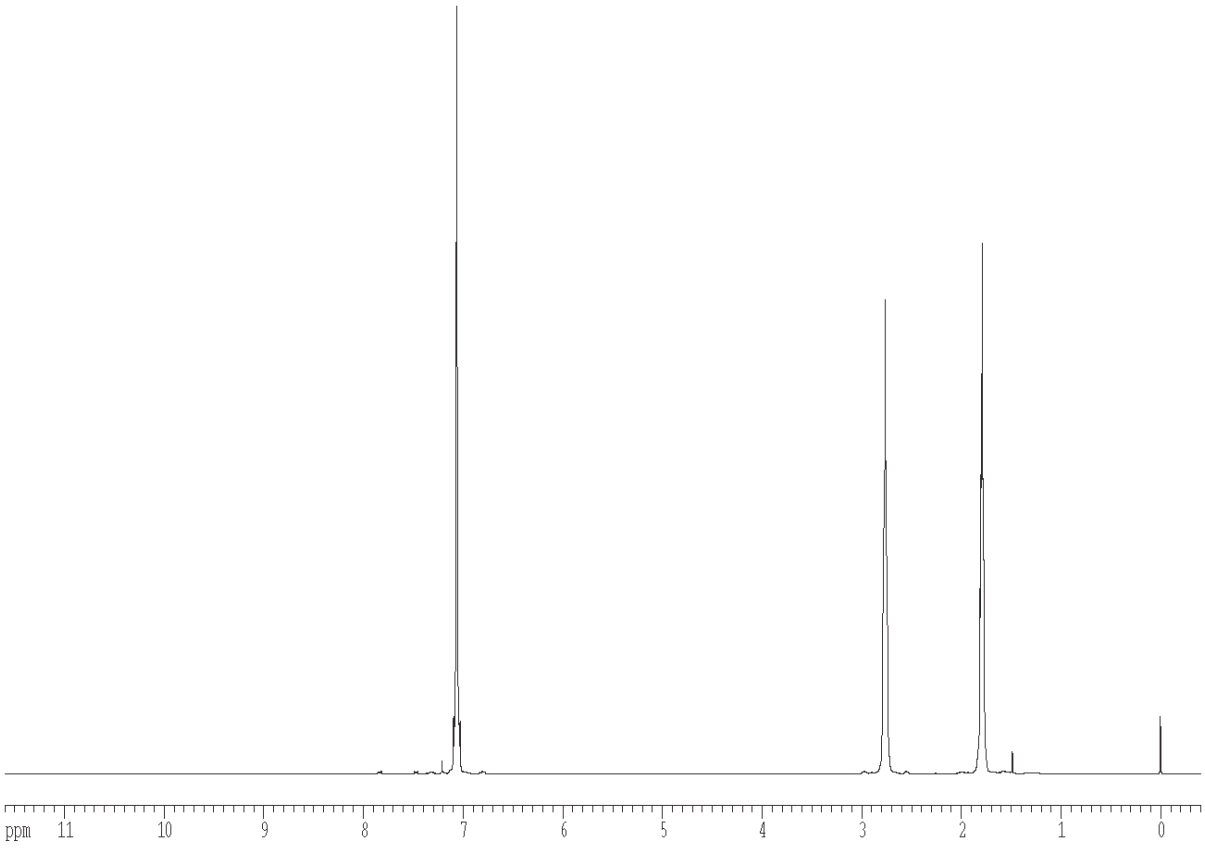

FIGURE J2
Proton Nuclear Magnetic Resonance Spectrum of Tetralin

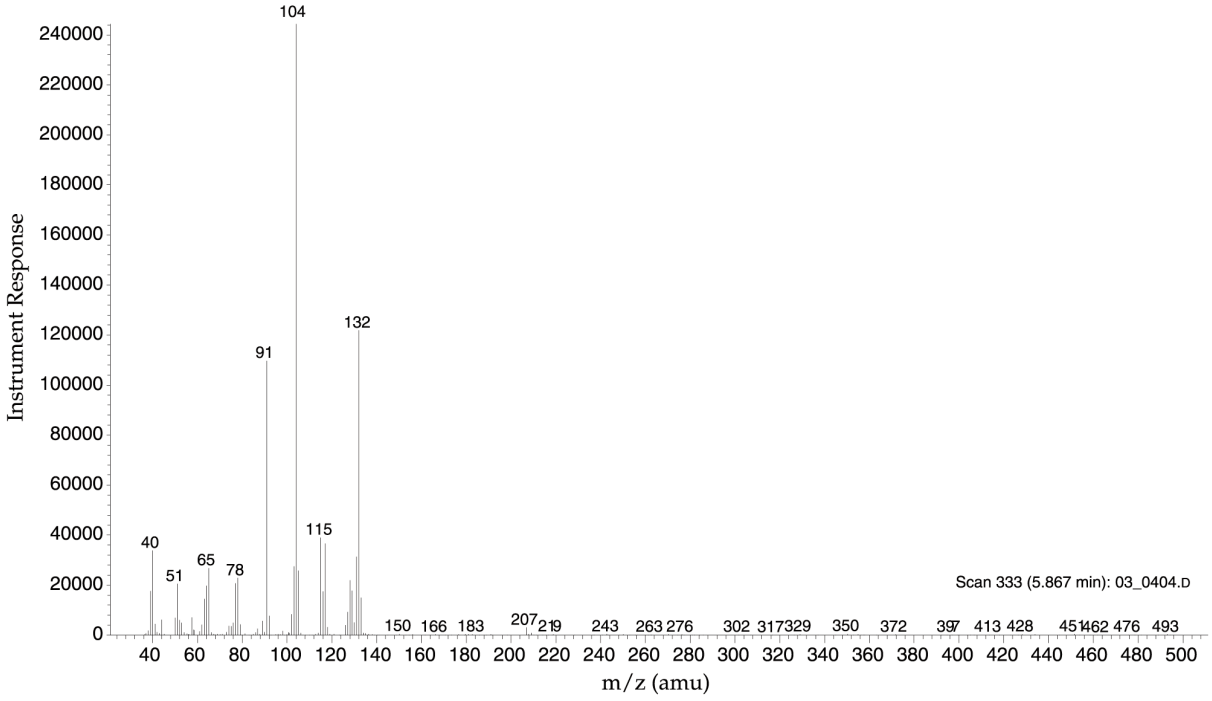

FIGURE J3
Low Resolution Mass Spectrum of Tetralin

TABLE J1
Gas Chromatography Systems Used in the Inhalation Studies of Tetralin[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry	DB-5, 30 m × 0.25 mm, 0.25-µm film (J&W Scientific, Folsom, CA)	Helium at 1.2 mL/minute	50° C for 0.5 minute, then 15° C/minute to 280° C
System B			
Flame ionization	Equity-5, 30 m × 0.32 mm, 0.25-µm film (Supelco, Bellefonte, PA)	Helium at 1.0 mL/minute	50° C for 0.5 minute, then 15° C/minute to 280° C, held for 24.2 minutes
System C			
Flame ionization	DB-5, 30 m × 0.25 mm, 1.0-µm film (J&W Scientific)	Helium at 24 psi head pressure	50° C for 1 minute, then 10° C/minute to 200° C
System D			
Flame ionization	DB-5, 30 m × 0.53 mm, 1.5-µm film (J&W Scientific)	Nitrogen at ~20 mL/minute	Valve oven 280° C Column oven 160° C
System E			
Flame ionization	Rtx-5 Amine, 15 m × 0.53 mm, 1.5-µm film (Restek, Bellefonte, PA)	Nitrogen at ~24 mL/minute (10 psi)	Valve oven 150° C Column oven 125° C
System F			
Flame ionization	DB-5, 30 m × 0.53 mm, 1.5-µm film (J&W Scientific)	Helium at 6 psi head pressure	60° C for 1 minute, then 16° C/minute to 200° C
System G			
Flame ionization	DB-5, 30 m × 0.25 mm, 1.0-µm film (J&W Scientific)	Helium at ~1.4 mL/minute	40° C for 1 minute, then 4° C/minute to 300° C
System H			
Mass spectrometry	Rtx-5, 30 m × 0.25 mm, 1.0-µm film (Restek)	Helium at 9 psi head pressure	120° C for 1 minute, then 15° C/minute to 280° C, held for 2 minutes

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA).

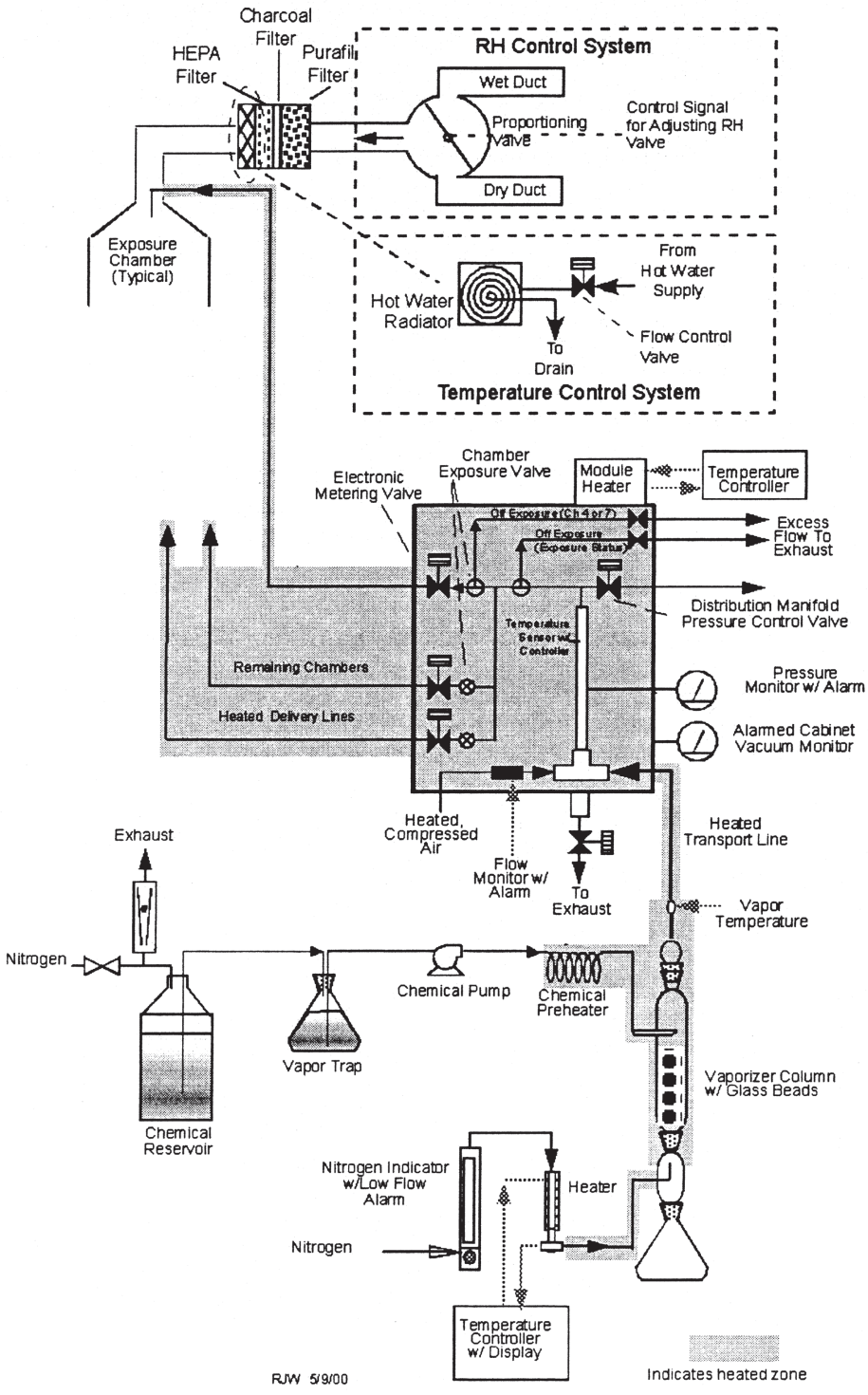

FIGURE J4
Vapor Generation and Delivery System Used in the Inhalation Studies of Tetralin

TABLE J2
Summary of Chamber Concentrations in the 2-Week Inhalation Studies of Tetralin

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
7.5	213	7 ± 1
15	214	14 ± 3
30	208	28 ± 5
60	220	56 ± 10
120	227	115 ± 20
Mouse Chambers		
7.5	229	7 ± 1
15	230	14 ± 3
30	224	29 ± 5
60	236	56 ± 10
120	246	115 ± 19

[a] Mean ± standard deviation

TABLE J3
Summary of Chamber Concentrations in the 3-Month Inhalation Studies of Tetralin

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
7.5	1,138	8 ± 0
15	1,135	15 ± 1
30	1,142	30 ± 2
60	1,144	60 ± 3
120	1,148	119 ± 5
Mouse Chambers		
7.5	1,172	8 ± 0
15	1,169	15 ± 1
30	1,176	30 ± 2
60	1,178	60 ± 3
120	1,182	119 ± 5

[a] Mean ± standard deviation

TABLE J4
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Tetralin

	Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	30	7,388	30 ± 1
	60	7,413	60 ± 2
	120	7,415	120 ± 4
Mouse Chambers			
	30	7,592	30 ± 1
	60	7,328	60 ± 2
	120	7,375	120 ± 4

[a] Mean ± standard deviation

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

TABLE K1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

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

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

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.9 ± 0.53	13.8 – 16.1	25
Crude fat (% by weight)	8.0 ± 0.37	7.4 – 9.0	25
Crude fiber (% by weight)	9.2 ± 0.45	8.2 – 9.9	25
Ash (% by weight)	5.0 ± 0.21	4.4 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.770 ± 0.070	0.670 – 0.970	18
Cystine	0.225 ± 0.023	0.150 – 0.250	18
Glycine	0.706 ± 0.043	0.620 – 0.800	18
Histidine	0.362 ± 0.082	0.310 – 0.680	18
Isoleucine	0.542 ± 0.046	0.430 – 0.660	18
Leucine	1.087 ± 0.066	0.960 – 1.240	18
Lysine	0.712 ± 0.118	0.310 – 0.840	18
Methionine	0.407 ± 0.051	0.260 – 0.490	18
Phenylalanine	0.626 ± 0.043	0.540 – 0.720	18
Threonine	0.500 ± 0.046	0.430 – 0.610	18
Tryptophan	0.142 ± 0.024	0.110 – 0.200	18
Tyrosine	0.388 ± 0.058	0.280 – 0.540	18
Valine	0.667 ± 0.045	0.550 – 0.730	18
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.243	3.49 – 4.54	18
Linolenic	0.30 ± 0.035	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	4,920 ± 1,210	3,360 – 8,900	25
Vitamin D (IU/kg	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	8.5 ± 3.66	5.9 – 25.2	25
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)	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.959 ± 0.046	0.873 – 1.030	25
Phosphorus (%)	0.589 ± 0.028	0.538 – 0.641	25
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.33 ± 0.158	0.14 – 0.50	25
Cadmium (ppm)	0.07 ± 0.021	0.04 – 0.10	25
Lead (ppm)	0.08 ± 0.026	0.05 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.20 ± 0.057	0.14 – 0.45	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	14.5 ± 4.33	10.00 – 24.4	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 0	10 – 10	25
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total Nitrosoamines (ppb)[e]	4.4 ± 2.04	2.3 – 8.5	25
N-Nitrosodimethylamine (ppb)[e]	2.6 ± 1.74	1.1 – 6.9	25
N-Nitrosopyrrolidine (ppb)[e]	1.8 ± 0.79	0.9 – 4.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCB	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.098 ± 0.111	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.189 ± 0.377	0.020 – 1.850	25
Endosulfan 1	<0.01		25
Endosulfan 2	<0.01		25
Endosulfane sulfate	<0.03		25

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

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

For the 2-week studies, serologic analyses were performed by the study laboratory on five male F344/N rats and five female NBR rats approximately 4 weeks after receipt and on five male and five female mice at the end of the study. During the 3-month studies, serologic analyses were performed by the study laboratory on five male and five female sentinel rats and mice during week 1 and by Microbiological Associates, Inc. (Rockville, MD), on five male and five female chamber control rats and mice at terminal sacrifice. During the 2-year studies, serologic analyses were performed by the study laboratory on five male and five female sentinel rats and mice during week 1. In addition, serum samples were collected from five male and five female sentinel rats and five male and three or five female sentinel mice at 6, 12, and 18 months and five male and five female 120 ppm rats and mice at study termination. Blood from each animal was collected and allowed to clot, and the serum was separated. The 6, 12, and 18 month and study termination samples from the 2-year studies were processed appropriately and sent to MA Bioservices/BioReliance (Rockville, MD) for determination of antibody titers. Fecal samples from mice were tested for *Helicobacter* at 18 months. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

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

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

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

RESULTS

All test results were negative.

APPENDIX M
SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

INTRODUCTION

Toxicokinetic studies were conducted in F344/N rats and B6C3F1 mice to estimate toxicokinetic parameters for the elimination of tetralin from blood. Male and female rats and mice received either a single intravenous dose of 2 or 20 mg tetralin/kg body weight or a single 6-hour inhalation exposure of 15, 60, or 120 ppm. Post-dose blood samples were analyzed for tetralin up to 24 hours after dosing and used to estimate the toxicokinetic parameters.

MATERIALS AND METHODS

Chemicals

Tetralin was obtained from Advanced Aromatics (Baytown, TX) in one lot (139699) and from Sigma Aldrich Fluka (St. Louis, MO) in two lots (00822JG and 07808LG). Lots 00822JG and 07808LG were combined by Research Triangle Institute (Research Triangle Park, NC) and assigned lot number 8359-80-01. Lot 139699 was used in the intravenous studies, and combined lot 8359-80-01 was used in the inhalation studies. The test article was stored at room temperature (lot 139699) or at room temperature (combined lot 8359-80-01) under a nitrogen headspace. Tetralin used in the intravenous and inhalation studies was 99.4% and 99% pure, respectively, as analyzed by gas chromatography (GC) with flame ionization detection (FID). On receipt, the structure and purity were confirmed by GC/FID, mass spectrometry (MS), infrared spectroscopy, and nuclear magnetic resonance spectroscopy. [$^2H_{12}$]-tetralin was obtained from Aldrich Chemical Company (Milwaukee, WI) and was used as the internal standard.

Intravenous Administration

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms (Germantown, NY), each with an indwelling catheter surgically implanted in the jugular vein by the supplier. Animals were housed in facilities accredited by the American Association for Accreditation of Laboratory Animal Care and were approximately 13 weeks old at study start. Animals were acclimated for 4 (males) or 5 (females) days before dosing and were housed individually in polycarbonate cages with hardwood chip bedding (Sani Chips®, P.J. Murphy Forest Products Corp., Montville, NJ). NTP-2000 feed (Zeigler Bros., Gardners, PA) and water were available *ad libitum*. Room environmental conditions included a light/dark cycle of 12 hours, room temperature of 73° ± 3° F and a relative humidity of 55% ± 15%. Animals were observed for mortality and moribundity twice daily during the study.

A total of 18 rats per sex per dose and 36 mice per sex per dose were assigned to each study. Dose formulations were prepared in a mixture of ethanol:Cremophor®:water [1:1:8 (v/v/v)]. Animals were administered a single, bolus dose of tetralin at a nominal dose of 2 and 20 mg tetralin/kg body weight through an indwelling jugular cannula using a calibrated, airtight syringe. Dosing volumes were 2 mL/kg body weight (rats) or 4 mL/kg body weight (mice).

At specified times following dosing, rats were anesthetized using approximately 70% CO_2, and blood was collected from the retroorbital sinus (3 rats per sex per dose per time point) in heparin. Blood was collected up to two times from each rat, alternating between the right and left eye. Mice were bled once by closed chest cardiac puncture under approximately 70% CO_2 anesthesia (3 mice per sex per dose per time point). All blood samples were inverted gently and placed in ice until stored at –70° C. Both rats and mice were sacrificed by CO_2 asphyxiation after the final bleeding.

Inhalation Exposure

Male and female F344/N rats and B6C3F1 mice were obtained from Charles River Laboratories (Raleigh, NC) and were acclimated to the facility for approximately 1 week before use. Animals were housed in humidity- and temperature-controlled, HEPA-filtered, mass air-displacement rooms in facilities accredited by the American Association for Accreditation of Laboratory Animal Care. NTP-2000 feed (Zeigler Bros., Gardners, PA) and water were available *ad libitum*. Room environmental conditions included a light/dark cycle of 12 hours, room temperature of 24° ± 2° C, and 55% ± 15% relative humidity. In preparation for dosing, animals were housed individually in compartments of Hazleton 2000 inhalation chamber cage units (Lab Products, Inc., Aberdeen, MD). Each chamber holds a maximum of six cage units. Each rat unit contained 24 individual compartments, and each mouse unit contained 40 individual compartments.

Tetralin was pumped into the top of a heated glass column filled with glass beads. Heated nitrogen entered the column from below, vaporized, and then carried tetralin vapor to a condenser column. The vapor-saturated nitrogen leaving the condenser was transported to the exposure room where it was mixed with heated air before it entered a short distribution manifold. From the distribution manifold, individual delivery lines carried the vapor to each exposure chamber. Flow to each chamber was controlled by compressed-air-driven vacuum pumps located at the chamber end of each delivery line. A three-way valve, mounted in the line from the distribution manifold to each chamber, was used to direct the vapor to the chamber exhaust until animal exposure was scheduled to begin. To begin the exposure, the valve was opened and vapor was injected into the chamber inlet duct where it was further diluted with conditioned chamber air to achieve the desired exposure concentration. Tetralin concentrations in the chambers were controlled by adjustment of the test article injection rate.

Chamber and room concentrations of tetralin were determined using an on-line Hewlett Packard (Palo Alto, CA) model 5890 gas chromatograph with flame ionization detection. The on-line standard was a constant concentration of tetralin produced by a diffusion generator. Chamber atmosphere samples, obtained using ORBO-101 (Supelco, Inc., Bellefonte, PA) charcoal sampling tubes and analyzed against gravimetrically prepared standards on an off-line gas chromatograph, were used to verify calibration of the on-line monitor.

Male and female F344/N rats (13 weeks old) with an average weight of 240 and 161 g, respectively, and male and female B6C3F1 mice (12 weeks old) with an average weight of 28.6 and 19.7 g, respectively, received whole-body inhalation exposures of 15, 60, or 120 ppm tetralin for 6 hours. During exposure, animals had access to water but not to food.

At specified times following dosing, animals were anesthetized using approximately 70% CO_2 and blood was collected in heparin from the retroorbital sinus (rats) or the supraorbital sinus (mice). Each animal was bled twice, once from each eye. Five female mice died after the first bleeding, so animals scheduled for later time points were substituted. As a result, the last sample collection point for the 15 ppm exposure group was eliminated, and one mouse was bled a third time to fill a 120 minute time point. Both rats and mice were sacrificed by CO_2 asphyxiation after the final bleeding. After collection, blood samples were stored at –70° C.

Analysis of Tetralin in Blood

Tetralin concentration in blood was determined by a validated GC/MS method using [$^2H_{12}$]-tetralin as the internal standard. Briefly, 0.1 µL of blood was diluted with 100 µL of 43 mM sodium bicarbonate buffer (~pH 11). After adding 115 nanomoles of [$^2H_{12}$]-tetralin, the sample was mixed and extracted with 0.5 mL cyclohexane. The cyclohexane layer was analyzed by GC/MS in electron ionization mode using a Hewlett-Packard 5971A mass selective detector coupled to a 5890 Series 11 gas chromatograph. The analyte separation was carried out on a fused-silica capillary column (DB-5MS; 30 m × 0.25 mm; film thickness, 0.25 µm; J&W Scientific, Folsom, CA). Two microliter volumes were injected in splitless mode using helium as the carrier gas at a head pressure of 10 psi. Injector and detector temperatures were 275° C and 300° C, respectively. The GC oven was maintained at 50° C for 0.5 minute, then ramped at 20° C/minute to 165° C, followed by 50° C/minute to 300° C. Ions m/z 104 and

m/z 144 were monitored for tetralin and [$^2H_{12}$]-tetralin, respectively. The limit of detection and experimental limit of quantitation were 0.0001 and 0.0006 µg tetralin/g of blood, respectively. Quality control samples were prepared at target concentrations of approximately 0.012 and 8.0 µg tetralin/g of blood and were analyzed with approximately every 10 study samples.

Toxicokinetic parameters for tetralin were estimated by fitting a bi-exponential elimination model to the tetralin blood concentrations using a nonlinear least-squares fitting program (Proc NLIN, SAS 8.2, SAS Institute Inc., Cary, NC).

RESULTS

Rats

Intravenous Administration

Blood concentration versus time following an intravenous dose of 2 or 20 mg tetralin/kg body weight and the model-fitted curves are provided in Figure M1. The profiles showed a characteristic bi-exponential elimination at both doses with an initial rapid elimination followed by a second slower elimination phase. In general, there was no significant difference in half-lives of initial ($t_{1/2\alpha}$) and terminal ($t_{1/2\beta}$) elimination phases between the sexes or dose groups. Toxicokinetic parameters estimated are given in Table M1.

Area under the blood concentration time profile increased more-than-proportional to the dose for both sexes, which was reflected by 1.6- to 1.8-fold increase in the dose normalized-area under the curve (AUC_∞/Dose) at 20 mg/kg compared to 2 mg/kg for males and females, respectively. There was no apparent difference in AUC_∞ between males and females after intravenous administration. The apparent volume of distribution (V_d) was greater than the total body water (~670 mL/kg; Davies and Morris, 1993), suggesting that tetralin binds to plasma proteins and that tissue intake occurs.

Inhalation Exposure

The blood tetralin concentrations versus time following a single 6-hour whole-body inhalation exposure to 15, 60, or 120 ppm and the model-fitted curves are provided in Figure M2. The profile showed a characteristic bi-exponential elimination for all doses with a rapid initial elimination phase representing elimination of tetralin from blood and rapidly perfused tissues, such as liver, lung, and kidney, followed by a slower elimination phase representing elimination from slowly perfused tissues, such as muscle and fat. Both $t_{1/2\alpha}$ and $t_{1/2\beta}$ in males and $t_{1/2\beta}$ in females increased as a function of exposure. However, the increase was not statistically significant in either males or females. No apparent change in $t_{1/2\alpha}$ was observed in females. There were no sex differences in $t_{1/2\alpha}$ or $t_{1/2\beta}$ over the exposure concentrations tested. Toxicokinetic parameters estimated are given in Table M2.

Estimates of the initial blood concentration of tetralin (C_0) revealed more-than-proportional increases as a function of exposure concentration in both sexes (Table M2). No statistically significant differences were observed between the sexes at exposure concentrations of 60 or 120 ppm. However, male rats had significantly higher initial blood concentrations (0.330 ± 0.019) than females (0.278 ± 0.025) in the 15 ppm exposure group.

Area under the curve for the blood concentration time profile (AUC_∞) increased more-than-proportional to the dose for both sexes as evidenced by the increase in AUC_∞/Exposure with increasing exposure concentration. The increase was statistically significant in both sexes (Table M2). This suggests that metabolic and/or elimination pathways are saturated after inhalation exposure at higher exposure concentrations in rats in both sexes. The AUC_∞/Exposure for male rats was significantly higher than for females at each exposure concentration, suggesting that female rats are better able to eliminate tetralin from systemic circulation than male rats after inhalation exposure.

Mice

Intravenous Administration

The tetralin blood concentration versus time following an intravenous dose of 2 or 20 mg/kg body weight and the model-fitted curves for mice are provided in Figure M3. The profiles showed a characteristic bi-exponential elimination at both doses with an initial rapid elimination followed by a second slower elimination phase. The $t_{1/2\alpha}$ was 5 to 6 minutes for both sexes at both doses, reflecting a rapid disposition of tetralin from mouse blood. There was neither dose- nor sex-dependent differences in $t_{1/2\alpha}$. Although not significant, there was a notable decrease in $t_{1/2\beta}$ with increasing dose in both sexes. This decrease in $t_{1/2\beta}$ was more noticeable in males (1.7-fold) compared to females (1.3-fold). Toxicokinetic parameters estimated are reported in Table M3.

Area under the curve for the blood concentration time profile increased more-than-proportional to the dose for both sexes, which was reflected by a 1.8- to 2.3-fold increase in AUC_c/Dose or AUC_∞/Dose at 20 mg/kg body weight for female and male mice, respectively, compared to those of the 2 mg/kg dose. There were no significant differences in AUC_∞ between the sexes at either dose. V_d was greater than the total body water (~670 mL/kg; Davies and Morris, 1993), suggesting that tetralin binds to plasma proteins and that tissue intake occurs.

Inhalation Exposure

Blood concentrations versus time following a single 6-hour whole-body inhalation exposure to 15, 60, or 120 ppm and the model-fitted curves are provided in Figure M4. This profile showed a characteristic bi-exponential elimination for all doses with a rapid initial clearance phase representing elimination of tetralin from blood and rapidly perfused tissues, such as liver, lung, and kidney, followed by a slower clearance phase representing clearance from slowly perfused tissues, such as muscle and fat. In male mice, the $t_{1/2\alpha}$ increased as a function of exposure. The $t_{1/2\alpha}$ for females increased as a function of exposure up to 60 ppm; however, at 120 ppm, the $t_{1/2\alpha}$ significantly decreased. As a consequence, a significantly shorter $t_{1/2\alpha}$ was observed in females compared to males at 120 ppm. The $t_{1/2\beta}$ increased with increasing exposure concentration in male mice but decreased with increasing exposure concentration in female mice. However, the differences were not statistically significant for males or females at any exposure concentration. Toxicokinetic parameters estimated are reported in Table M4.

Estimates of C_0 revealed proportional increases as a function of exposure concentration in male mice (Table M4). Although, the increase in female mice was more-than-proportional to dose, the differences were not statistically significant. Higher C_0 was observed in male mice compared to female mice at 15 and 60 ppm, but the differences were not statistically significant.

Area under the curve for the blood concentration time profile increased more-than-proportional to the dose for both sexes as evidenced by the increase in AUC_∞/Exposure with increasing exposure concentration, and the increase was statistically significant in both sexes (Table M4). This suggests that metabolic and/or elimination pathways are saturated at higher exposure concentrations in mice in both sexes. No statistically significant differences were apparent between sexes indicating male and female mice eliminated tetralin from systemic circulation with similar efficiencies at all exposure concentrations.

DISCUSSION

The present studies were conducted to evaluate the toxicokinetic parameters of tetralin in mice and rats after intravenous administration and after whole body inhalation exposure.

In general, the bi-exponential model provided a good fit to the blood concentration time profile at all doses after both routes of exposure with an initial rapid elimination phase followed by a second slower elimination phase. Tetralin was eliminated from blood following dose-dependent nonlinear kinetics in both sexes and species after intravenous injection and whole body inhalation exposure.

After intravenous administration to rats, there was no significant difference in $t_{1/2\alpha}$ or $t_{1/2\beta}$ between doses or sexes. After intravenous administration to mice, neither dose- nor sex-dependent differences in $t_{1/2\alpha}$ were observed. However, there was a notable dose-related decrease in $t_{1/2\beta}$ with increasing dose. The decrease in $t_{1/2\beta}$ was more noticeable (1.7-fold) in male mice compared to female mice (1.3-fold).

In rats, unlike after intravenous injection, differences between males and females in elimination kinetics were observed following inhalation exposure; females consistently had shorter $t_{1/2\alpha}$ and $t_{1/2\beta}$ values than males at all exposure concentrations. A decrease in $t_{1/2\beta}$ similar to that observed after intravenous administration was noted in female mice receiving a single 6-hour inhalation exposure with increasing exposure concentration. The $t_{1/2\alpha}$ was also shorter at 120 ppm compared to the two lower exposure concentrations. In male mice, in contrast to what was observed after intravenous administration, both $t_{1/2\alpha}$ and $t_{1/2\beta}$ increased as the exposure concentration increased.

Initial tetralin blood concentrations (C_0) were similar among rats (1.33 to 22.8 µg/g) and mice (0.955 to 20.3 µg/g) after intravenous administration of 2 or 20 mg tetralin/kg body weight. In mice receiving a single 6-hour inhalation exposure of 15, 60, or 120 ppm tetralin, the estimated C_0 values (0.242 to 15.3 µg/g) were also comparable to the single intravenous administration in rats and mice. However, in rats receiving a similar whole body inhalation exposure, estimated C_0 ranged from 0.278 to 4.58 µg/g. C_0 values in rats from a single intravenous dose of 2 mg/kg (1.49 µg/g for males and 1.33 µg/g for females) was comparable to that in rats following a single inhalation exposure to 60 ppm for 6 hours (1.68 µg/g for males and 1.65 µg/g for females).

There were more-than-proportional increases in AUC_∞ with increasing dose in both species and sexes following both exposure scenarios. As noted above, in rats at a 2 mg/kg intravenous dose, C_0 values were comparable to those following a 6 hour exposure to 60 ppm. Comparing these two groups, AUCs were generally much higher (~6 fold) for both sexes following inhalation exposure versus bolus intravenous dosing. Since terminal elimination rates were significantly slower for intravenous dosing compared to inhalation exposure for both sexes, this difference in AUCs between the routes of exposure is most likely due to the fact that tetralin has more time to equilibrate with slowly perfused tissues (e.g., fat) after the inhalation exposure. Hence, with inhalation exposure, the elimination of greater amounts from these tissues causes an overall increased time for elimination from blood and significantly increased AUCs.

When comparing the elimination kinetics of tetralin in mice to rats receiving equivalent doses by two exposure routes (inhalation and intravenous), mice eliminated tetralin more rapidly than rats. Elimination half-lives were shorter, and AUC_∞ values were lower in mice compared to rats at similar doses and exposure concentrations.

Overall, the results of both the single administration inhalation and intravenous studies indicate that dose-, sex-, and route-of-exposure-related effects apparently influence tetralin elimination in rats and mice.

REFERENCE

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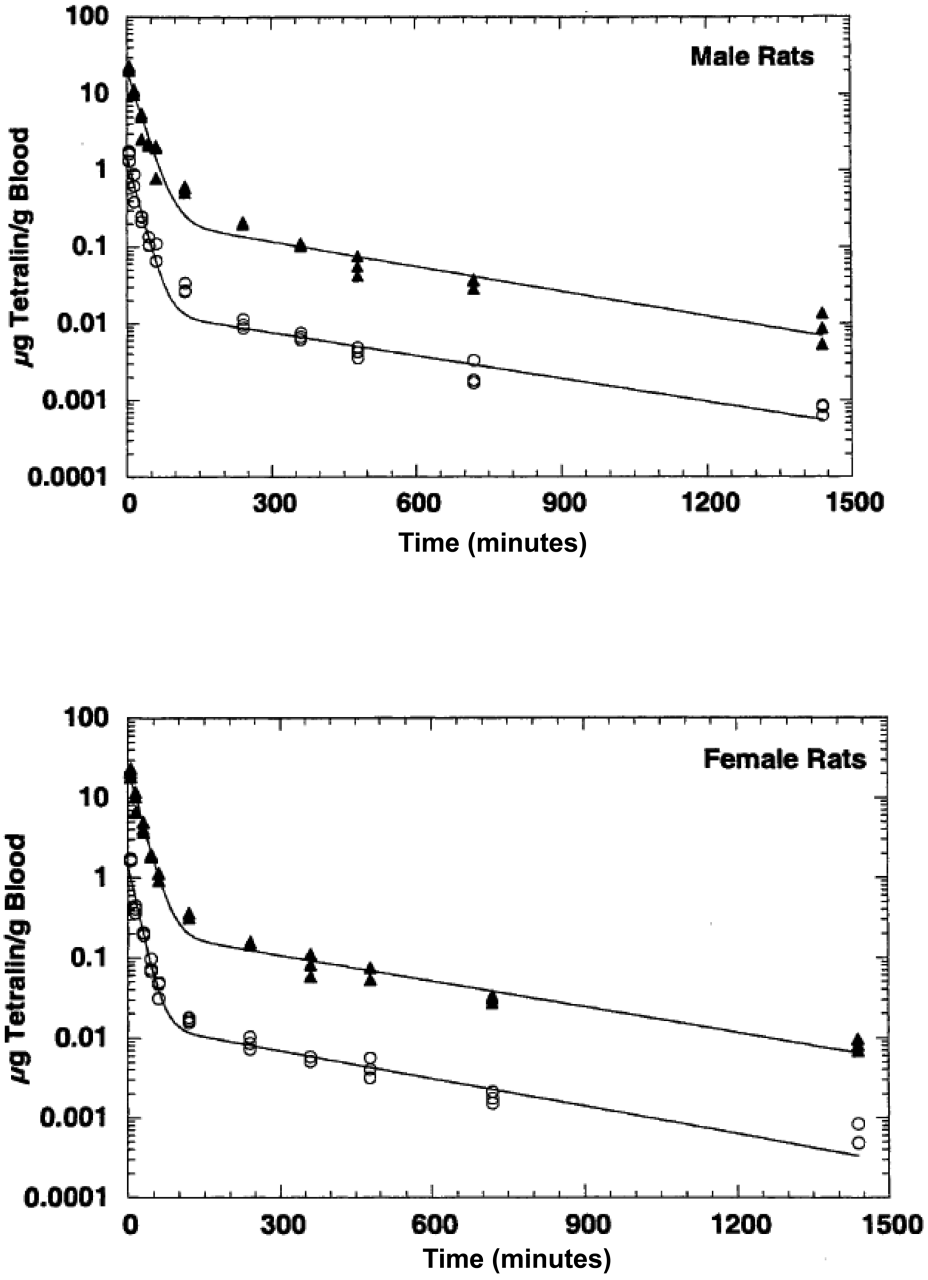

FIGURE M1
Blood Elimination Profiles for Rats Following a Single Intravenous Dose of 2 (open circles) or 20 (solid triangles) mg Tetralin/kg Body Weight

TABLE M1
Toxicokinetic Parameter Estimates in Rats after a Single Intravenous Dose of Tetralin[a]

	2 mg/kg	20 mg/kg
Male		
A_0 (μg/g)	1.48 ± 0.29	19.7 ± 4.0
B_0 (μg/g)	0.0152 ± 0.0025	0.248 ± 0.047
α (minute^{-1})	0.0563 ± 0.0058	0.0464 ± 0.0056
β (minute^{-1})	0.00230 ± 0.00025	0.00249 ± 0.00027
AUC_α (μg·minute/g)	26.3 ± 3.1	425 ± 49
AUC_β (μg·minute/g)	6.58 ± 0.59	99.8 ± 10.7
AUC_c (μg·minute/g)	32.8 ± 3.1	525 ± 50
AUT_∞ (μg·minute/g)	39.2 ± 1.8	632 ± 20
C_0 (μg/g)	1.49 ± 0.29	20.0 ± 4.0
$t_{1/2\alpha}$ (minute)	12.3 ± 1.3	14.9 ± 1.8
$t_{1/2\beta}$ (minute)	301 ± 32	279 ± 31
Cl_{ss} (mL/kg/minute)	57.5 ± 5.5	35.9 ± 3.4
V_d (mL/kg)	1,020 ± 97	775 ± 73
AUC_c/Dose [(μg·minute/g)/(mg/kg)]	16.4 ± 1.6	26.3 ± 2.5
AUT_∞/Dose [(μg·minute/g)/(mg/kg)]	19.6 ± 0.90	31.6 ± 1.0
C_0/Dose [(μg/g)/(mg/kg)]	0.745 ± 0.15	1.00 ± 0.20
Female		
A_0 (μg/g)	1.32 ± 0.23	22.6 ± 3.0
B_0 (μg/g)	0.0153 ± 0.0024	0.225 ± 0.027
α (minute^{-1})	0.0655 ± 0.0055	0.0550 ± 0.0038
β (minute^{-1})	0.00266 ± 0.00028	0.00247 ± 0.00018
AUC_α (μg·minute/g)	20.1 ± 2.3	410 ± 34
AUC_β (μg·minute/g)	5.75 ± 0.46	91.0 ± 6.4
AUC_c (μg·minute/g)	25.9 ± 2.3	501 ± 35
AUT_∞ (μg·minute/g)	32.3 ± 0.7	560 ± 19
C_0 (μg/g)	1.33 ± 0.23	22.8 ± 3.0
$t_{1/2\alpha}$ (minute)	10.6 ± 0.9	12.6 ± 0.9
$t_{1/2\beta}$ (minute)	260 ± 27	281 ± 20
Cl_{ss} (mL/kg/minute)	72.8 ± 6.5	37.6 ± 2.6
V_d (mL/kg)	1,112 ± 99	684 ± 48
AUC_c/Dose [(μg·minute/g)/(mg/kg)]	13.0 ± 1.2	25.1 ± 1.8
AUT_∞/Dose [(μg·minute/g)/(mg/kg)]	16.2 ± 0.35	28.0 ± 0.95
C_0/Dose [(μg/g)/(mg/kg)]	0.665 ± 0.12	1.14 ± 0.15

[a] All values are reported as the mean ± standard error.
A_0 and B_0 = intercepts on the y-axis of the extrapolated initial and terminal elimination phases, respectively;
α and β = hybrid elimination rate constants for the initial and terminal elimination phases, respectively;
AUC_α and AUC_β = area under the initial and terminal phase blood concentration curves as functions of A and α and B and β, respectively;
AUC_c = area under the blood concentration versus time curve to the last time point (T);
AUT_∞ = trapezoidal area under the blood concentration versus time curve using C_0 at 0 and extrapolation to infinity using β (C_T/C);
C_0 = initial blood concentration (= A_0 + B_0);
$t_{1/2\alpha}$ and $t_{1/2\beta}$ = elimination half-lives for the initial and terminal elimination phases, respectively ($t_{1/2\alpha}$ = ln2/α; $t_{1/2\beta}$ = ln2/β);
Cl_{ss} = total system clearance = [CL_s (kg/kg$_\beta$/min) × 1000 g/kg]/1.06 g/ml where CL_s = Dose/AUC_c and 1.06 g/ml is specific gravity of blood in rats and mice;
V_d = apparent volume of distribution (= Cl_{ss}/α).

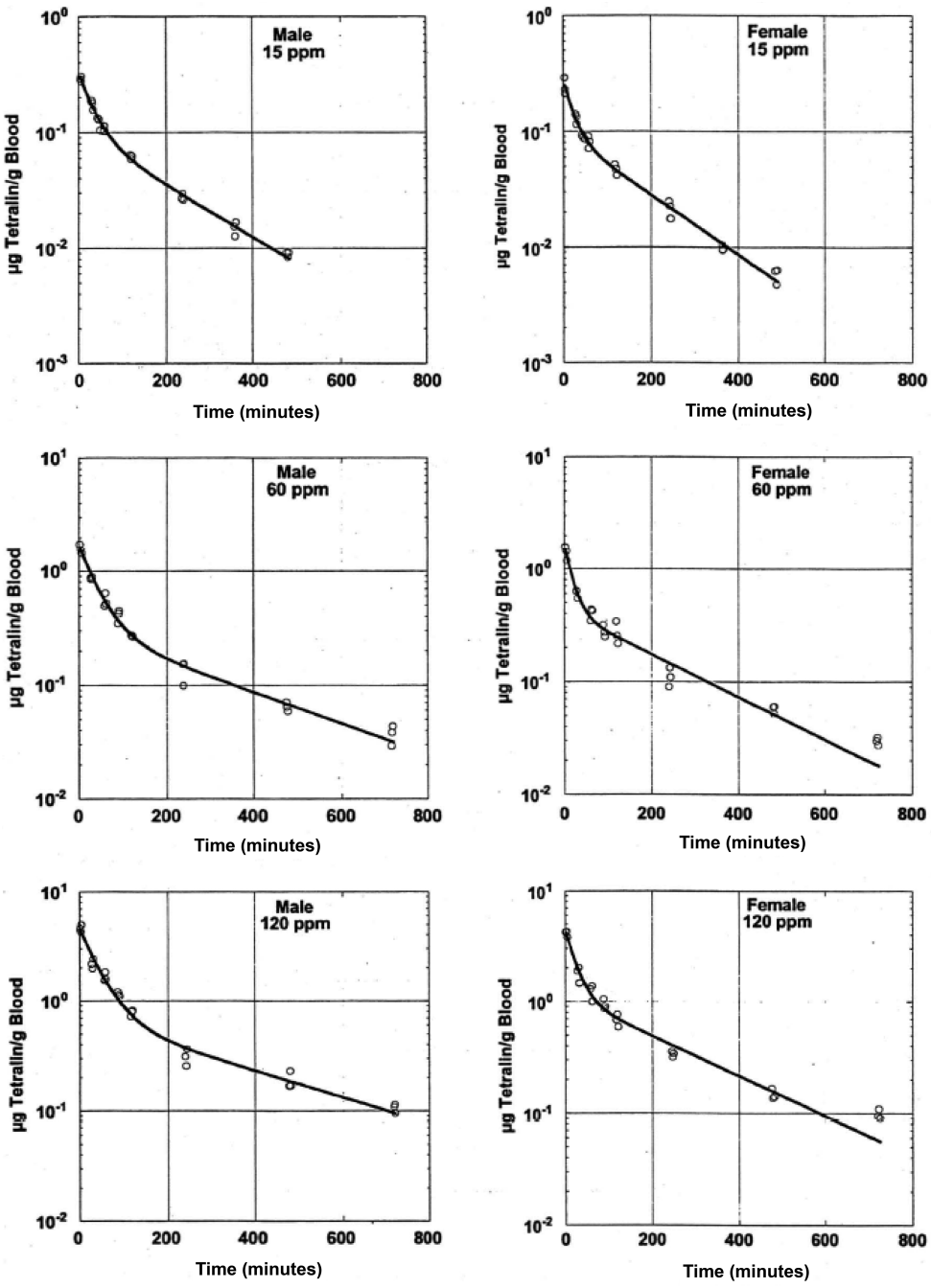

FIGURE M2
Blood Elimination Profiles for Rats Following a Single 6-Hour Inhalation Exposure to Tetralin

TABLE M2
Toxicokinetic Parameter Estimates in Rats after a Single 6-Hour Inhalation Exposure to Tetralin[a]

	15 ppm	60 ppm	120 ppm
Male			
C_0 (μg/g)	0.330 ± 0.019	1.68 ± 0.13	4.58 ± 0.47
α (minute^{-1})	0.0314 ± 0.0063	0.0257 ± 0.0054	0.0238 ± 0.0061
$t_{1/2\alpha}$ (minute)	22.1 ± 4.4	27.0 ± 5.7	29.1 ± 7.5
β (minute^{-1})	0.00518 ± 0.00092	0.00317 ± 0.0010	0.00279 ± 0.0014
$t_{1/2\beta}$ (minute)	134 ± 24	219 ± 69	249 ± 130
Postexposure AUC_∞ (μg·minute/g)	27.7 ± 0.69	156 ± 7.9	431 ± 26
AUC_∞/Exposure [(μg·minute/g)/(ppm)]	1.85 ± 0.046	2.60 ± 0.13	3.59 ± 0.21
Female			
C_0 (μg/g)	0.278 ± 0.025	1.65 ± 0.19	4.43 ± 0.40
α (minute^{-1})	0.0445 ± 0.015	0.0534 ± 0.016	0.0418 ± 0.011
$t_{1/2\alpha}$ (minute)	15.6 ± 5.1	13.0 ± 3.9	16.6 ± 4.4
β (minute^{-1})	0.00592 ± 0.0012	0.00434 ± 0.00099	0.00410 ± 0.00095
$t_{1/2\beta}$ (minute)	117 ± 23	160 ± 37	169 ± 39
Postexposure AUC_∞ (μg·minute/g)	20.7 ± 1.0	127 ± 7.3	369 ± 15
AUC_∞/Exposure [(μg·minute/g)/ppm)]	1.38 ± 0.069	2.12 ± 0.12	3.08 ± 0.12

[a] All values are reported as the estimate ± 0.5 of the 95% confidence interval.
A_0 and B_0 = intercepts on the y-axis of the extrapolated initial and terminal elimination phases, respectively;
C_0 = initial tetralin blood concentration (= A_0 + B_0);
α and β = hybrid elimination rate constants for the initial and terminal elimination phases, respectively;
$t_{1/2\alpha}$ and $t_{1/2\beta}$ = elimination half-lives for the initial and terminal elimination phases, respectively ($t_{1/2\alpha}$ = ln2/α; $t_{1/2\beta}$ = ln2/β);
AUC_α = area under the blood concentration versus time curve using C_0 at 0 and extrapolation to infinity using β (C_T/β).

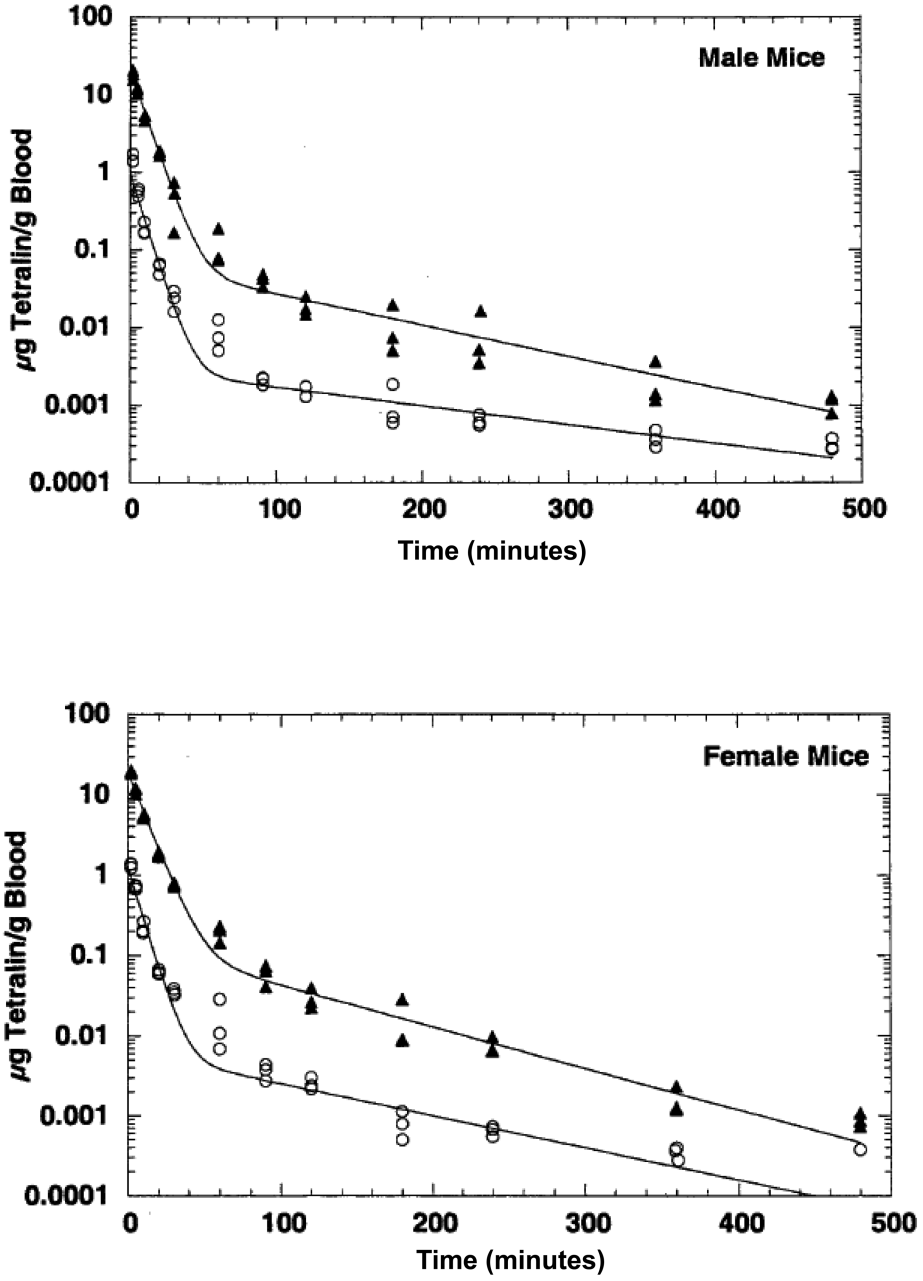

FIGURE M3
Blood Elimination Profiles for Mice Following a Single Intravenous Dose of 2 (open circles) or 20 (solid triangles) mg Tetralin/kg Body Weight

TABLE M3
Toxicokinetic Parameter Estimates in Mice after a Single Intravenous Dose of Tetralin[a]

	2 mg/kg	20 mg/kg
Male		
A_0 (μg/g)	0.952 ± 0.221	20.3 ± 4.3
B_0 (μg/g)	0.00297 ± 0.00064	0.0678 ± 0.0161
α (minute^{-1})	0.136 ± 0.014	0.124 ± 0.012
β (minute^{-1})	0.00553 ± 0.00084	0.00921 ± 0.00090
AUC_α (μg·minute/g)	7.02 ± 1.05	164 ± 24
AUC_β (μg·minute/g)	0.538 ± 0.056	7.36 ± 1.15
AUC_c (μg·minute/g)	7.55 ± 1.06	171 ± 24
AUT_∞ (μg·minute/g)	10.4 ± 0.4	187 ± 7
C_0 (μg/g)	0.955 ± 0.221	20.3 ± 4.3
$t_{1/2\alpha}$ (minute)	5.11 ± 0.54	5.59 ± 0.55
$t_{1/2\beta}$ (minute)	125 ± 19	75.2 ± 7.4
Cl_{ss} (mL/kg/minute)	250 ± 35	110 ± 15
V_d (mL/kg)	1,838 ± 257	890 ± 122
AUC_c/Dose [(μg·minute/g)/(mg/kg)]	3.78 ± 0.53	8.55 ± 1.2
AUT_∞/Dose [(μg·minute/g)/(mg/kg)]	5.20 ± 0.20	9.35 ± 0.35
C_0/Dose [(μg/g)/(mg/kg)]	0.478 ± 0.11	1.02 ± 0.22
Female		
A_0 (μg/g)	1.18 ± 0.31	18.3 ± 3.1
B_0 (μg/g)	0.00640 ± 0.00218	0.140 ± 0.027
α (minute^{-1})	0.143 ± 0.019	0.110 ± 0.010
β (minute^{-1})	0.00924 ± 0.00168	0.0119 ± 0.0008
AUC_α (μg·minute/g)	8.22 ± 1.37	166 ± 18
AUC_β (μg·minute/g)	0.693 ± 0.127	11.8 ± 1.6
AUC_c (μg·minute/g)	8.92 ± 1.38	177 ± 18
AUT_∞ (μg·minute/g)	11.3 ± 0.4	198 ± 4
C_0 (μg/g)	1.18 ± 0.31	18.4 ± 3.1
$t_{1/2\alpha}$ (minute)	4.84 ± 0.63	6.28 ± 0.57
$t_{1/2\beta}$ (minute)	75.0 ± 13.6	58.4 ± 3.7
Cl_{ss} (mL/kg/minute)	211 ± 33	107 ± 11
V_d (mL/kg)	1,478 ± 231	969 ± 103
AUC_c/Dose [(μg·minute/g)/(mg/kg)]	4.46 ± 0.69	8.85 ± 0.90
AUT_∞/Dose [(μg·minute/g)/(mg/kg)]	5.65 ± 0.20	9.90 ± 0.02
C_0/Dose [(μg/g)/(mg/kg)]	0.590 ± 0.16	0.920 ± 0.16

[a] All values are reported as the mean ± standard error.
A_0 and B_0 = intercepts on the y-axis of the extrapolated initial and terminal elimination phases, respectively;
α and β = hybrid elimination rate constants for the initial and terminal elimination phases, respectively;
AUC_α and AUC_β = area under the initial and terminal phase blood concentration curves as functions of A and α and B and β, respectively;
AUC_c = area under the blood concentration versus time curve to the last time point (T);
AUT_∞ = trapezoidal area under the blood concentration versus time curve using C_0 at 0 and extrapolation to infinity using β (C_T/C);
C_0 = initial blood concentration (= A_0 + B_0);
$t_{1/2\alpha}$ and $t_{1/2\beta}$ = elimination half-lives for the initial and terminal elimination phases, respectively ($t_{1/2\alpha}$ = ln2/α; $t_{1/2\beta}$ = ln2/β);
Cl_{ss} = total system clearance = [CL_s (kg/kg$_\beta$/min) × 1000 g/kg]/1.06 g/ml where CL_s = Dose/AUC_c and 1.06 g/ml is specific gravity of blood in rats and mice;
V_d = apparent volume of distribution (= Cl_{ss}/α).

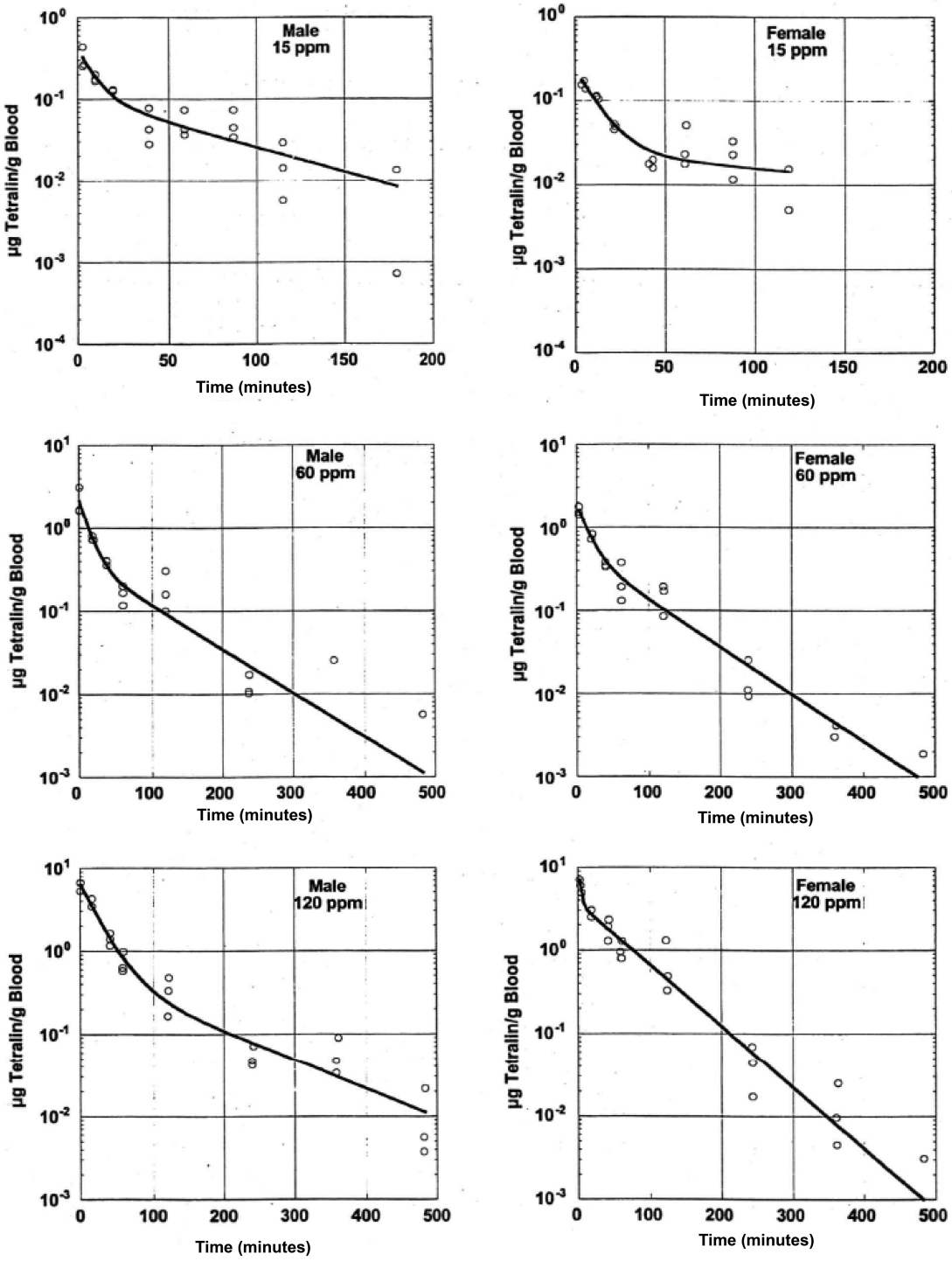

FIGURE M4
Blood Elimination Profiles for Mice Following a Single 6-Hour Inhalation Exposure to Tetralin

TABLE M4
Toxicokinetic Parameter Estimates in Mice after a Single 6-Hour Inhalation Exposure to Tetralin[a]

	15 ppm	60 ppm	120 ppm
Male			
C_0 (μg/g)	0.423 ± 0.16	2.26 ± 0.52	6.56 ± 0.70
α (minute^{-1})	0.117 ± 0.098	0.0730 ± 0.052	0.0421 ± 0.011
$t_{1/2\alpha}$ (minute)	5.92 ± 4.9	9.49 ± 6.7	16.5 ± 4.4
β (minute^{-1})	0.0140 ± 0.0094	0.0121 ± 0.011	0.00801 ± 0.0062
$t_{1/2\beta}$ (minute)	49.5 ± 33	57.2 ± 50	86.6 ± 67
Postexposure AUC_∞ (μg·minute/g)	10.7 ± 1.6	72.6 ± 14	234 ± 20
AUC_∞/Exposure [(μg·minute/g)/(ppm)]	0.713 ± 0.10	1.21 ± 0.23	1.95 ± 0.17
Female			
C_0 (μg/g)	0.242 ± 0.085	1.93 ± 0.38	15.3 ± 22
α (minute^{-1})	0.0906 ± 0.055	0.0639 ± 0.041	0.393 ± 0.53
$t_{1/2\alpha}$ (minute)	7.65 ± 4.6	10.8 ± 7.0	1.76 ± 2.4
β (minute^{-1})	0.00437 ± 0.016	0.0131 ± 0.0061	0.0170 ± 0.0046
$t_{1/2\beta}$ (minute)	159 ± 560	53.0 ± 25	40.8 ± 11
Postexposure AUC_∞ (μg·minute/g)	7.46 ± 9.1	67.9 ± 7.5	293 ± 66
AUC_∞/Exposure [(μg·minute/g)/(ppm)]	0.497 ± 0.60	1.13 ± 0.12	2.44 ± 0.55

[a] All values are reported as the estimate ± 0.5 of the 95% confidence interval.
A_0 and B_0 = intercepts on the y-axis of the extrapolated initial and terminal elimination phases, respectively;
C_0 = initial tetralin blood concentration (= A_0 + B_0);
α and β = hybrid elimination rate constants for the initial and terminal elimination phases, respectively;
$t_{1/2\alpha}$ and $t_{1/2\beta}$ = elimination half-lives for the initial and terminal elimination phases, respectively ($t_{1/2\alpha}$ = ln2/α; $t_{1/2\beta}$ = ln2/β);
AUC_α = area under the blood concentration versus time curve using C_0 at 0 and extrapolation to infinity using β (C_T/β).

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