NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF α-METHYLSTYRENE

(CAS NO. 98-83-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

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

November 2007

NTP TR 543

NIH Publication No. 08-4474

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

FOREWORD

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

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

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.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF α-METHYLSTYRENE

(CAS NO. 98-83-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

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

November 2007

NTP TR 543

NIH Publication No. 08-4474

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

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

M.E. Wyde, Ph.D., Study Scientist
R.C. Sills, D.V.M., Ph.D., Study Pathologist
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
L.T. Burka, Ph.D.
R.S. Chhabra, Ph.D.
M.L. Cunningham, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
R.R. Maronpot, D.V.M.
S.D. Peddada, Ph.D.
J.H. Roycroft, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S.

Battelle Toxicology Northwest

Conducted studies and evaluated pathology findings

J.A. Dill, Ph.D., Principal Investigator
S.L. Grumbein, D.V.M., Ph.D.
B.K. Hayden
R.A. Renne, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology review

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

Dynamac Corporation

Prepared quality assessment audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides and prepared pathology report on rats (June 9, 2005)

W.G. Lieuallen, D.V.M., Ph.D., Chairperson
Pathology Associates, A Division of Charles River Laboratories, Inc.
K.J. Cimon, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
National Toxicology Program
L. Kooistra, D.V.M., Ph.D., Observer
Pathology Associates, A Division of Charles River Laboratories, Inc.
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
G. Pearse, B.V.M. & S.
National Toxicology Program
A.K. Remick, D.V.M., Observer
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

Evaluated slides and prepared pathology report on mice (March 8, 2005)

W.G. Lieuallen, D.V.M., Ph.D., Chairperson
Pathology Associates, A Division of Charles River Laboratories, Inc.
M.F. Cesta, D.V.M.
National Toxicology Program
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
National Toxicology Program
D. Lewis, Observer
North Carolina State University
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
D.L. Morgan, Ph.D.
National Toxicology Program
G. Pearse, B.V.M. & S.
National Toxicology Program
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

Constella Group, Inc.
Provided statistical analyses

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

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
D.C. Serbus, Ph.D.
G.E. Simmons, M.A.

CONTENTS

SUMMARY

Background

α-Methylstyrene is used to make heat-resistant acrylonitrile-butadiene-styrene resins and polymers. We studied the effects of α-methylstyrene on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We exposed groups of 50 male and female rats to atmospheres containing 100, 300, or 1,000 parts per million (ppm) of α-methylstyrene 6 hours per day, 5 days a week for 2 years. Groups of 50 male and female mice were similarly exposed to atmospheres containing 100, 300, or 600 ppm α-methylstyrene. Control animals were housed in exposure chambers for similar periods but with no test chemical in their air. At the end of the study, tissues from more than 40 sites were examined for every animal.

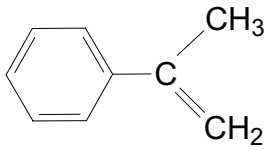
Results

Survival was similar for animals receiving α-methylstyrene and the controls, but the average body weight for each animal group receiving the highest concentration of α-methylstyrene was less than that for the control group. Male rats exposed to α-methylstyrene had increased rates of tumors of the kidney and a slightly increased rate of mononuclear cell leukemia. Female mice exposed to α-methylstyrene had increased rates of a variety of liver cancers, and male mice also had slightly increased rates of liver tumors.

Conclusions

We conclude that exposure to α-methylstyrene in the air caused kidney tumors, and possibly mononuclear cell leukemia, in male rats. We conclude that α-methylstyrene caused liver cancer in female mice, and also possibly in male mice.

ABSTRACT



α-METHYLSTYRENE

CAS No. 98-83-9

Chemical Formula: C_9H_{10} Molecular Weight: 118.2

Synonyms: Isopropenylbenzene, 2-phenylpropylene, 1-methylethenyl benzene

α-Methylstyrene is used in the production of acrylonitrile-butadiene-styrene resins and copolymers, which improve the impact and heat-resistant properties of polymers, specialty grades of plastics, rubber, and protective coatings. α-Methylstyrene also moderates polymerization rates and improves product clarity in coatings and resins. Low molecular weight liquid polymers are used as plasticizers in paints, waxes, adhesives, and plastics. α-Methylstyrene was nominated by the U.S. Environmental Protection Agency for toxicologic evaluation and genotoxicity studies based on its high production volume and limited information available on its toxicity. Male and female F344/N rats and B6C3F$_1$ mice were exposed to α-methylstyrene (99.5% pure) by inhalation for 3 months or 2 years. Inhalation studies were conducted because the primary route of human exposure is via inhalation. Genetic toxicology studies were conducted in *Salmonella typhimurium,* cultured Chinese hamster ovary cells, and mouse peripheral blood erythrocytes.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed by whole-body inhalation to α-methylstyrene at concentrations of 0, 75, 150, 300, 600, or 1,000 ppm for 6 hours per day, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. All rats survived to the end of the study, and mean body weights of all exposed groups were similar to those of the chamber controls. Kidney weights were significantly increased in 1,000 ppm males and 600 and 1,000 ppm females. Statistically significant increases in liver weights occurred in 150 ppm or greater males and 600 and 1,000 ppm females. The incidences of renal hyaline droplet accumulation were similar between exposed groups and chamber control groups, but the severity of hyaline droplet accumulation in 600 and 1,000 ppm males was greater than in chamber controls. Consistent with the hyaline droplet accumulation, an exposure-related increase in α2u-globulin was detected in the kidneys of males exposed to α-methylstyrene. Morphologic changes were not detected in the liver.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed by whole-body inhalation to α-methylstyrene at concentrations of 0, 75, 150, 300, 600, or 1,000 ppm for 6 hours

per day, 5 days per week for 14 weeks. Two female mice in the 1,000 ppm group died before exposure on day 3. Final mean body weights of 600 and 1,000 ppm males and 75, 300, and 1,000 ppm females were significantly less than those of the chamber controls; final mean body weight gains of mice exposed to 300 ppm or greater were also significantly less. Moderate to severe sedation (males only) and ataxia were observed in 1,000 ppm mice. The absolute liver weights of 600 and 1,000 ppm females and the relative liver weights of 300, 600, and 1,000 ppm males and females were significantly increased. The estrous cycle lengths of 600 and 1,000 ppm female mice were significantly longer than that of the chamber controls. Minimal to mild centrilobular hypertrophy was present in the livers of male and female mice exposed to 600 or 1,000 ppm α-methylstyrene. The incidences of exposure-related nasal lesions, including atrophy and hyperplasia of Bowman's glands and atrophy and metaplasia of the olfactory epithelium, were significantly increased in all exposed groups of males and females. The incidences of hyaline degeneration, characterized by the accumulation of eosinophilic globules in the cytoplasm of the respiratory epithelium, were significantly increased in females exposed to 150 ppm or greater.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed by whole body inhalation to α-methylstyrene at concentrations of 0, 100, 300, or 1,000 ppm for 6 hours per day, 5 days per week, except holidays, for 105 weeks. Survival rates of exposed male and female rats were similar to those of the chamber controls. The mean body weights of 1,000 ppm males and females were less than those of the chamber control groups during year 2 of the study.

Two 1,000 ppm males and one 300 ppm male had renal tubule carcinomas, and one 300 ppm male had a renal tubule adenoma. Because of the neoplasms observed in 300 and 1,000 ppm males at the end of the 2-year study and the finding of α2u-globulin accumulation in the kidneys at 3 months, which is often associated with kidney neoplasms, additional step sections of kidney were prepared; additional males with focal hyperplasia or adenoma were identified. The incidences of renal tubule adenoma and carcinoma (combined) in the 1,000 ppm males were significantly greater than those in the chamber controls when the single and step sections were combined. The incidence of mineralization of the renal papilla was significantly increased in 1,000 ppm males. The incidence of mononuclear cell leukemia in 1,000 ppm males was significantly increased compared to the chamber controls. In the nose, the incidences of basal cell hyperplasia were significantly increased in all exposed groups of males and females, and the incidences of degeneration of the olfactory epithelium were increased in 1,000 ppm males and females and 300 ppm females.

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed by whole body inhalation to α-methylstyrene at concentrations of 0, 100, 300, or 600 ppm for 6 hours per day, 5 days per week, except holidays, for 105 weeks. Survival of all exposed male and female mice was similar to that of the chamber control groups. Mean body weights of 600 ppm males were less than those of the chamber control group throughout the study, and those of 600 ppm females were less after week 13. The mean body weights of 300 ppm males and females were less than those of the chamber controls during much of the study, but these groups recovered by the end of the study.

The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in the 100 and 600 ppm males and in all exposed groups of females. The incidences of hepatocellular adenoma were significantly increased in all exposed groups of females, and the incidences in all exposed groups of males and females exceeded the historical range for chamber controls. The incidences of hepatocellular carcinoma and eosinophilic foci of the liver were significantly increased in 600 ppm females. The incidences of olfactory epithelial metaplasia and hyperplasia of the glands overlying the olfactory epithelium were significantly increased in all exposed groups of males and females. In addition, atrophy of the olfactory epithelium was significantly increased in 300 and 600 ppm males. The incidence and severity of nephropathy were increased in 600 ppm females compared to chamber controls. Epithelial hyperplasia of the forestomach also was present in male mice.

Genetic Toxicology

α-Methylstyrene was not mutagenic in four strains of *Salmonella typhimurium*, with or without rat or hamster liver metabolic activation enzymes (S9). α-Methylstyrene did not induce chromosomal

aberrations in cultured Chinese hamster ovary cells, with or without S9 activation, but did significantly increase the frequency of sister chromatid exchanges in cultures exposed in the presence of S9. *In vivo*, no significant increases in the frequencies of micronucleated erythrocytes were seen in blood samples of male mice obtained at the conclusion of the 3-month study. However, in female mice from the 3-month study, a significant increase in micronucleated erythrocytes was observed in the 1,000 ppm group.

CONCLUSIONS

Under the conditions of this 2-year inhalation study, there was *some evidence of carcinogenic activity** of α-methylstyrene in male F344/N rats based on increased incidences of renal tubule adenomas and carcinomas (combined). The increased incidence of mononuclear cell leukemia in 1,000 ppm male F344/N rats may have been related to α-methylstyrene exposure. There was *no evidence of carcinogenic activity* of α-methylstyrene in female F344/N rats exposed to 100, 300, or 1,000 ppm. There was *equivocal evidence of carcinogenic activity* of α-methylstyrene in male $B6C3F_1$ mice based on marginally increased incidences of hepatocellular adenoma or carcinoma (combined). There was *clear evidence of carcinogenic activity* of α-methylstyrene in female $B6C3F_1$ mice based on increased incidences of hepatocellular adenomas and carcinomas.

Exposure of rats to α-methylstyrene resulted in kidney toxicity, which in males exhibited some features of α2u-globulin nephropathy. Exposure to α-methylstyrene resulted in nonneoplastic lesions of the nose in male and female rats and mice and of the liver and kidney in female mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of α-Methylstyrene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in air	0, 100, 300, or 1,000 ppm	0, 100, 300, or 1,000 ppm	0, 100, 300, or 600 ppm	0, 100, 300, or 600 ppm
Body weights	1,000 ppm group less than the chamber control group	1,000 ppm group less than the chamber control group	600 ppm group less than the chamber control group	600 ppm group less than the chamber control group
Survival rates	27/50, 32/50, 23/50, 22/50	27/50, 24/50, 36/50, 26/50	35/50, 32/50, 40/50, 36/50	39/50, 38/50, 37/50, 37/50
Nonneoplastic effects	Kidney: papilla, mineralization (12/50, 16/50, 10/50, 33/50); renal tubule, hyperplasia (standard evaluation - 0/50, 0/50, 0/50, 0/50) (standard and extended evaluations combined - 1/50, 0/50, 1/50, 4/50) Nose: olfactory epithelium, hyperplasia, basal cell (0/50, 17/50, 18/50, 43/50); olfactory epithelium, degeneration (1/50, 3/50, 3/50, 16/50)	Nose: olfactory epithelium, hyperplasia, basal cell (0/49, 14/49, 30/50, 49/50); olfactory epithelium, degeneration (1/49, 1/49, 7/50, 24/50)	Nose: olfactory epithelium, metaplasia (6/50, 47/50, 49/50, 49/50); olfactory epithelium, glands, hyperplasia (4/50, 50/50, 50/50, 50/50); olfactory epithelium, atrophy (0/50, 2/50, 8/50, 12/50)	Liver: Eosinophilic focus (2/50, 5/50, 7/50, 12/50) Nose: olfactory epithelium, metaplasia (2/49, 49/49, 47/50, 50/50); olfactory epithelium, glands, hyperplasia (3/49, 49/49, 50/50, 50/50) Kidney: nephropathy (16/50, 21/49, 12/50, 26/50); severity of nephropathy (1.1, 1.3, 1.0, 1.6)
Neoplastic effects	Kidney: renal tubule adenoma (standard evaluation - 0/50, 0/50, 1/50, 0/50) (standard and extended evaluations combined - 1/50, 2/50, 2/50, 5/50); renal tubule adenoma or carcinoma (standard evaluation - 0/50, 0/50, 2/50, 2/50) (standard and extended evaluations combined - 1/50, 2/50, 3/50, 7/50)	None	None	Liver: hepatocellular adenoma (10/50, 20/50, 21/50, 23/50); hepatocellular carcinoma (3/50, 9/50, 6/50, 18/50); hepatocellular adenoma or carcinoma (13/50, 26/50, 24/50, 33/50)
Equivocal findings	Mononuclear cell leukemia (26/50, 32/50, 29/50, 38/50)	None	Liver: hepatocellular adenoma or carcinoma (28/50, 36/50, 33/50, 37/50)	None

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of α-Methylstyrene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Level of evidence of carcinogenic activity	Some evidence	No evidence	Equivocal evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535 with and without S9			
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro:*	Positive with S9, negative without S9			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro:*	Negative with and without S9			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*	Negative in male mice, positive in female mice			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on α-methylstyrene on June 12, 2006, 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.

Charlene A. McQueen, Ph.D., Chairperson
College of Pharmacy
University of Arizona
Tucson, AZ

Diane F. Birt, Ph.D.
Department of Food Science and Human Nutrition
Iowa State University
Ames, IA

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

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

George P. Daston, Ph.D.
Miami Valley Laboratories
The Procter and Gamble Company
Cincinnati, OH

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

John P. Giesy, Jr., Ph.D.*
Department of Zoology
Michigan State University
East Lansing, MI

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

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

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

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

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

Special Ad Hoc Reviewers

Paul Cooke, Ph.D.
Department of Veterinary Biosciences
University of Illinois
Urbana, IL

Leena Hilakivi-Clarke, Ph.D.
Lombardi Comprehensive Cancer Center
Georgetown University
Washington, DC

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 12, 2006, the draft Technical Report on the carcinogenesis studies of α-methylstyrene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.E. Wyde, NIEHS, introduced the toxicology and carcinogenesis studies of α-methylstyrene by describing the uses of the chemical, the study design for the short- and long-term studies, the effects of the chemical on the kidney and liver in the short-term studies, and the effects on the kidney, liver, and nose in the long-term studies. The proposed conclusions were: *some evidence of carcinogenic activity* of α-methylstyrene in male F344/N rats, *no evidence of carcinogenic activity* of α-methylstyrene in female F344/N rats, *equivocal evidence of carcinogenic activity* of α-methylstyrene in male B6C3F$_1$ mice, and *clear evidence of carcinogenic activity* of α-methylstyrene in female B6C3F$_1$ mice.

Dr. Birt, the first principal reviewer, thought the study was performed and reported carefully, and she agreed with the proposed conclusions. She suggested that descriptions of ataxia in the 3-month studies, the chamber buildup and decay times, and the body weight effects could be described more fully.

Dr. Kerkvliet, the second principal reviewer, said she would like to have seen measurements of hepatic cytochrome P450 activities given the known action of styrene. She suggested mentioning more prominently that the inhalation exposure was whole-body rather than nose-only and asked if the forestomach lesions would have any other effects on the animals' health. She also thought that it would be useful to discuss the differences in action between styrene and α-methylstyrene and asked for more details on the procedures for vaginal cytology.

Dr. Deininger, the third principal reviewer, thought the report was well written and noted the difficulty in drawing conclusions for mononuclear cell leukemia with high background incidences and variability.

Dr. Wyde replied that details concerning the body weights, chamber conditions, and vaginal cytology would be amplified and noted that ataxia was not observed in the 2-year studies. He also agreed to expand the comparison of styrene and α-methylstyrene, including their metabolic pathways.

Dr. R.C. Sills, NIEHS, said the forestomach lesions were focal and mild in severity and likely did not affect the animals' eating or digestion.

Dr. Daston asked if the conclusion statements regarding the kidney tumors in rats might carry some qualifying statement about a possible relationship with α2u-globulin accumulation.

Dr. A. Chappelle, representing Sunoco Corporation, said that the results of the 3-month and 2-year studies supported an α2u-globulin nephropathy mediated mechanism for the induction of kidney tumors in male rats. She also thought the increase in liver tumors in female mice was due to a lower than normal incidence in the control group. She also asserted that a maximum tolerated dose was exceeded in some animal groups.

Dr. Walker noted the blood erythrocyte micronucleus data for mice indicated the chemical had a clastogenic effect, and thus one could not say its mechanism of action in rats was solely due to α2u-globulin. Regarding liver tumors in female mice, he also noted that the first comparison in a study is with concurrent controls, and the increase in this study was marked, and also the control rate was not the lowest seen among the historical database.

In consideration of the conclusions, Dr. Daston suggested that a statement about association with α2u-globulin accumulation be added to the first paragraph after mention of the kidney tumors in male rats.

Dr. R.C. Sills, NIEHS, presented an overview of the spectrum of kidney lesions observed, some of which might be associated with α2u-globulin, while other cytotoxic effects may be due directly to the chemical.

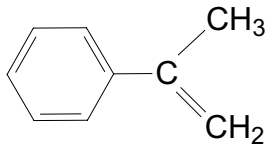
Dr. J.R. Bucher, NIEHS, added that there was not enough evidence to support a statement that all the male kidney tumors were due to this response and not to a direct effect of the chemical.

Dr. Daston maintained that some suggestion of the possibility of a contribution by α2u-globulin in the conclusion would be useful. Dr. Sills replied that while some aspects of the classic α2u-globulin syndrome were seen, some others, such as cell proliferation, single cell necrosis, and granular casts were not. He also noted a recent study of *p*-nitrobenzoic acid in which α2u-globulin accumulation and cell proliferation were seen but kidney tumors were not.

Dr. Daston moved, and Dr. Kerkvliet seconded, to add a statement in the first paragraph of the conclusions that the male rat kidney tumors were seen in association with α2u-globulin accumulation. Drs. Bucher and Wyde noted that the α2u-globulin is typically observed in the 3-month studies but not late in the 2-year studies. The motion was defeated with one yes vote (Dr. Daston), eight no votes, and one abstention (Dr. Deininger).

Dr. Birt then moved, and Dr. Bradfield seconded, that the conclusions be accepted as originally written. Dr. Walker proposed changing the word "accumulation" to "nephropathy" in the second paragraph, and Dr. Birt so modified her motion. The motion was passed unanimously with 10 votes.

INTRODUCTION



α-METHYLSTYRENE

CAS No. 98-83-9

Chemical Formula: C_9H_{10} Molecular Weight: 118.2

Synonyms: Isopropenylbenzene, 2-phenylpropylene, 1-methylethenyl benzene

CHEMICAL AND PHYSICAL PROPERTIES

α-Methylstyrene is a volatile aromatic hydrocarbon that is structurally similar to styrene. It is a colorless liquid with a boiling point of 165° C, a specific gravity of 0.906 at 25° C, and a vapor pressure of 2.3 mm Hg at 20° C (Verschueren, 1977; Lewis, 1997). α-Methylstyrene is combustible and has a flash point of 53.9° C (Lewis, 1997). It is soluble in organic solvents and has a log octanol:water partition coefficient of 3.48 at 25° C (Hansch *et al.*, 1995).

PRODUCTION, USE, AND HUMAN EXPOSURE

α-Methylstyrene is primarily formed as a by-product in the manufacture of phenol from cumene via oxidation and cleavage in an acidic medium (Lewis *et al.*, 1983). Alkylation of benzene with propylene followed by dehydrogenation of isopropylbenzene to α-methylstyrene was practiced commercially by Dow until 1977 (*Kirk-Othmer*, 1997). The primary use of α-methylstyrene is in the production of acrylonitrile-butadiene-styrene resins and copolymers, which improve the impact and heat-resistant properties of polymers, specialty grades of plastics, rubber, and protective coatings (Lewis *et al.*, 1983). α-Methylstyrene also moderates polymerization rates and improves product clarity in coatings and resins (Lewis *et al.*, 1983). Low molecular weight liquid polymers are used as plasticizers in paints, waxes, adhesives, and plastics (Santodonato *et al.*, 1980).

α-Methylstyrene is a high production volume chemical with a reported domestic production volume of between 34 and 192 million pounds in 1977 (USEPA, 1985) and 10 to 48 million pounds between 1980 and 1983 according to the U.S. International Trade Commission (1981a, 1982, 1983a, 1984). The estimated annual domestic production capacity was 113 million pounds in 1985 (SRI, 1985), and the reported import volumes were 22,046 and 2.2 million pounds in 1980 and 1982, respectively (USITC, 1981b, 1983b).

Human exposure occurs primarily in occupational settings via inhalation or dermal exposure. Occupational exposure limits for airborne concentrations of α-methylstyrene have been established by the American Conference of Governmental Industrial Hygienists (2005) based on irritation, dermatitis, and central nervous system effects. The 8-hour time-weighted average threshold limit value is 50 ppm (240 g/m^3), and the

15-minute short-term exposure limit is 100 ppm (485 mg/m^3). The National Institute of Occupational Safety and Health (NIOSH) exposure limit is 50 ppm, and the Occupational Safety and Health Administration (OSHA) permissible exposure limit is 100 ppm (NIOSH, 2003). Both NIOSH and OSHA have established 5,000 ppm (24 g/m^3) as the concentration that is immediately dangerous to life and health (Sittig, 1991). There is no specific information available on consumer exposure. However, α-methylstyrene is reportedly an outgassing product of polystyrene insulation materials (Halacy, 1983).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Limited information is available on the absorption, distribution, metabolism, and excretion of α-methylstyrene. De Costa *et al.* (2001) investigated the tissue distribution, metabolism, and excretion of α-methylstyrene in male F344/N rats and characterized the profile of metabolites produced in human liver slices. These studies demonstrated that α-methylstyrene is readily metabolized and is excreted primarily in the urine with only 0.3% of the administered dose present in the tissues. Although atrolactic acid has been proposed as a major metabolite in humans, dogs, rats, and guinea pigs (Bardodej and Bardodejova, 1970), the predominant metabolite following intravenous administration was 2-phenyl-1,2-propanediol glucuronide, which accounted for 50% of the urinary metabolites identified (De Costa *et al.*, 2001). 2-Phenyl-1,2-propanediol was the primary metabolite produced by human liver slices. S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine was present at 13% in the urine and was consistent with depletion of hepatic glutathione observed following inhalation exposure in B6C3F$_1$ mice (De Costa *et al.*, 2001). The metabolites formed by the human liver slices were the same as those excreted in rat urine (De Costa *et al.*, 2001). Intravenous administration of radiolabeled α-methylstyrene to male F344/N rats resulted in the excretion of 76% in the first 24 hours and 86% after 72 hours (De Costa *et al.*, 2001). Exhalation of volatile organics and carbon dioxide accounted for 2% and 0.02% of the dose, respectively.

TOXICITY

Experimental Animals

Early studies of α-methylstyrene were conducted by Wolf *et al.* (1956). These studies reported an LD$_{50}$ of 4.9 g/kg in the White rat and a lowest lethal concentration of 3,000 ppb in the rat and the guinea pig following 7-hour exposures for 3 to 4 days. Application to the conjunctival sac of white rabbits induced slight conjunctival irritation without corneal injury. Repeat application to the ear and abdominal skin for up to 4 weeks induced moderate-to-marked irritation and slight necrosis. These studies were performed with an undiluted liquid of an unspecified purity. In chronic experiments lasting up to 212 days in rats, guinea pigs, rabbits, and rhesus monkeys, the only effects observed were increased liver weights in guinea pigs and increased liver and kidney weights in rats exposed to 600 ppm α-methylstyrene.

Morgan *et al.* (1999) conducted 12-day studies designed to compare the toxicity of α-methylstyrene to that of styrene in B6C3F$_1$ mice and F344 rats. B6C3F$_1$ mice did not exhibit chemically induced signs of toxicity when exposed to up to 500 ppm of α-methylstyrene, exposure concentrations that previously induced toxicity in styrene-treated mice. At exposure concentrations of 600, 800, and 1,000 ppm, mortality was observed in female mice after the first exposure. No mortality was observed in males throughout the study, and no further mortality was observed in females after the initial deaths following the first exposure. Decreased hepatic glutathione and increased relative liver weights were observed in both sexes, but no histopathologic lesions were observed in mice. F344 rats exposed to 600 or 1,000 ppm α-methylstyrene for 12 days had increased relative liver weights in both sexes, and hyaline droplet accumulation was observed in male kidneys. Hyaline droplet accumulation was confirmed in male F344 rats at concentrations of 250 ppm and greater but was not observed in female F344 or NBR rats, both of which are α2u-globulin deficient.

Gagnaire and Langlais (2005) demonstrated that α-methylstyrene is weakly ototoxic in Sprague-Dawley rats. Exposure to α-methylstyrene induced a loss of outer hair cells in the area of the cochlea responsive to medium frequencies.

Humans

No specific toxicity studies of α-methylstyrene in humans were found in the literature. Wolf *et al.* (1956) reported limited exposures of human subjects. In those studies, the detectable odor limit was 50 ppm, and eye and nasal irritation were reported at concentrations of 600 ppm and higher.

CARCINOGENICITY

No information regarding the carcinogenic effects of α-methylstyrene in experimental animals or epidemiology studies in humans was found in the literature.

α-Methylstyrene is structurally related to styrene but differs from styrene in the presence of a methyl group on the α carbon. Styrene is oxidized to styrene-7,8-oxide (Leibman, 1975), which has been shown to be a direct rodent carcinogen (Ponomarkov *et al.*, 1984; Lijinsky, 1986; Conti *et al.*, 1988) and is reasonably anticipated to be a human carcinogen (NTP, 2002). Styrene induces lung tumors in mice but not rats (Cruzan *et al.*, 1998, 2001). Given the structural similarity to styrene, α-methylstyrene was initially expected to elicit similar toxic effects.

GENETIC TOXICITY

The mutagenic activity of α-methylstyrene has not been extensively studied. Published results of the limited number of assays conducted indicate that the compound is not mutagenic in *Salmonella typhimurium*, with or without exogenous metabolic activation (Zeiger *et al.*, 1992). It is a weak inducer of sister chromatid exchanges in cultured human lymphocytes in the absence of exogenous metabolic activation (Norppa and Vainio, 1983).

The mutagenicity of styrene, which is structurally similar to α-methylstyrene, and styrene oxide, an active metabolite of styrene, has been studied extensively. Although positive results have been reported for styrene in a variety of standard *in vivo* and *in vitro* genotoxicity tests (Norppa and Sorsa, 1993; Speit and Henderson, 2005), there are some conflicting data sets, particularly in human exposure studies where polymorphisms in key metabolism enzymes, such as CYP450 2E1, may alter the profiles of active metabolites among members of a population (Henderson and Speit, 2005). The mutagenicity of styrene is dependent upon its conversion to the active metabolite styrene oxide by CYP2E1 (Wenker *et al.*, 2001; Norppa and Sorsa, 1993). For example, styrene was nonmutagenic in *S. typhimurium*, with or without activation (Zeiger *et al.*, 1988), but styrene oxide induced mutations in strain TA100, with and without S9 (Zeiger *et al.*, 1992). Styrene induces sister chromatid exchanges *in vivo* and *in vitro* (Kligerman *et al.*, 1993). These results are consistent with those from other DNA damage studies with styrene and styrene oxide in mice (Vaghef and Hellman, 1998). Neither styrene nor styrene oxide has been shown to induce chromosomal damage (Kligerman *et al.*, 1993; Preston and Abernethy, 1993; Scott and Preston, 1994; Speit and Henderson, 2005).

STUDY RATIONALE

α-Methylstyrene was nominated by the U.S. Environmental Protection Agency for toxicologic evaluation, subchronic, and genotoxicity studies based on its high production volume and the limited availability of information on its toxicity. Inhalation studies were conducted because inhalation is the primary route of human exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF α-METHYLSTYRENE

α-Methylstyrene, stabilized with 4-*tert*-butyl catechol to inhibit oxidation and polymerization during storage, was obtained in one lot from Acros Organics (Fair Lawn, NJ) by the analytical chemistry laboratory, Research Triangle Institute (RTI) (Research Triangle Park, NC), and shipped to the study laboratory, Battelle Toxicology Northwest (Richland, WA), in two shipments that were reassigned lot numbers BNW 13871-4 and BNW 13871-54. Lot BNW 13871-4 was used in the 3-month and 2-year studies; lot BNW 13871-54 was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory (BNW 13871-4 only), the study laboratory, and Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO); stability analyses were also conducted by the study laboratory. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN), and Oneida Research Services (Whitesboro, NY; data not used) (Appendix I). Reports on analyses performed in support of the α-methylstyrene studies are on file at the National Institute of Environmental Health Sciences.

Both shipments of the chemical, a colorless liquid with a sharp, sweet, aromatic odor, were identified as α-methylstyrene by the analytical chemistry and study laboratories using infrared and proton nuclear magnetic resonance spectroscopy. The purity was determined by elemental analyses, moisture analysis, and gas chromatography (GC). The concentration of 4-*tert*-butyl catechol was determined by high performance liquid chromatography (HPLC), and polymer concentration was determined by a turbidity assay using ultraviolet/visible (UV/Vis) spectroscopy. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for α-methylstyrene. Karl Fischer titration indicated 514 ppm water for lot BNW 13871-4 and 141 ppm water for lot BNW 13871-54. GC by one system indicated one major peak and several minor peaks, one of which had a relative peak area greater than 0.05% (0.066%); the purity was determined to be greater than 99%. GC by two systems indicated one major peak and seven impurities, one of which had an area greater than 0.1% (0.21%) of the total peak area. The 0.21% impurity was determined to be *sec*-butylbenzene. Concentrations of 4-*tert*-butyl catechol were well above the 8 ppm action criteria as a polymerization inhibitor set by the study laboratory. Polymer concentration was less than 10 ppm. The overall purities of lots BNW 13871-4 and BNW 13871-54 were estimated at 99.5%.

Periodic purity reanalyses of the bulk chemical were performed by the study laboratory using GC, HPLC to determine 4-*tert*-butyl catechol concentration, and UV/Vis to determine polymer concentration. The purity reanalyses of the bulk chemical were performed at the beginning and end of each study and every 26 weeks during the 2-year studies. To ensure stability, the bulk chemical was stored at controlled room temperature in the original containers (55 gallon metal drums). No degradation of the chemical was detected, and 4-*tert*-butyl catechol and polymer concentrations remained within the study laboratory criteria.

VAPOR GENERATION AND EXPOSURE SYSTEM

α-Methylstyrene was held in an 8-gallon stainless steel chemical reservoir. α-Methylstyrene was pumped through a preheater and into the top of a heated glass column filled with glass beads to increase the surface area for evaporation. Heated nitrogen entering the column from below vaporized the chemical as it conveyed it out of the generator. The vapor was transported to the exposure room at an elevated temperature to prevent condensation. In the distribution manifold cabinet, the vapor was mixed with additional heated air before it entered a short vapor distribution manifold. The pressure in the distribution manifold was fixed to ensure constant flow through the manifold and into the chambers as the flow of vapor to each chamber was adjusted.

Electronically actuated metering valves controlled the flow to each chamber. In addition, an exposure-shutoff valve controlled vapor delivery to each chamber. Vapor was diverted to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. To start the exposure, the valves were opened to allow the flow of vapor to reach the chamber-metering valves and move into individual temperature-controlled delivery lines to each chamber. The vapor was then injected into the chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber 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 was used to count the particles in all chambers before and during generation. No particle counts greater than 200 particles/cm^3 were detected.

Vapor Concentration Monitoring

The α-methylstyrene concentrations in the exposure chambers were monitored by an online GC. Samples were drawn from each exposure chamber approximately every 20 (3-month studies) or 24 (2-year studies) minutes during each 6-hour exposure period using a 16-port stream select valve. Summaries of the chamber vapor concentrations are given in Tables I2 and I3. The online GC was checked throughout the day for instrument drift against an online standard of α-methylstyrene in nitrogen supplied by a diffusion standard generator.

The online GC was calibrated monthly by a comparison of chamber concentration data to data from grab samples, which were collected with graphitized carbon black sampling tubes, extracted with toluene containing butylbenzene as an internal standard, and analyzed by an offline GC. The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. The offline GC was calibrated with gravimetrically prepared standard solutions of α-methylstyrene containing butylbenzene as an internal standard in toluene.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. Based on experimental data, a T_{90} value of 12 minutes was selected for the studies.

The uniformity of α-methylstyrene vapor concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured once during the 3-month studies and every 3 months during the 2-year studies. The vapor concentration was measured using an online GC. Chamber concentration uniformity was maintained throughout the studies.

The persistence of α-methylstyrene in the chamber after vapor delivery ended was determined by monitoring the concentration with animals present in the 1,000 ppm chambers (mice and rats) in the 3-month studies and in the 600 ppm chamber (mice) and the 1,000 ppm chamber (rats) in the 2-year studies. In the 3-month studies, the concentration decreased to 1% of the target concentration within approximately 46 minutes in both 1,000 ppm chambers. In the 2-year studies, the concentration decreased to 1% of the target concentration within approximately 38 minutes in the 600 ppm chamber (mice) and 41 minutes in the 1,000 ppm chamber (rats).

In the 3-month studies, samples of α-methylstyrene were collected, with and without animals present, from the distribution line, 75 and 1,000 ppm exposure chambers (rats and mice), generator reservoir, and vapor trap and analyzed by GC. No evidence of degradation was detected, and no impurities were detected that were not present in the bulk material. HPLC was used to determine the concentration of 4-*tert*-butyl catechol in exposure chambers; none was detected. Polymer concentration was determined using UV/Vis; the concentration was less than 10 ppm. These results indicated that α-methylstyrene was stable for up to 7 weeks in the generator reservoir.

During the 2-year studies, samples were collected from the distribution line, 100 and 600 ppm exposure chambers (mice), and 100 and 1,000 ppm exposure chambers (rats) and analyzed by GC. Samples from the generator reservoir and vapor trap were collected at 26 weeks and

analyzed by GC. No evidence of degradation was detected, and no impurities were detected that were not present in the bulk material. The 1,000 ppm rat exposure chamber sample was analyzed by HPLC for 4-*tert*-butyl catechol; none was detected. Polymer concentration, determined by UV/Vis, was less than 10 ppm. These results indicated that α-methylstyrene was stable for up to 26 weeks in the generator reservoir.

3-Month Studies

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

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 (male rats and male and female mice) or 12 (female rats) 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 by the study laboratory on five male and five female sentinel rats and mice 3 weeks after arrival at the study laboratory. At terminal sacrifice, serum was collected from five male and five female chamber control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and 10 male and 10 female mice were exposed to α-methylstyrene at concentrations of 0, 75, 150, 300, 600, or 1,000 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. One additional exposure day was scheduled during the last exposure week to give male rats 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. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

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 red cell volume; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using an ABX Cobas Helios hematology analyzer (ABX Co., Irvine, CA). Manual hematocrit values were determined using a microcentrifuge (Heraeus Haemofuge, Germany) and a Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison to Helios values for packed cell volume. Blood smears for mice and rats were stained with Romanowsky-type aqueous stain in a Wescor 7100 Aerospray Slide Stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts for mice and rats 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, Branchburg, NJ). Table 1 lists the parameters measured.

After three (males) or four (females) consecutive exposure days 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 appearance, 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, Branchburg, NJ). Table 1 lists the parameters measured. A microscopic analysis of the urinary sediment was then performed.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on rats and mice exposed to 0, 300, 600, and

1,000 ppm. The parameters evaluated are listed in Table 1. 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. Four sperm morphology slides were prepared for each animal evaluated. 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. 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 with toluidine blue. 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).

The left kidney was removed from all male and female core study rats at terminal necropsy, sectioned in half longitudinally, placed in a cassette, and fixed with 10% neutral buffered formalin for approximately 24 hours. After fixation, one half of the left kidney was processed and embedded in paraffin. A cross-section of small intestine was included in the embedding paraffin as a positive control for the cell proliferation study. After embedding, the left kidney was cut into three 5-µm-thick sections. The first section was stained with hematoxylin and eosin for histopathology (males and females). The second section was stained with Mallory-Heidenhain for evaluation for hyaline droplets (males and females). The third section was stained with proliferating cell nuclear antigen (PCNA) complexed with avidin and biotin for determination of cell proliferation indices (males). The right kidneys of all core study male rats were frozen and stored at approximately –70° C.

For male rats, the slides stained with PCNA were evaluated to determine the labeling index of cells in the *S*-phase in the proximal tubules. Evaluation was done using a 20× objective and an ocular grid. Counting started at the second grid in from the outer edge of the cortex of the kidney. After one grid was counted, the slide was moved toward the medulla, and every other field encountered by the grid was counted. This procedure was repeated until at least 2,000 proximal tubular nuclei (labeled and unlabeled) were counted. After 2,000 proximal tubular nuclei were counted but the entire grid had not been counted, the remainder of the grid was counted. If 2,000 proximal tubular nuclei had not been counted by the time the outer medulla was reached, the slide was moved two grids laterally, and the counting process was resumed at the second grid in from the edge of the cortex.

The frozen kidneys from core study male rats were evaluated for α2u-globulin and soluble protein. 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 an Ultra-Turrax tissue homogenizer (Tekmar Co., Cincinnati, OH). The homogenate was centrifuged at approximately 3,000 g for 15 minutes at 4° C. The protein content of each supernatant was measured in a 1:50 dilution (in phosphate-buffered saline-Tween) using the Bicinchoninic Acid Protein Assay Reagent kit (Pierce Chemical Co., Rockford, IL).

Analysis of α2u-globulin in supernatants prepared from kidney homogenates was conducted using a competitive indirect enzyme-linked immunosorbent assay (ELISA). Ascites fluid containing anti-α2u-globulin monoclonal antibodies was developed by the Chemical Industry Institute of Toxicology (Research Triangle Park, NC). 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 and study samples were assayed in triplicate.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered

formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Testes were fixed in plastic and sectioned to a thickness of 1 µm. Complete histopathologic examinations were performed on all chamber control and 1,000 ppm animals, and tissues were examined to a no-effect level in the remaining exposed groups. Gross lesions found at necropsy or trimming were examined in all exposure groups. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to α-methylstyrene at concentrations of 0, 100, 300, 600 (mice only), or 1,000 (rats only) ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week, except holidays, for 105 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 11 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 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 K).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Cages, racks, and chambers were changed weekly. Cages were rotated weekly in chambers. Refer to Table 1 for more information about animal maintenance. Refer to Appendix J for information about feed composition and contaminants.

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.

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 and female rats were step sectioned at 1 mm intervals to obtain three to four additional sections from each kidney with a maximum of eight additional sections per animal (Eustis *et al.*, 1994). 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 kidney and nose of rats and mice; liver of female rats and male and female mice; pituitary gland of rats; eye, larynx, and testis of male rats; adrenal gland, lung, pleura, and spleen of female rats; and adrenal gland and forestomach of male mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the

laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of α-Methylstyrene

3-Month Studies	2-Year Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	
F344/N rats B6C3F$_1$ mice	F344/N rats B6C3F$_1$ mice
Animal Source	
Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 11 days	11 days
Average Age When Studies Began	
6 weeks	6 weeks
Date of First Exposure	
Rats: August 28 (males) or 29 (females), 2000 Mice: August 28, 2000	Rats: August 6, 2001 Mice: July 30, 2001
Duration of Exposure	
6 hours plus T_{90} (12 minutes) per day, 5 days per week, excluding holidays, for 14 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, excluding holidays, for 105 weeks
Date of Last Exposure	
Rats: November 27 (males) or 28 (females), 2000 Mice: November 29 (males) or 30 (females), 2000	Rats: August 6, 2003 Mice: July 31, 2003
Necropsy Dates	
Rats: November 28 (males) or 29 (females), 2000 Mice: November 30 (males) or December 1 (females), 2000	Rats: August 4 to 7, 2003 Mice: July 28 to August 1, 2003
Average Age at Necropsy	
19 weeks	110 weeks
Size of Study Groups	
Rats: 10 males and 10 females (core study), 10 males and 10 females (clinical pathology study) Mice: 10 males and 10 females	50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
1	1
Method of Animal Identification	
Tail tattoo	Tail tattoo

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of α-Methylstyrene

3-Month Studies	2-Year Studies
Diet	
NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum* (except during animal exposure and urine collection periods); changed weekly	NTP-2000 irradiated wafer rodent feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* (except during exposure periods); changed weekly
Water	
Tap water (Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI); available *ad libitum*	Same as 3-month studies
Cages	
Stainless steel, wire bottom (Lab Products, Inc., Seaford, DE); changed weekly	Same as 3-month studies; changed weekly; rotated weekly in chambers
Cageboard	
Untreated paper cage pan liner; changed daily	Untreated paper cage pan liner (Techboard, Sheperd Specialty Papers, Kalamazoo, MI); changed daily
Chambers	
Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE); chambers changed weekly; excreta pans changed daily	Same as 3-month studies
Chamber Air Supply Filters	
Single HEPA (Environmental Filter, Santa Rosa, CA), changed annually; charcoal (RSE, INC., New Baltimore, MI), new at study start; Purafil (Environmental Systems, Lynwood, WA), new at study start	Same as 3-month studies, except single HEPA was open stock
Chamber Environment	
Temperature: 75° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Air changes: 15 ± 2/hour	Temperature: 75° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Air changes: 15 ± 2/hour
Exposure Concentrations	
0, 75, 150, 300, 600, and 1,000 ppm	Rats: 0, 100, 300, and 1,000 ppm Mice: 0, 100, 300, and 600 ppm
Type and Frequency of Observation	
All animals were observed twice daily. Clinical findings were recorded weekly. Animals were weighed initially, weekly, and at the end of the studies.	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.
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus.	Necropsies were performed on all animals.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of α-Methylstyrene

3-Month Studies	2-Year Studies
Clinical Pathology	
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. Core study rats were placed in metabolism cages for 16-hour urine collection during week 12. ***Hematology:*** hematocrit; packed red cell volume; hemoglobin; erythrocyte, reticulocyte, and platelet counts; Howell-Jolly bodies (mice); mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile acids ***Urinalysis:*** creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, γ-glutamyltransferase, *N*-acetyl-β-D-glucosaminidase, volume, and specific gravity	None
Histopathology	
Complete histopathology was performed on all core study chamber control and 1,000 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, 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.	Complete histopathology was performed on all core study 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 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	
Epididymal sperm concentration and motility; spermatid heads/testis; and left cauda, epididymis, and testis weights were evaluated in male rats and mice from the 0, 300, 600, and 1,000 ppm exposure groups at terminal sacrifice. For 12 consecutive days prior to scheduled terminal sacrifice, vaginal cytology slides were prepared for all surviving female rats and mice in the 0, 300, 600, and 1,000 ppm exposure groups. 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).	None
Renal Toxicity	
At the end of the studies, left kidneys of core study rats were evaluated for histopathology (males and females) and cell proliferation (males). Right kidneys of core study male rats were evaluated for α2u-globulin and soluble protein.	None

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B3, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

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

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and $B6C3F_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each 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, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used

to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed up to the present. 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 α-methylstyrene was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium,* sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, 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

3-MONTH STUDY

All rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of all exposed groups were similar to those of the chamber controls. No clinical findings related to α-methylstyrene exposure were observed.

The hematology, clinical chemistry, and urinalysis data for rats are listed in Tables 3 and F1. At study termination, an exposure-related decrease in the erythron, evidenced by a decrease in the hematocrit, hemoglobin, and erythrocyte count values, occurred in males exposed to

TABLE 2
Survival and Body Weights of Rats in the 3-Month Inhalation Study of α-Methylstyrene

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	95 ± 4	330 ± 7	235 ± 6	
75	10/10	87 ± 5	338 ± 10	251 ± 9	102
150	10/10	94 ± 5	334 ± 6	240 ± 5	101
300	10/10	91 ± 4	329 ± 6	238 ± 5	100
600	10/10	88 ± 4	327 ± 5	240 ± 7	99
1,000	10/10	90 ± 5	313 ± 5	223 ± 6	95
Female					
0	10/10	90 ± 3	201 ± 5	111 ± 4	
75	10/10	90 ± 4	203 ± 5	114 ± 4	101
150	10/10	90 ± 4	203 ± 5	113 ± 5	101
300	10/10	89 ± 3	198 ± 4	108 ± 3	99
600	10/10	89 ± 4	202 ± 4	113 ± 4	100
1,000	10/10	90 ± 4	192 ± 4	102 ± 3	96

[a] Number of animals surviving at 3 months/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 3
Selected Clinical Chemistry and Urinalysis Data for F344/N Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10	10	10
Male						
Clinical Chemistry						
Bile acids (μmol/L)						
Day 3	32.8 ± 1.9	38.8 ± 3.5	34.3 ± 1.9	37.8 ± 1.6	43.6 ± 1.5**	44.0 ± 2.0**
Day 23	24.2 ± 1.7	26.9 ± 0.6	29.7 ± 1.0**	35.7 ± 1.0**	39.8 ± 1.5**	41.1 ± 1.2**
Week 14	31.1 ± 2.3	32.4 ± 2.3	29.7 ± 1.1	35.8 ± 1.7	35.1 ± 1.8	42.2 ± 1.7**
Urinalysis						
Creatinine (mg/dL)						
Week 12	74.00 ± 12.21	40.90 ± 4.30	58.70 ± 11.45	47.60 ± 5.44	34.60 ± 4.10**	33.60 ± 3.41**
Protein/creatinine ratio						
Week 12	0.65 ± 0.04	0.68 ± 0.02	0.72 ± 0.03	0.77 ± 0.03*	0.94 ± 0.04**	1.17 ± 0.03**
Alkaline phosphatase/creatinine ratio						
Week 12	3.05 ± 0.28	2.99 ± 0.14	3.15 ± 0.22	3.67 ± 0.20*	3.78 ± 0.11**	3.88 ± 0.18**
Aspartate aminotransferase/creatinine ratio						
Week 12	0.09 ± 0.01	0.08 ± 0.02	0.12 ± 0.01	0.26 ± 0.02**	0.39 ± 0.05**	0.48 ± 0.02**
Lactate dehydrogenase/creatinine ratio						
Week 12	0.54 ± 0.02	0.60 ± 0.02*	0.70 ± 0.04**	0.95 ± 0.02**	1.38 ± 0.09**	1.59 ± 0.05**
γ-Glutamyltransferase/creatinine ratio						
Week 12	19.10 ± 0.84	21.56 ± 0.67	20.09 ± 0.72	21.66 ± 0.65	20.57 ± 0.63	22.09 ± 0.95
N-acetyl-β-D-glucosaminidase/creatinine ratio						
Week 12	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.21 ± 0.01**	0.22 ± 0.01**	0.26 ± 0.01**
Volume (mL/16 hours)						
Week 12	12.0 ± 1.9	22.5 ± 2.5	17.9 ± 3.5	18.5 ± 2.5	24.7 ± 3.0**	25.0 ± 2.6**
Female						
Clinical Chemistry						
Bile acids (μmol/L)						
Day 3	25.7 ± 1.0	25.8 ± 2.0	27.1 ± 1.5	29.6 ± 1.4	32.5 ± 1.3**	36.1 ± 3.5**
Day 23	25.9 ± 1.6	24.4 ± 0.7	26.0 ± 0.9	28.5 ± 1.2	30.3 ± 1.5*	31.7 ± 1.9**
Week 14	25.7 ± 1.1	30.7 ± 3.8	30.5 ± 2.9	28.1 ± 1.3	25.2 ± 0.8	33.5 ± 1.8**
Urinalysis						
Creatinine (mg/dL)						
Week 12	37.70 ± 3.01	48.10 ± 7.05	41.50 ± 6.75	30.60 ± 2.70	29.70 ± 3.37	41.70 ± 2.77
Protein/creatinine ratio						
Week 12	0.08 ± 0.01	0.09 ± 0.01[b]	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.01**	0.10 ± 0.01*
Alkaline phosphatase/creatinine ratio						
Week 12	2.05 ± 0.10	1.97 ± 0.07	1.97 ± 0.13	2.29 ± 0.14	2.87 ± 0.10**	3.22 ± 0.15**
Aspartate aminotransferase/creatinine ratio						
Week 12	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Lactate dehydrogenase/creatinine ratio						
Week 12	0.31 ± 0.02	0.34 ± 0.03[b]	0.38 ± 0.03*	0.46 ± 0.04**	0.60 ± 0.04**	0.69 ± 0.02**
γ-Glutamyltransferase/creatinine ratio						
Week 12	8.24 ± 0.51	7.70 ± 0.47	10.34 ± 0.77	9.65 ± 0.45	13.63 ± 1.36**	13.89 ± 0.91**

TABLE 3
Selected Clinical Chemistry and Urinalysis Data for F344/N Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10	10	10
Female (continued)						
Urinalysis (continued)						
N-acetyl-β-D-glucosaminidase/creatinine ratio						
Week 12	0.12 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01*	0.16 ± 0.01**
Volume (mL/16 hours)						
Week 12	13.5 ± 1.4	11.3 ± 1.5	12.0 ± 1.4	15.8 ± 1.9	16.8 ± 1.8	10.5 ± 0.7

* Significantly different (P≤0.05) from the chamber control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are given as mean ± standard error. Ratios were calculated and statistical tests were performed on unrounded data.
[b] n=9

150 ppm or greater; female rats were unaffected. The erythron effect in males was minimal, represented by an approximate 5% decrease in the erythron variables of 1,000 ppm males. The mechanism for this erythron change in the male rats was unknown, but since there was no erythropoietic response to the erythron decrease, either the effect was too minimal to stimulate a response or there may have been some minimal suppression of erythropoiesis.

Serum chemistry evaluations demonstrated an exposure concentration-related increase in bile acid concentrations that was minimal but consistent in the 1,000 ppm male and female rats at all time points. The mechanism for the bile acid increase was unknown but could suggest a cholestatic or hepatocellular effect. However, serum alkaline phosphatase activity, another marker of cholestasis, was unaffected. Also, serum alanine aminotransferase and sorbitol dehydrogenase activities, which are typically increased with hepatocellular injury, were either unaffected or decreased.

Urine chemistry evaluations at week 12 demonstrated exposure concentration-related increases in the following ratios: protein/creatinine, alkaline phosphatase/creatinine, aspartate aminotransferase/creatinine, lactate dehydrogenase/creatinine, γ-glutamyltransferase/creatinine, and *N*-acetyl-β-glucosaminidase/creatinine; these were consistent with an insult to the renal tubular epithelium. Increases in the ratios occurred in both sexes and primarily involved the 300 ppm males and 600 ppm or greater males and females. The 600 and 1,000 ppm males demonstrated a two-fold increase in timed urine volume.

No exposure-related gross lesions were observed. Statistically significant increases in absolute and relative kidney weights were seen in 1,000 ppm males and 600 and 1,000 ppm females (Tables 4 and G1). Statistically significant increases in absolute and relative liver weights were seen in 150 ppm and greater males and 600 and 1,000 ppm females. Exposure to α-methylstyrene had no effect on reproductive endpoints of male or female rats (Tables H1 and H2).

The incidences of hyaline droplet accumulation were similar between exposed groups and chamber control groups (Table 5). Hyaline droplet accumulation in male rats is frequently associated with the accumulation of α2u-globulin, a male-rat-specific low molecular weight protein. Chemicals that induce the accumulation of α2u-globulin in the renal tubules are associated with increased renal tubule epithelial degeneration, necrosis and regeneration, and subsequent renal carcinogenicity. Kidney sections from male and female rats were stained with hematoxylin and eosin and the Mallory-Heidenhain method to better visualize and assess hyaline droplet accumulation. Generally, hyaline droplets in the

TABLE 4
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	330 ± 7	338 ± 10	334 ± 6	329 ± 6	327 ± 5	313 ± 5
R. Kidney						
Absolute	1.002 ± 0.025	1.071 ± 0.038	1.064 ± 0.019	1.070 ± 0.024	1.088 ± 0.024	1.104 ± 0.021*
Relative	3.037 ± 0.035	3.166 ± 0.047*	3.188 ± 0.027**	3.254 ± 0.034**	3.324 ± 0.035**	3.526 ± 0.040**
Liver						
Absolute	10.24 ± 0.25	10.75 ± 0.36	11.40 ± 0.22*	11.28 ± 0.36*	12.65 ± 0.39**	13.56 ± 0.38**
Relative	31.031 ± 0.235	31.775 ± 0.388	34.159 ± 0.332**	34.266 ± 0.673**	38.612 ± 0.740**	43.269 ± 0.852**
Female						
Necropsy body wt	201 ± 5	203 ± 5	203 ± 5	198 ± 4	202 ± 4	192 ± 4
R. Kidney						
Absolute	0.665 ± 0.015	0.673 ± 0.014	0.679 ± 0.013	0.673 ± 0.012	0.713 ± 0.017*	0.717 ± 0.014*
Relative	3.313 ± 0.055	3.319 ± 0.038	3.362 ± 0.060	3.408 ± 0.058	3.526 ± 0.027**	3.729 ± 0.036**
Liver						
Absolute	5.856 ± 0.178	5.929 ± 0.167	6.074 ± 0.126	6.001 ± 0.168	6.572 ± 0.208**	7.129 ± 0.183**
Relative	29.108 ± 0.414	29.190 ± 0.379	30.135 ± 0.907	30.376 ± 0.783	32.459 ± 0.464**	37.061 ± 0.561**

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

chamber control, 75, 150, and 300 ppm groups were characterized by the presence of small, round eosinophilic droplets of uniform size in the cytoplasm of proximal convoluted tubules. Hyaline droplets in the 600 and 1,000 ppm groups were larger and more variable in shape, which contributed to a slightly higher severity of hyaline droplet accumulation in these groups. Consistent with the hyaline droplet accumulation, an exposure-related increase in α2u-globulin was detected in the kidneys of males exposed to α-methylstyrene (Table 6). The increased labeling index was observed in renal tubule cells of male rats exposed to 150 ppm or greater (Table 6). There was no evidence of granular casts within renal tubules; granular casts are sometimes seen as a result of necrosis of epithelial cells due to abnormal hyaline droplet accumulation and are generally considered the most severe manifestation of hyaline droplet nephropathy. Morphological changes were not detected in the liver.

Exposure Concentration Selection Rationale: α-Methylstyrene had no effect on survival or body weights of male or female rats. Liver and kidney weights were increased; however, no corresponding histopathologic lesions were observed in the liver. In the kidney, the severity of renal hyaline droplet accumulation was minimal to mild at 1,000 ppm, and an exposure concentration-related increase in renal cell proliferation and α2u-globulin concentration was observed. Therefore, the exposure concentrations selected for the 2-year inhalation study were 300, 600, and 1,000 ppm.

TABLE 5
Incidences of Selected Nonneoplastic Lesions of the Renal Cortex in Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Renal Tubule, Accumulation, Hyaline Droplet[a]	9 (1.1)[b]	10 (1.2)	10 (1.3)	10 (1.1)	10 (1.8)	10 (1.7)
Renal Tubule, Regeneration	8 (1.0)	4 (1.3)	9 (1.0)	5 (1.0)	9 (1.2)	8 (1.1)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Renal Tubule, Regeneration	0	0	3 (1.0)	3 (1.0)	2 (1.0)	1 (1.0)

[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 6
Renal Toxicity Data for Male Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10	10	10
Cells labeled	50.00 ± 2.94	64.60 ± 6.42	66.60 ± 5.87*	73.20 ± 4.41**	65.10 ± 3.77**	85.80 ± 7.17**
Cells counted	2,131 ± 43	2,137 ± 31	2,142 ± 39	2,197 ± 36	2,130 ± 22	2,173 ± 44
Labeling index (%)[b]	2.339 ± 0.110	3.032 ± 0.316	3.083 ± 0.226*	3.353 ± 0.230**	3.050 ± 0.159**	3.935 ± 0.307**
Soluble protein (mg/mL)	22.54 ± 1.52	27.96 ± 1.81	39.19 ± 1.01**	40.64 ± 1.49**	38.22 ± 1.78**	42.28 ± 0.94**
α2u-Globulin (nmol/g kidney)	195.2 ± 23.9	349.0 ± 56.7*	497.0 ± 47.1**	689.0 ± 69.5**	724.0 ± 116.4**	749.4 ± 85.4**
α2u-Globulin (ng/μg soluble protein)	81.32 ± 8.87	115.46 ± 16.81	119.29 ± 11.28*	160.82 ± 16.51**	176.02 ± 26.18**	167.42 ± 20.50**

* Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test
** $P \leq 0.01$
[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.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 7 and in the Kaplan-Meier survival curves (Figure 1). Survival rates of exposed male and female rats were similar to those of the chamber controls.

Body Weights and Clinical Findings

The mean body weights of 1,000 ppm males and females were 5% to 10% less than those of the chamber control groups during year 2 of the study (Tables 8 and 9; Figure 2). No clinical findings related to chemical exposure were observed. A few exposed male rats (0/50, 3/50, 1/50, 5/50) and a limited number of chamber control and exposed female rats (2/50, 2/50, 3/50, 9/50) had seizures. The seizures were clonic and of short duration. They were most frequently observed and recorded during daily animal care activities. The first seizure episode was observed in a female rat at 49 weeks of exposure. No evidence of brain lesions was found to account for the cause or effect of the clonic seizures noted clinically in exposed and chamber control animals. 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 7
Survival of Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	1	1
Moribund	19	15	21	25
Natural deaths	4	3	5	2
Animals surviving to study termination	27	32	23	22[b]
Percent probability of survival at end of study[c]	54	64	47	45
Mean survival (days)[d]	680	700	679	663
Survival analysis[e]	P=0.182	P=0.327N	P=0.767	P=0.529
Female				
Animals initially in study	50	50	50	50
Accidental deaths	2	0	0	0
Moribund	15	21	12	20
Natural deaths	6	5	2	4
Animals surviving to study termination	27	24	36	26
Percent probability of survival at end of study	56	48	72	52
Mean survival (days)	647	680	701	685
Survival analysis	P=0.948	P=0.582	P=0.147N	P=0.901

[a] Censored from survival analyses
[b] Includes one animal that died during the last week of the study
[c] Kaplan-Meier determinations
[d] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[e] 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 lower mortality in an exposed group is indicated by **N**.

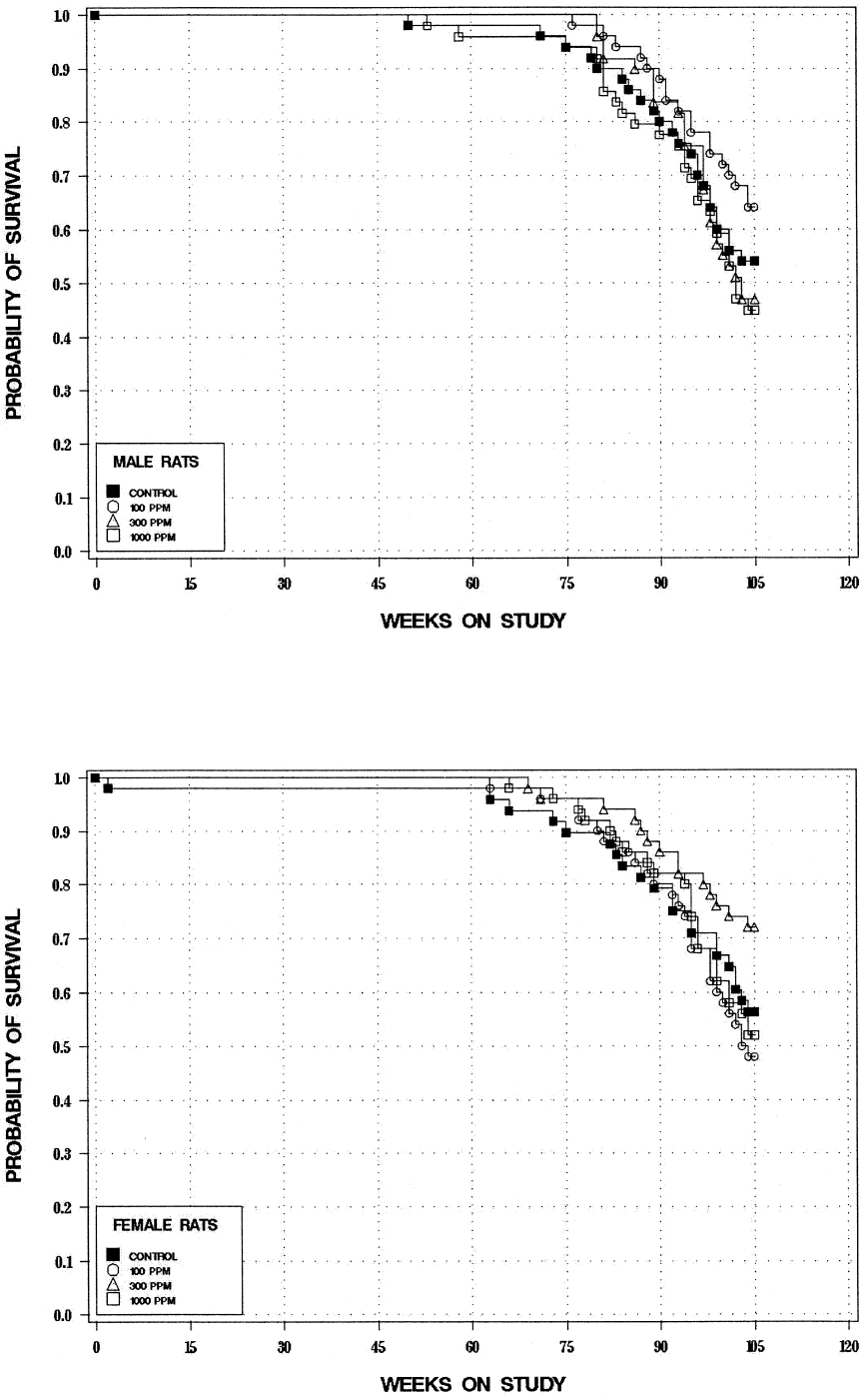


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to α-Methylstyrene for 2 Years

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of α-Methylstyrene

Days on Study	Chamber Control		100 ppm			300 ppm			1,000 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	76	50	76	100	50	75	98	50	74	98	50
9	110	50	110	101	50	107	98	50	100	91	50
16	139	50	141	102	50	137	99	50	127	92	50
23	167	50	172	103	50	168	101	50	157	94	50
30	191	50	198	104	50	194	102	50	184	96	50
37	211	50	219	104	50	215	101	50	204	97	50
44	232	50	238	103	50	233	100	50	224	96	50
51	248	50	254	102	50	249	100	50	240	97	50
58	263	50	268	102	50	263	100	50	255	97	50
65	277	50	283	102	50	277	100	50	270	98	50
72	290	50	294	101	50	289	99	50	283	98	50
79	301	50	306	102	50	300	100	50	295	98	50
86	312	50	316	102	50	311	100	50	305	98	50
114	350	50	355	101	50	349	100	50	343	98	50
142	377	50	381	101	50	375	100	50	367	97	50
170	400	50	403	101	50	397	99	50	383	96	50
198	419	50	423	101	50	416	99	50	406	97	50
226	435	50	438	101	50	427	98	49	418	96	49
254	449	50	449	100	50	439	98	49	428	95	49
282	461	50	460	100	50	453	98	49	438	95	49
310	470	50	469	100	50	462	98	49	450	96	49
338	481	50	480	100	50	473	98	49	457	95	49
366	489	49	490	100	50	479	98	49	463	95	49
394	493	49	496	101	50	486	99	49	468	95	48
422	500	49	500	100	50	493	99	49	473	95	47
450	508	49	504	99	50	500	99	49	479	94	47
478	514	49	510	99	50	507	99	49	480	93	47
506	520	48	513	99	50	509	98	49	482	93	47
534	530	47	522	99	49	514	97	49	485	92	46
562	532	45	519	98	49	516	97	47	481	90	45
590	536	44	524	98	47	523	98	45	489	91	40
618	530	42	526	99	45	525	99	44	490	92	39
646	531	39	523	99	42	526	99	41	490	92	38
660	529	38	521	99	41	526	99	37	493	93	35
674	534	34	521	98	39	509	95	37	486	91	32
688	526	32	525	100	37	520	99	30	475	90	31
702	523	30	515	99	36	527	101	27	473	91	28
716	511	28	508	99	34	513	100	25	474	93	23
Mean for weeks											
1-13	217		221	102		217	100		209	96	
14-52	427		429	100		421	99		410	96	
53-103	519		513	99		511	98		480	93	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of α-Methylstyrene

Days on Study	Chamber Control		100 ppm			300 ppm			1,000 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	73	50	73	100	50	71	98	50	72	98	50
9	96	50	96	100	50	92	96	50	89	93	50
16	112	48	113	101	50	109	97	50	104	93	50
23	126	48	128	101	50	125	99	50	120	95	50
30	136	48	139	103	50	137	101	50	132	97	50
37	146	48	148	101	50	146	100	50	142	97	50
44	155	48	157	102	50	154	100	50	150	97	50
51	162	48	163	101	50	160	99	50	157	97	50
58	167	48	169	102	50	167	100	50	163	98	50
65	171	48	174	102	50	171	100	50	168	98	50
72	176	48	180	102	50	177	100	50	173	98	50
79	180	48	185	103	50	182	101	50	178	99	50
86	185	48	191	103	50	187	101	50	182	98	50
114	199	48	207	104	50	203	102	50	196	99	50
142	208	48	216	104	50	212	102	50	204	98	50
170	217	48	227	105	50	221	102	50	209	96	50
198	225	47	238	106	50	231	103	50	217	97	50
226	233	47	246	105	50	237	102	50	223	96	50
254	242	47	254	105	50	245	101	50	229	95	50
282	251	47	263	105	50	253	101	50	235	94	50
310	262	47	273	104	50	265	101	50	245	93	50
338	274	47	288	105	50	278	101	50	255	93	50
366	284	47	299	105	50	286	100	50	262	92	50
394	293	47	305	104	50	292	100	50	273	93	50
422	302	47	316	105	50	303	101	50	283	94	50
450	311	46	323	104	49	311	100	50	291	94	50
478	320	45	332	104	49	320	100	49	299	94	49
506	327	45	339	104	48	328	100	48	307	94	49
534	333	43	342	103	48	333	100	48	310	93	48
562	335	43	349	104	44	335	100	48	313	94	46
590	341	40	355	104	44	343	100	47	319	94	43
618	344	39	352	102	41	348	101	44	321	93	42
646	351	36	357	102	39	351	100	43	317	91	41
660	347	36	355	103	37	353	102	41	318	92	40
674	352	34	359	102	34	353	100	41	328	93	34
688	349	33	362	104	30	352	101	39	323	93	33
702	347	32	357	103	29	350	101	38	325	94	30
716	351	29	358	102	26	346	99	37	326	93	29
Mean for weeks											
1-13	145		147	102		144	99		141	97	
14-52	235		246	105		238	102		223	95	
53-103	330		341	103		332	100		307	93	

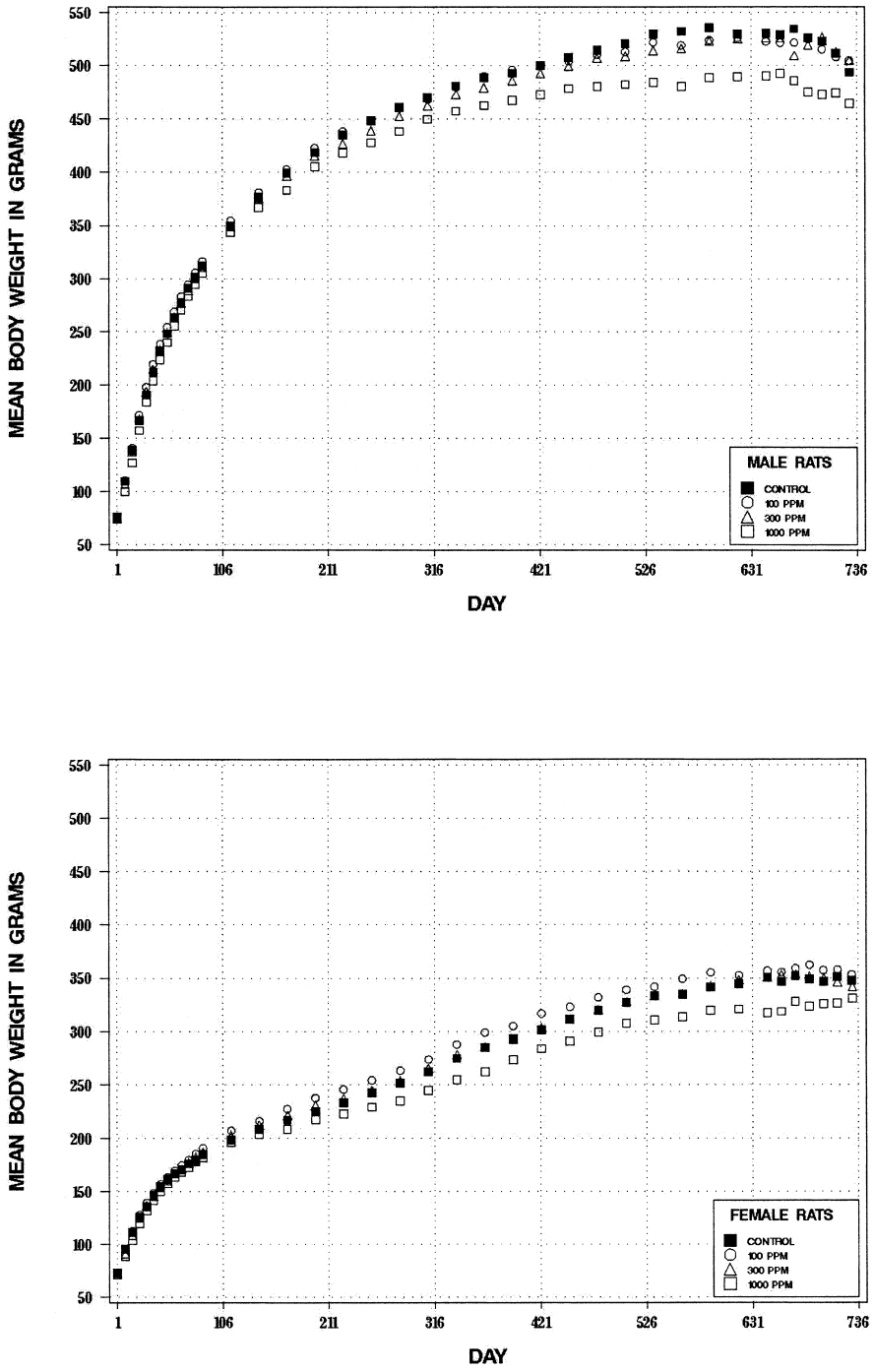


FIGURE 2
Growth Curves for Male and Female Rats
Exposed to α-Methylstyrene by Inhalation for 2 Years

Pathology and Statistical Analyses

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

Kidney: Two 1,000 ppm males and one 300 ppm male had renal tubule carcinomas, and one 300 ppm male had a renal tubule adenoma (Tables 10 and A1). Microscopically, the renal tubule adenoma was a well circumscribed, discrete mass of neoplastic epithelial cells greater than five tubules in diameter (Plates 1 and 2). The neoplastic cells were typically arranged in solid aggregates, small nests, or tubule-like structures without obvious lumens. Microscopically, renal tubule carcinomas were invasive and composed of a mixture of round cells with large vesicular nuclei and abundant, pale eosinophilic cytoplasm and a lesser number of vacuolated cells, forming sheets and large nests resembling tubules with areas of necrosis surrounded by a thin fibrous stroma. Cellular atypia and pleomorphism were present (Plate 3), and in two rats, metastasis to the lung occurred (Plate 4).

Initially, a single hematoxylin and eosin-stained section of each kidney was prepared. Because of the neoplasms observed in 300 and 1,000 ppm males at the end of the 2-year study, the hyaline droplet nephropathy with α2u-globulin accumulation detected at the end of the 3-month study, and the known association between α2u-globulin accumulation and renal neoplasms, additional kidney step sections (three to four from each kidney) were prepared from the remaining formalin-fixed tissues at 1 mm intervals for each male. Additional males with focal hyperplasia or adenoma were identified. The incidences of these proliferative lesions in the step sections and in the single and step sections combined are shown in Tables 10 and A2. The incidences of renal tubule adenoma and carcinoma (combined) in the 1,000 ppm males were significantly greater than those in chamber controls when the single and step sections were combined (Tables 10 and A2).

Renal tubule hyperplasia, as defined in the current study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered a preneoplastic lesion. Renal tubule hyperplasia, adenoma, and carcinoma are part of a morphologic continuum. Hyperplasia was generally a focal, minimal to mild lesion consisting of single or multiple adjacent tubule profiles containing atypical epithelial cells that partially or completely filled the lumen. The epithelial cells were variably enlarged with distinct cell borders, expanded eosinophilic cytoplasm, variable nuclear size, and multiple enlarged nucleoli. The affected tubules were generally, but not always, larger than normal tubules.

The incidence of mineralization of the renal papilla, a common lesion associated with α2u-globulin nephropathy, was significantly increased in 1,000 ppm males (Tables 10 and A4). The mineral was most frequently present as elongated profiles within tubules (linear mineralization) and is characteristic of α2u-globulin nephropathy. Minimal mineralization of the renal papilla, characterized by laminated concretions, was present in a few exposed females and was significant at 300 and 1,000 ppm when compared to chamber controls (Tables 10 and B3). In 1,000 ppm females, there was a decreased incidence of mineralization of the transitional epithelium within the pelvis; the significance of this finding in females is unclear. Minimal to mild mineralization of the transitional epithelium is common in females and may occur at an early age in rats.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Male				
Single Sections (Standard Evaluation)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia[a]	0	0	0	0
Papilla, Mineralization	12 (1.1)[b]	16 (1.0)	10 (1.0)	33** (1.4)
Nephropathy	41 (2.2)	46 (2.3)	46 (2.4)	45 (2.4)
Renal Tubule, Adenoma[c]	0	0	1	0
Renal Tubule, Carcinoma[d]	0	0	1	2
Renal Tubule, Adenoma or Carcinoma[e]	0	0	2	2
Step Sections (Extended Evaluation)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	1 (1.0)	0	1 (1.0)	4 (2.3)
Renal Tubule, Adenoma, Multiple	0	0	0	2
Renal Tubule, Adenoma (includes multiple)				
Overall rate[f]	1/50 (2%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
Adjusted rate[g]	2.4%	4.5%	2.4%	12.4%
Terminal rate[h]	1/27 (4%)	1/32 (3%)	1/23 (4%)	3/22 (14%)
First incidence (days)	729 (T)	723	729 (T)	653
Poly-3 test[i]	P=0.033	P=0.524	P=0.761	P=0.091
Single and Step Sections (Combined)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	1 (1.0)	0	1 (1.0)	4 (2.3)
Renal Tubule, Adenoma, Multiple	0	0	0	2
Renal Tubule, Adenoma (includes multiple)	1	2	2	5
Renal Tubule, Adenoma or Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	7/50 (14%)
Adjusted rate	2.4%	4.5%	7.1%	17.2%
Terminal rate	1/27 (4%)	1/32 (3%)	2/23 (9%)	3/22 (14%)
First incidence (days)	729 (T)	723	716	653
Poly-3 test	P=0.006	P=0.524	P=0.305	P=0.026

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Female				
Number Examined Microscopically	49	50	50	50
Papilla, Mineralization	1 (1.0)	6 (1.0)	8* (1.0)	7* (1.0)
Pelvis, Transitional Epithelium, Mineralization	31 (1.5)	26 (1.0)	31 (1.1)	16** (1.0)
Nephropathy	34 (1.6)	27 (1.3)	35 (1.5)	31 (1.8)

(T) Terminal sacrifice
* 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): 3/399 (0.8% ± 1.0%); range 0%-2%
[d] Historical incidence: 1/399 (0.3% ± 0.7%); range 0%-2%
[e] Historical incidence: 4/399 (1.0% ± 1.1%); range 0%-2%
[f] Number of animals with neoplasm per number of animals with kidney examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] 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.

Testis: The incidences of interstitial cell adenoma in all exposed groups of male rats were increased compared to the chamber controls (chamber control, 33/50; 100 ppm, 44/50; 300 ppm, 41/50; 1,000 ppm, 44/50; Table A2). The incidences in the 100 and 1,000 ppm groups were slightly outside of the historical range for chamber controls in inhalation studies given NTP-2000 diet (Table A3b). Given the lower than expected response in chamber controls, the lack of an exposure response, and that adenomas of the testis are one of the most common neoplasms in F344/N rats in 2-year studies, this response was considered unrelated to α-methylstyrene exposure.

Mononuclear Cell Leukemia: The incidence of mononuclear cell leukemia in 1,000 ppm males was significantly increased compared to the chamber controls (Tables 11, A1, and A2). The incidence was slightly outside the historical chamber control incidence for inhalation studies (Tables 11 and A3c). Mononuclear cell leukemia is one of the most common neoplasms in F344/N rats in 2-year studies.

Nose: The incidences of basal cell hyperplasia were significantly increased in all exposed groups of males and females (Tables 12, A4, and B3). Basal cell hyperplasia was characterized by crowding of basal cells along the basement membrane of the olfactory epithelium (Plates 5 and 6). Significantly increased incidences of degeneration of the olfactory epithelium occurred in 1,000 ppm males and females and in 300 ppm females. Degeneration was characterized by decreased cellularity and a vacuolated appearance to the olfactory epithelium (Plates 5 and 6).

TABLE 11
Incidences of Mononuclear Cell Leukemia in Male Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Overall rate[a, b]	26/50 (52%)	32/50 (64%)	29/50 (58%)	38/50 (76%)
Adjusted rate[c]	58.7%	67.7%	61.9%	80.2%
Terminal rate[d]	17/27 (63%)	23/32 (72%)	12/23 (52%)	14/22 (64%)
First incidence (days)	495	562	558	401
Poly-3 test[e]	P=0.018	P=0.239	P=0.459	P=0.016

[a] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 188/399 (47.1% ± 10.3%); range 32%-66%
[b] Number of animals with mononuclear cell leukemia per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the 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.

TABLE 12
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	50	49
Olfactory Epithelium, Hyperplasia, Basal Cell[a]	0	17** (1.0)[b]	18** (1.2)	43** (1.8)
Olfactory Epithelium, Degeneration	1 (2.0)	3 (1.3)	3 (1.7)	16** (1.0)
Female				
Number Examined Microscopically	49	49	50	50
Olfactory Epithelium, Hyperplasia, Basal Cell	0	14** (1.0)	30** (1.0)	49** (1.6)
Olfactory Epithelium, Degeneration	1 (1.0)	1 (1.0)	7* (1.3)	24** (1.1)

* 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

MICE

3-MONTH STUDY

All male mice survived to the end of the study (Table 13). Two of 10 female mice in the 1,000 ppm group died before exposure on day 3; all other female mice survived to the end of the study. Final mean body weights of 600 and 1,000 ppm males and 75, 300, and 1,000 ppm females were significantly less than those of the chamber controls; final mean body weight gains of mice exposed to 300 ppm or greater were significantly less than those of the chamber controls. Moderate to severe sedation (males only) and ataxia were observed in 1,000 ppm mice. No other clinical findings related to α-methylstyrene exposure were observed.

The hematology data for mice are listed in Table F2. At study termination, a minimal (approximately 4%) decrease in the erythron, evidenced by a decrease in the hemoglobin concentration and erythrocyte count values, occurred in 1,000 ppm females and may represent a change similar to what occurred in the 3-month male rat study.

No exposure-related gross lesions were observed. Exposure-related changes in organ weights were seen in the liver. Statistically significant increases in absolute liver weight were seen in 600 and 1,000 ppm females, and there was an increase, although not statistically significant, in the 1,000 ppm males (Tables 14 and G2). Statistically significant increases in relative liver weights were also seen in 150 ppm males and 300, 600, and 1,000 ppm males and females. In male mice, decreased epididymal weights were observed, but exposure to α-methylstyrene had no effect on reproductive endpoints (Table H3). In females, the estrous cycle

TABLE 13
Survival and Body Weights of Mice in the 3-Month Inhalation Study of α-Methylstyrene

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.2 ± 0.3	38.7 ± 0.9	15.5 ± 0.8	
75	10/10	23.4 ± 0.3	37.8 ± 0.8	14.4 ± 0.7	98
150	10/10	23.5 ± 0.3	38.5 ± 1.2	15.0 ± 1.1	99
300	10/10	23.5 ± 0.3	36.8 ± 0.7	13.3 ± 0.8*	95
600	10/10	23.5 ± 0.4	33.7 ± 0.6**	10.2 ± 0.6**	87
1,000	10/10	23.4 ± 0.3	32.3 ± 0.6**	8.9 ± 0.4**	83
Female					
0	10/10	19.3 ± 0.4	31.0 ± 0.8	11.7 ± 0.8	
75	10/10	18.7 ± 0.4	28.3 ± 0.6*	9.6 ± 0.5	91
150	10/10	19.8 ± 0.3	30.7 ± 0.7	10.9 ± 0.7	99
300	10/10	19.3 ± 0.3	28.3 ± 0.5*	9.0 ± 0.4*	91
600	10/10	19.4 ± 0.2	29.7 ± 0.5	10.3 ± 0.5*	96
1,000	8/10[c]	19.5 ± 0.4	27.7 ± 0.7**	8.0 ± 0.5**	89

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

TABLE 14
Liver Weights for Mice in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	38.7 ± 0.9	37.8 ± 0.8	38.5 ± 1.2	36.8 ± 0.7	33.7 ± 0.6**	32.3 ± 0.6**
Liver						
Absolute	1.484 ± 0.048	1.496 ± 0.034	1.582 ± 0.070	1.572 ± 0.038	1.551 ± 0.044	1.633 ± 0.031
Relative	38.368 ± 1.024	39.618 ± 0.741	40.954 ± 0.688*	42.822 ± 0.878**	46.039 ± 0.865**	50.622 ± 0.676**
Female						
n	10	10	10	10	10	8
Necropsy body wt	31.0 ± 0.8	28.3 ± 0.6*	30.7 ± 0.7	28.3 ± 0.5*	29.7 ± 0.5	27.7 ± 0.7**
Liver						
Absolute	1.357 ± 0.034	1.277 ± 0.031	1.373 ± 0.031	1.332 ± 0.033	1.568 ± 0.025**	1.596 ± 0.055**
Relative	43.807 ± 0.543	45.130 ± 0.600	44.804 ± 0.680	47.132 ± 0.899**	52.813 ± 0.660**	57.645 ± 1.035**

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

lengths in the 600 and 1,000 ppm groups were significantly longer than in the chamber control group (Table H4).

Minimal to mild centrilobular hypertrophy was present in the livers of male and female mice exposed to 600 and 1,000 ppm α-methylstyrene (Table 15). Microscopically, centrilobular hypertrophy was characterized by hepatocytes that had increased cytoplasmic volume, with diffuse pale eosinophilia and a reduction in vacuolation compared to unaffected hepatocytes. Centrilobular hypertrophy contributed to the increased liver weights found at terminal sacrifice.

Multiple exposure-related nasal lesions were present in all exposed groups of males and females (Table 15). Two females that died early in the study had necrosis of the olfactory epithelium characterized by sloughed olfactory epithelium, with pyknotic nuclei and disrupted organization of cell layers. Mice that survived to terminal sacrifice had atrophy and metaplasia of the olfactory epithelium and atrophy and hyperplasia of Bowman's glands. Atrophy and metaplasia of the olfactory epithelium were slightly more severe in animals exposed to 300 ppm or greater; this change was characterized by decreased layers of neuronal cells and loss of associated axons, with replacement by simple columnar ciliated respiratory epithelium. Atrophy and hyperplasia of Bowman's glands were also more severe at 300 ppm or greater. Atrophy was characterized by loss of Bowman's glands adjacent to the olfactory mucosa changes, and the normal glandular epithelium was replaced by hyperplastic epithelium characterized by increased numbers of cuboidal cells, distended with mucin, cell debris, and inflammatory cells. Hyaline degeneration of the respiratory epithelium was significantly increased in 150 ppm or greater females and was characterized by the accumulation of eosinophilic globules in the apical cytoplasm at the junction of the olfactory and respiratory epithelium on the nasal septum. There was no apparent exposure-related effect on the severity of this lesion. The accumulation of intracellular hyaline globules is commonly

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male						
Liver[a]	10	10	10	10	10	10
Hypertrophy, Centrilobular[b]	0	0	0	0	4* (1.0)[c]	10** (1.9)
Nose	10	10	10	10	10	10
Bowman's Glands, Atrophy,	0	7** (1.0)	10** (1.3)	10** (1.9)	10** (2.0)	10** (2.0)
Bowman's Glands, Hyperplasia	0	9** (1.1)	10** (1.6)	10** (2.3)	10** (2.9)	10** (2.7)
Olfactory Epithelium, Atrophy	0	10** (1.1)	10** (1.4)	10** (2.0)	10** (2.0)	10** (2.1)
Olfactory Epithelium, Metaplasia	0	5* (1.2)	10** (1.4)	10** (2.0)	10** (2.0)	10** (2.0)
Respiratory Epithelium, Hyaline Degeneration	0	1 (1.0)	2 (1.0)	1 (1.0)	2 (1.0)	0
Female						
Liver	10	10	10	10	10	10
Hypertrophy, Centrilobular	0	0	0	0	5* (1.0)	8** (1.6)
Nose	10	10	10	10	10	10
Bowman's Glands, Atrophy	0	8** (1.0)	9** (1.3)	10** (2.0)	10** (2.0)	8** (2.5)
Bowman's Glands, Hyperplasia	0	5* (1.0)	10** (1.7)	10** (2.3)	10** (2.6)	8** (2.6)
Olfactory Epithelium, Atrophy	0	10** (1.0)	10** (1.6)	10** (2.0)	10** (2.0)	8** (2.0)
Olfactory Epithelium, Metaplasia	0	4* (1.0)	9** (1.7)	10** (2.0)	10** (2.0)	8** (2.0)
Olfactory Epithelium, Necrosis	0	0	0	0	0	2 (3.0)
Respiratory Epithelium, Hyaline Degeneration	0	2 (2.0)	6** (1.3)	9** (1.6)	8** (1.4)	4* (1.0)

* 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

observed in inhalation studies. This response is considered an adaptive or protective response to inhalation of irritants.

Exposure Concentration Selection Rationale: At 1,000 ppm, final mean body weights were decreased 17% and 11% and mean body weight gains were decreased 43% and 32% in males and females, respectively. Moderate to severe sedation in males and ataxia in both sexes were observed at this exposure concentration. At 600 ppm, decreased final mean body weight and mean body weight gains were not as severe, and no clinical findings of toxicity were observed. The increased incidences and severity of nasal lesions observed in all exposed groups is a commonly observed response in the nose following chemical exposure. Based on these findings, the exposure concentrations selected for the 2-year inhalation study were 100, 300, and 600 ppm.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 3). Survival of all exposed male and female mice was similar to that of the chamber control groups.

Body Weights and Clinical Findings

Mean body weights of 600 ppm males were less than those of the chamber control groups throughout the study, and those of 600 ppm females were less after week 13 (Tables 17 and 18; Figure 4). The 300 ppm male and female mice had mean body weights that were less than those of the chamber controls during much of the study. Mean body weights were maximally decreased by 18% at 600 ppm and 12% at 300 ppm midway through the study. However, final body weights of the survivors were 99% and 95% of the final body weights of the chamber controls, respectively. No clinical findings related to chemical exposure were observed.

TABLE 16
Survival of Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	5	8	7	9
Natural deaths	10	10	3	5
Animals surviving to study termination	35	32	40	36
Percent probability of survival at end of study[a]	70	64	80	72
Mean survival (days)[b]	698	669	713	681
Survival analysis[c]	P=0.583N	P=0.566	P=0.285N	P=1.000N
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	0	1	0
Moribund	8	9	8	11
Natural deaths	3	3	4	2
Animals surviving to study termination	39	38	37	37
Percent probability of survival at end of study	78	76	76	74
Mean survival (days)	701	696	676	707
Survival analysis	P=0.766	P=0.960	P=0.937	P=0.819

[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 exposed group is indicated by **N**.
[d] Censored from survival analyses

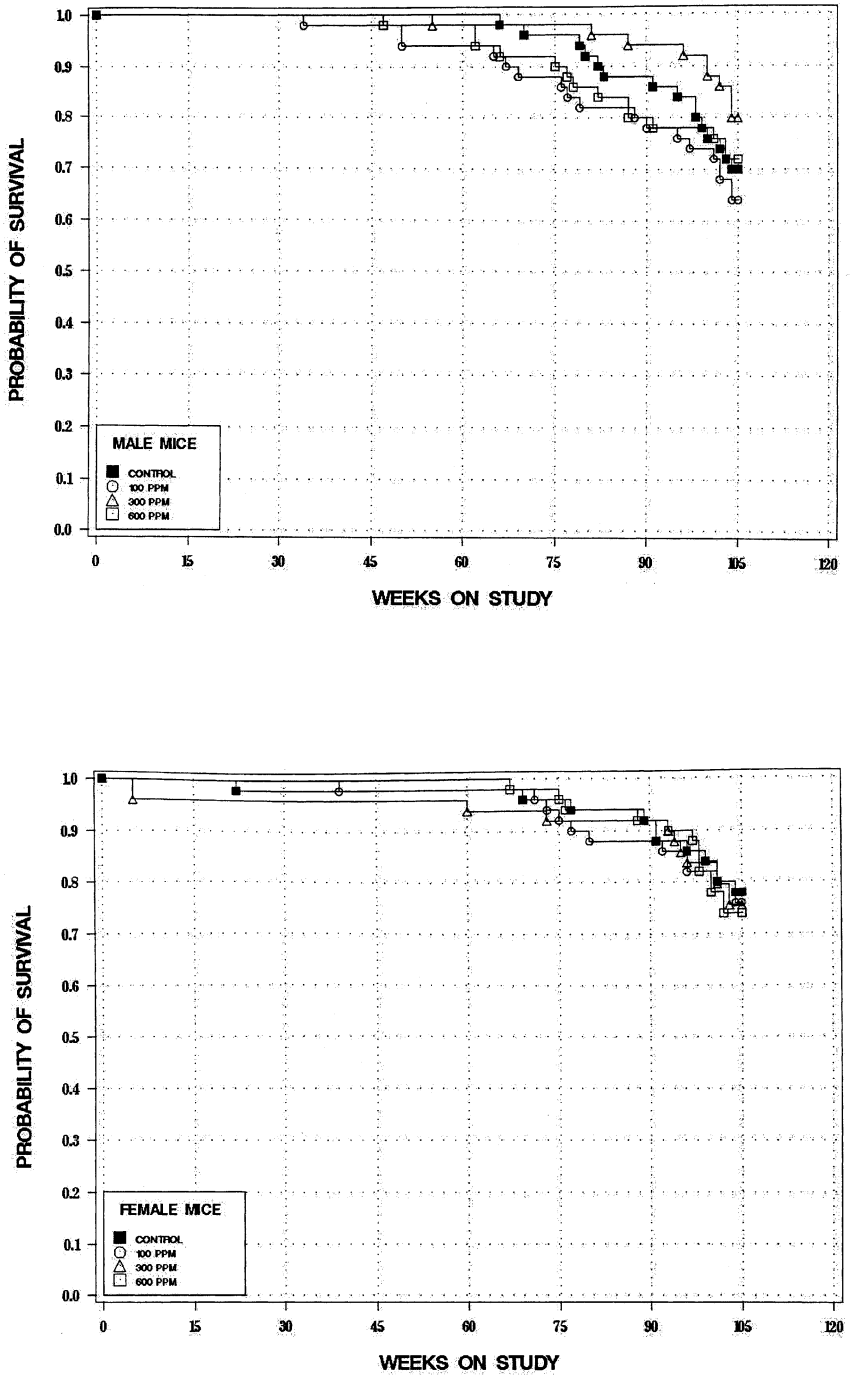


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to α-Methylstyrene for 2 Years

TABLE 17
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of α-Methylstyrene

Days on Study	Chamber Control		100 ppm			300 ppm			600 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	21.2	50	20.8	98	50	21.1	99	50	20.8	98	50
12	25.6	50	25.2	99	50	25.2	98	50	24.3	95	50
19	27.5	50	27.1	99	50	26.9	98	50	26.0	95	50
26	28.7	50	28.3	99	50	27.9	97	50	26.9	94	50
33	30.0	50	29.3	98	50	28.7	96	50	27.6	92	50
40	31.0	50	30.4	98	50	29.5	95	50	28.5	92	50
47	31.3	50	31.0	99	50	30.1	96	50	28.9	92	50
54	32.3	50	31.8	98	50	30.7	95	50	29.3	91	50
61	33.3	50	32.5	97	50	31.4	94	50	29.9	90	50
68	34.1	50	33.1	97	50	32.0	94	50	30.3	89	50
75	34.8	50	33.6	97	50	32.5	93	50	30.8	89	50
82	35.5	50	34.5	97	50	32.8	92	50	31.3	88	50
89	36.3	50	34.9	96	50	33.4	92	50	31.7	87	50
117	39.2	50	37.5	96	50	35.5	91	50	33.8	86	50
145	42.4	50	40.4	95	50	37.6	89	50	35.4	84	50
173	45.2	50	42.8	95	50	39.6	88	50	37.4	83	50
201	47.3	50	44.8	95	50	41.4	88	50	38.9	82	50
229	49.2	50	46.6	95	50	43.3	88	50	40.7	83	50
257	50.8	50	48.6	96	49	45.1	89	50	41.9	83	50
285	51.8	50	49.6	96	49	46.7	90	50	43.3	84	50
313	52.8	50	51.0	97	49	48.3	91	50	45.3	86	50
341	53.6	50	51.9	97	49	49.6	93	50	47.0	88	49
369	53.7	50	52.9	99	47	50.5	94	50	47.5	88	49
397	54.3	50	53.5	99	47	51.2	94	49	48.2	89	49
425	54.4	50	54.2	100	47	52.2	96	49	48.4	89	49
453	54.6	50	54.6	100	46	53.0	97	49	48.7	89	47
481	54.6	49	54.5	100	45	53.7	98	49	49.2	90	46
509	54.9	48	55.3	101	44	54.1	99	49	49.3	90	46
537	54.8	48	55.3	101	43	54.9	100	49	49.9	91	45
565	54.6	46	55.9	102	41	54.6	100	49	50.8	93	43
593	54.7	44	55.6	102	41	55.1	101	48	51.0	93	42
621	54.1	44	55.3	102	40	55.3	102	47	51.0	94	40
649	53.8	43	54.7	102	39	54.5	101	47	50.7	94	39
663	53.2	42	54.3	102	38	54.1	102	47	50.6	95	39
677	52.9	42	54.3	103	37	53.7	102	46	50.7	96	39
691	53.3	39	53.8	101	37	53.3	100	46	49.6	93	39
705	53.1	38	53.3	100	36	53.5	101	44	49.2	93	39
719	53.0	36	53.8	102	34	52.6	99	43	48.9	92	37
Mean for weeks											
1-13	30.9		30.2	98		29.4	95		28.2	92	
14-52	48.0		49.5	96		43.0	89		40.4	84	
53-103	54.0		54.5	101		53.5	99		49.6	92	

TABLE 18
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of α-Methylstyrene

Days on Study	Chamber Control		100 ppm			300 ppm			600 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	18.0	50	17.7	98	50	17.8	99	50	17.9	99	50
12	20.5	50	20.2	99	50	20.5	100	50	20.2	99	50
19	21.3	50	21.5	101	50	21.9	102	50	21.7	102	50
26	22.3	50	22.6	102	50	22.7	102	50	22.4	101	50
33	23.2	50	23.7	102	50	24.1	104	48	23.5	101	50
40	24.3	50	24.7	102	50	24.8	102	48	24.3	100	50
47	25.0	50	25.6	102	50	25.4	101	48	24.9	99	50
54	25.6	50	26.2	103	50	25.9	101	48	25.2	99	50
61	26.0	50	26.4	102	50	26.6	102	48	25.6	98	50
68	26.6	50	27.2	103	50	27.2	102	48	25.9	98	50
75	27.3	50	27.9	102	50	27.7	102	48	26.6	98	50
82	27.5	50	28.3	103	50	28.1	102	48	27.3	99	50
89	28.0	50	28.7	102	50	28.6	102	48	27.3	97	50
117	30.9	50	30.9	100	50	30.6	99	48	29.1	94	50
145	34.2	50	33.8	99	50	32.7	96	48	31.0	91	50
173	37.4	49	36.4	97	50	34.7	93	48	32.1	86	50
201	39.2	49	38.3	98	50	35.9	91	48	33.8	86	50
229	41.6	49	40.3	97	50	37.9	91	48	35.0	84	50
257	44.3	49	42.4	96	50	39.1	88	48	36.5	82	50
285	46.2	49	44.9	97	49	41.3	89	48	38.1	83	50
313	47.9	49	47.1	98	49	43.0	90	47	40.3	84	50
341	50.4	49	49.0	97	49	44.6	89	47	41.7	83	50
369	51.3	49	50.8	99	49	46.3	90	47	42.8	83	50
397	52.9	49	52.2	99	49	46.9	89	47	44.0	83	50
425	54.3	49	53.9	99	49	48.8	90	46	45.6	84	50
453	55.9	49	55.8	100	49	49.9	89	46	46.3	83	50
481	56.1	49	56.0	100	49	50.2	89	46	47.0	84	49
509	57.2	48	56.3	99	48	51.2	90	45	48.0	84	49
537	56.8	48	58.5	103	46	52.5	92	45	49.3	87	47
565	57.5	47	60.0	105	44	53.2	93	45	49.2	86	47
593	57.8	47	60.5	105	44	53.2	92	45	49.1	85	47
621	58.3	47	61.2	105	44	54.3	93	45	49.3	85	46
649	58.2	44	59.4	102	43	52.9	91	45	49.1	84	45
663	57.6	44	58.2	101	43	52.8	92	43	48.5	84	45
677	57.5	43	58.8	102	41	54.2	94	41	48.5	84	45
691	56.4	42	57.8	102	41	53.8	95	41	48.9	87	41
705	55.9	41	56.9	102	41	53.8	96	40	48.6	87	39
719	56.0	40	56.3	101	40	53.1	95	39	48.4	86	37
Mean for weeks											
1-13	24.3		24.7	102		24.7	102		24.1	99	
14-52	41.3		40.3	98		37.8	92		35.3	86	
53-103	56.2		57.0	101		51.7	92		47.7	85	

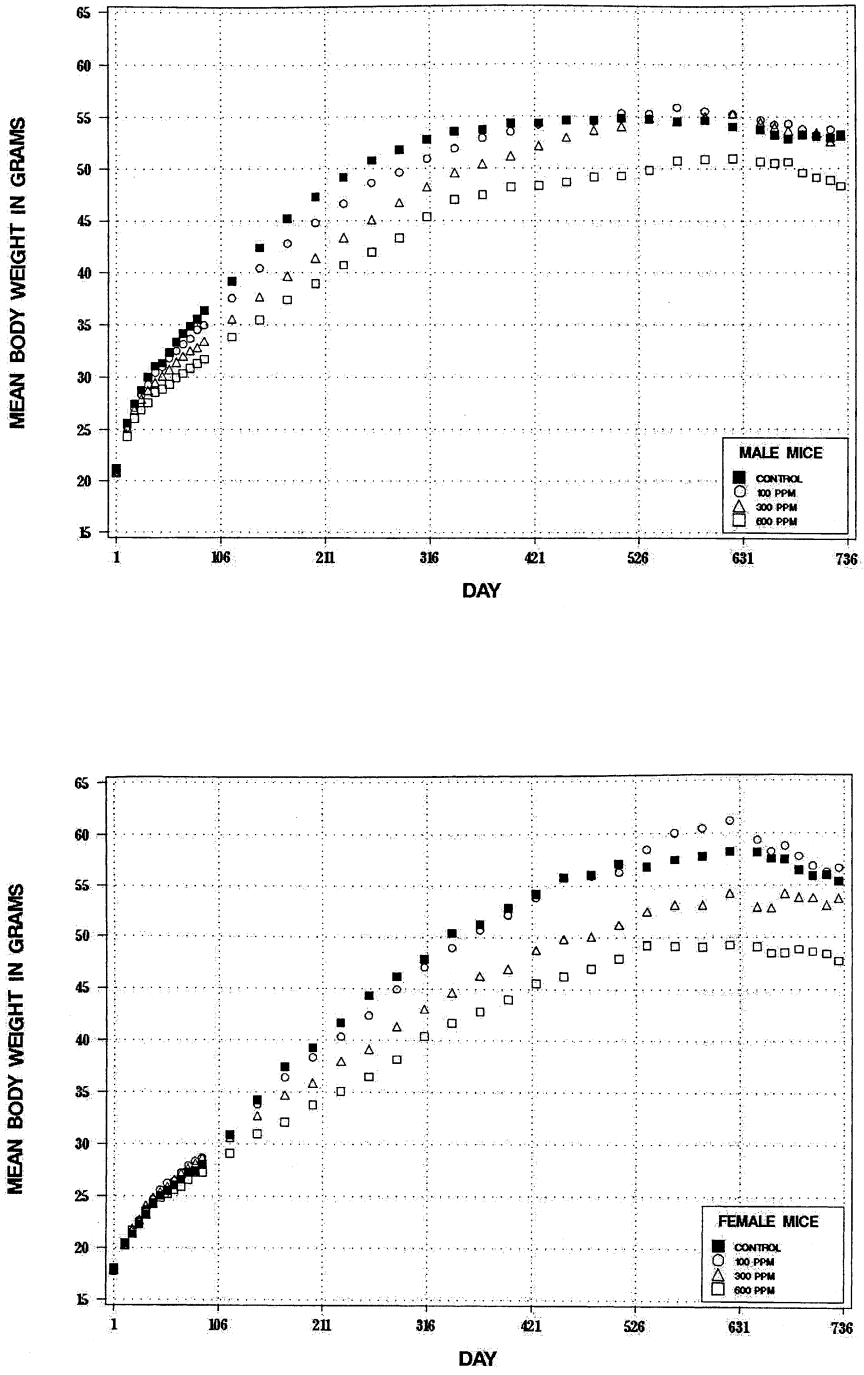


FIGURE 4
Growth Curves for Male and Female Mice Exposed to α-Methylstyrene by Inhalation for 2 Years

Pathology and Statistical Analyses

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

Liver: The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in the 100 and 600 ppm males and in all exposed groups of females (Tables 19, C1, C2, D1, and D2). The incidences of hepatocellular adenoma were significantly increased in all exposed groups of females. Significantly higher incidences of multiple hepatocellular adenomas were observed in the 300 and 600 ppm females. The incidence of hepatocellular carcinoma was significantly increased in 600 ppm females. The incidence of eosinophilic foci was significantly increased in 600 ppm females (Tables 19 and D4).

Microscopically, foci, hepatocellular adenoma, and hepatocellular carcinoma represent a continuum. These lesions had an appearance typical of that seen in $B6C3F_1$ mice. Eosinophilic foci were small to moderately large lesions composed of hepatocytes with eosinophilic cytoplasm; generally, these hepatocytes were enlarged (Plate 7). The hepatocytes were arranged in normal hepatic cords that merged with the surrounding normal hepatocytes. Most foci had little or no compression of the surrounding normal hepatocytes, although some degree of compression was present in some larger foci. Adenomas were discrete masses with distinct borders that caused compression of the surrounding normal hepatic parenchyma (Plate 8). Adenomas usually were composed of hepatocytes that appeared similar to those seen in eosinophilic foci, except that in adenomas, the normal lobular architecture was not apparent and plates of hepatocytes intersected the surrounding normal hepatocytes at sharp angles rather than merging with them as in foci. Carcinomas were discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding normal parenchyma. The neoplastic hepatocytes often were somewhat atypical, but the major distinguishing feature of carcinomas was the presence of abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly, the neoplastic cells formed glandular structures or solid masses (Plates 9 and 10).

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Male				
Number Examined Microscopically	50	50	50	50
Hepatocellular Adenoma, Multiple[a]	9	11	14	13
Hepatocellular Adenoma (includes multiple)[b]				
Overall rate[c]	24/50 (48%)	27/50 (54%)	27/50 (54%)	25/50 (50%)
Adjusted rate[d]	50.3%	59.7%	55.3%	55.3%
Terminal rate[e]	16/35 (46%)	19/32 (59%)	23/40 (58%)	22/36 (61%)
First incidence (days)	486	453	383	429
Poly-3 test[f]	P=0.453	P=0.238	P=0.385	P=0.389
Hepatocellular Carcinoma, Multiple	2	2	4	3
Hepatocellular Carcinoma (includes multiple)[g]				
Overall rate	10/50 (20%)	12/50 (24%)	11/50 (22%)	17/50 (34%)
Adjusted rate	21.2%	27.4%	22.7%	36.3%
Terminal rate	3/35 (9%)	5/32 (16%)	7/40 (18%)	8/36 (22%)
First incidence (days)	549	537	565	429
Poly-3 test	P=0.081	P=0.329	P=0.529	P=0.082
Hepatocellular Adenoma or Carcinoma[h]				
Overall rate	28/50 (56%)	36/50 (72%)	33/50 (66%)	37/50 (74%)
Adjusted rate	57.7%	77.4%	66.7%	76.7%
Terminal rate	17/35 (49%)	23/32 (72%)	26/40 (65%)	26/36 (72%)
First incidence (days)	486	453	383	429
Poly-3 test	P=0.093	P=0.031	P=0.239	P=0.035
Female				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus	2	5	7	12**
Hepatocellular Adenoma, Multiple	4	4	12*	16**
Hepatocellular Adenoma (includes multiple)[i]				
Overall rate	10/50 (20%)	20/50 (40%)	21/50 (42%)	23/50 (46%)
Adjusted rate	21.7%	43.9%	47.5%	48.7%
Terminal rate	9/39 (23%)	16/38 (42%)	21/37 (57%)	19/37 (51%)
First incidence (days)	725	640	731 (T)	464
Poly-3 test	P=0.014	P=0.018	P=0.007	P=0.005
Hepatocellular Carcinoma, Multiple	0	2	0	1
Hepatocellular Carcinoma (includes multiple)[j]				
Overall rate	3/50 (6%)	9/50 (18%)	6/50 (12%)	18/50 (36%)
Adjusted rate	6.5%	19.6%	13.1%	37.8%
Terminal rate	1/39 (3%)	6/38 (16%)	4/37 (11%)	11/37 (30%)
First incidence (days)	634	537	416	612
Poly-3 test	P<0.001	P=0.056	P=0.234	P<0.001

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Female (continued)				
Hepatocellular Adenoma or Carcinoma[k]				
Overall rate	13/50 (26%)	26/50 (52%)	24/50 (48%)	33/50 (66%)
Adjusted rate	27.9%	56.0%	52.5%	68.0%
Terminal rate	10/39 (26%)	20/38 (53%)	22/37 (60%)	24/37 (65%)
First incidence (days)	634	537	416	464
Poly-3 test	P<0.001	P=0.004	P=0.012	P<0.001

(T) Terminal sacrifice
* 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] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 134/350 (38.3% ± 6.3%); range, 30%-46%
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] 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.
[g] Historical incidence: 85/350 (24.3% ± 4.8%); range 18%-32%
[h] Historical incidence: 196/350 (56.0% ± 6.2%); range 50%-68%
[i] Historical incidence: 78/347 (22.5% ± 8.1%); range 12%-35%
[j] Historical incidence: 37/347 (10.7% ± 1.8%); range 8%-12%
[k] Historical incidence: 108/347 (31.1% ± 6.8%); range 22%-39%

Nose: The incidences of metaplasia of the olfactory epithelium and hyperplasia of the submucosal glands were significantly increased in all exposed groups of males and females compared to chamber controls (Tables 20, C4, and D4). In addition, atrophy of the olfactory epithelium was significantly increased in 300 and 600 ppm males. Metaplasia of the olfactory epithelium involved the dorsal meatus of nasal Levels II and III (Plates 11 and 12) and the upper one-third to one-half of the nasal septum in Level III (Plates 13 and 14), as well as the tips of ethmoturbinates adjacent to the affected septum. Metaplasia was characterized by replacement of the pseudostratified columnar olfactory epithelium with a single layer of ciliated columnar respiratory epithelium, and there was also loss of underlying nerve bundles and Bowman's glands. Other areas of Level III sometimes had fewer neuronal cells in the olfactory mucosa. Normal Bowman's glands were lost and replaced by hyperplastic glands that were usually dilated, lined by ciliated columnar epithelium, and filled with cell debris, mucus, inflammatory cells, or proteinaceous material.

Kidney: The incidence of nephropathy was increased in 600 ppm females compared to chamber controls (Tables 20 and D4). Nephropathy was characterized by the presence of focal to multifocal cortical tubules which had cytoplasmic basophilia, nuclear crowding, and thickened basement membranes. Also as the severity of nephropathy increased, thickening and hypercellularity of the glomerular tufts and infiltration of mononuclear cells around affected tubules was observed. Nephropathy is a spontaneously occurring lesion in the $B6C3F_1$ mouse, which is generally more severe in males than females. The greater incidence of nephropathy in 600 ppm females was considered related to α-methylstyrene.

Forestomach: The incidences of epithelial hyperplasia were significantly increased in 300 and 600 ppm males compared to chamber controls; chronic inflammation often was associated with the hyperplasia (Tables 20 and C4). Hyperplasia was a focal change characterized by an increase in the number of cell layers in the epithelium. α-Methylstyrene was most likely ingested during

TABLE 20
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Male				
Nose[a]	50	50	50	50
Olfactory Epithelium, Metaplasia[b]	6 (1.2)[c]	47** (2.7)	49** (3.0)	49** (3.0)
Olfactory Epithelium, Glands, Hyperplasia	4 (1.0)	50** (2.8)	50** (3.0)	50** (3.1)
Olfactory Epithelium, Atrophy	0	2 (2.5)	8** (1.8)	12** (1.7)
Stomach, Forestomach	50	49	49	48
Epithelium, Hyperplasia	1 (2.0)	4 (2.5)	7* (2.3)	11** (2.0)
Inflammation	0	2 (2.0)	1 (2.0)	5* (2.0)
Adrenal Cortex	50	49	49	50
Hypertrophy	25 (2.0)	13* (2.0)	14* (1.7)	13* (2.0)
Female				
Nose	49	49	50	50
Olfactory Epithelium, Metaplasia	2 (1.0)	49** (2.7)	47** (3.0)	50** (3.0)
Olfactory Epithelium, Glands, Hyperplasia	3 (1.0)	49** (2.9)	50** (2.9)	50** (3.0)
Olfactory Epithelium, Atrophy	1 (1.0)	6 (1.2)	4 (1.5)	3 (1.0)
Kidney	50	49	50	50
Nephropathy	16 (1.1)	21 (1.3)	12 (1.0)	26* (1.6)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 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

grooming and consequently resulted in hyperplasia of the forestomach epithelium.

Adrenal Gland: The incidences of hypertrophy of the adrenal cortex were significantly decreased in all exposed groups of males compared to chamber controls (Tables 20 and C4). The severity was mild across control and exposed groups. Hypertrophy is not considered a preneoplastic change and might reflect a functional change due to α-methylstyrene exposure.

Metastatic Neoplasms: The metastatic neoplasms in the α-methylstyrene study were due primarily to hepatocellular carcinomas in 600 ppm female mice which metastasized to the lung, a common site of metastasis of liver tumors (chamber control, 1/50; 100 ppm, 5/50; 300 ppm, 3/50; 600 ppm, 13/50; Table D1). The metastatic liver neoplasms in females were consistent with the evidence of carcinogenic activity and reflect the malignant behavior of these neoplasms where there is a progression of hepatocellular neoplasms from benign adenomas to malignant carcinomas that often metastasize. The only other metastatic neoplasms which were relevant to α-methylstyrene were two renal carcinomas that metastasized to the lung in 1,000 ppm male rats (Table A1).

GENETIC TOXICOLOGY

α-Methylstyrene tested over a concentration range of 1 to 3,333 µg/plate was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, in either the presence or the absence of 10% or 30% rat or hamster liver S9 metabolic activation enzymes (Table E1; Zeiger *et al.*, 1992). In cytogenetic tests with cultured Chinese hamster ovary cells, α-methylstyrene significantly increased the frequency of sister chromatid exchanges (SCEs) in cells exposed to concentrations of 50 to 149.9 µg/mL in the presence of S9 (Table E2); without S9, no significant increases in SCEs were observed. α-Methylstyrene in concentrations up to 251.3 µg/mL did not induce chromosomal aberrations (Table E3), with or without S9 activation. *In vivo*, no significant increases in the frequencies of micronucleated erythrocytes were seen in blood samples of male mice obtained at the conclusion of the 3-month exposure study. However, in female mice from the 3-month study, a significant increase in micronucleated normochromatic erythrocytes was observed at the highest exposure concentration of 1,000 ppm (Table E4), resulting in a negative call for male mice and a positive call in this assay for female mice. Reticulocytes (polychromatic immature erythrocytes; PCEs) were also scored for frequency of micronucleated cells in male and female mice. No increase in micronucleated PCEs was observed in either sex at the highest exposure concentration of 1,000 ppm, indicating that the damage observed in the mature erythrocyte population in 1,000 ppm females was reflective of long-term accumulation of damage and was not detectable immediately after exposure by analyzing recently-formed (within 48 hours) reticulocytes.

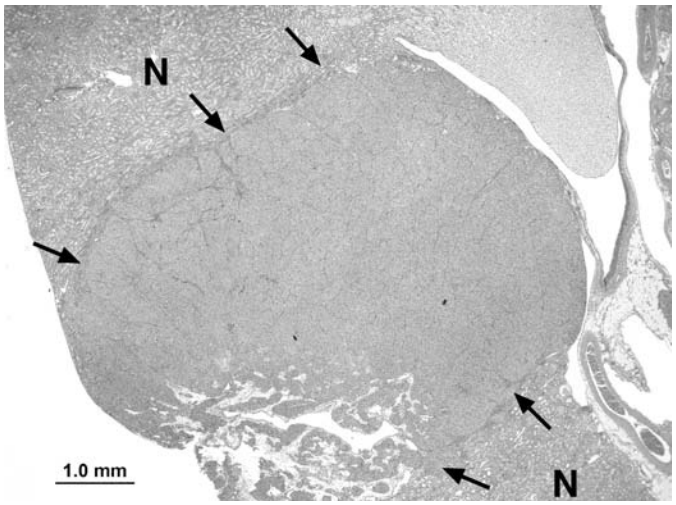


PLATE 1
Renal tubule adenoma in a male F344/N rat exposed to 300 ppm α-methylstyrene by inhalation for 2 years. Note the well circumscribed, discrete mass of neoplastic cells (arrows) that has displaced the normal renal parenchyma (N). H&E; 2×

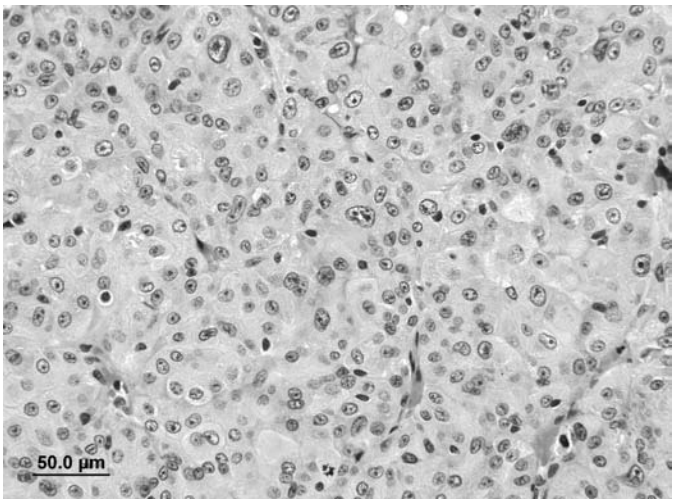


PLATE 2
Higher magnification of the renal tubule adenoma in Plate 1. Note the neoplastic cells are arranged in clusters and contain vesicular nuclei with prominent nucleoli and abundant eosinophilic cytoplasm. H&E; 40×

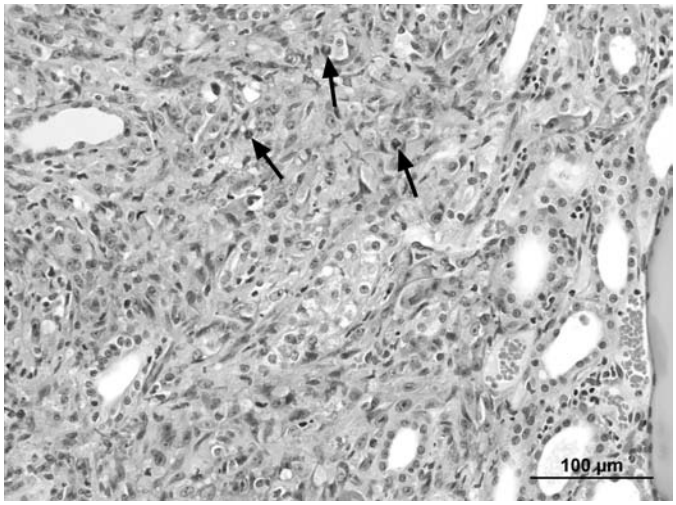


PLATE 3
Renal tubule carcinoma in a male F344/N rat exposed to 1,000 ppm α-methylstyrene by inhalation for 2 years. Note the cellular atypia and prominent mitotic figures (arrows). Many of the nuclei are hyperchromatic and vary in size and shape. H&E; 32×

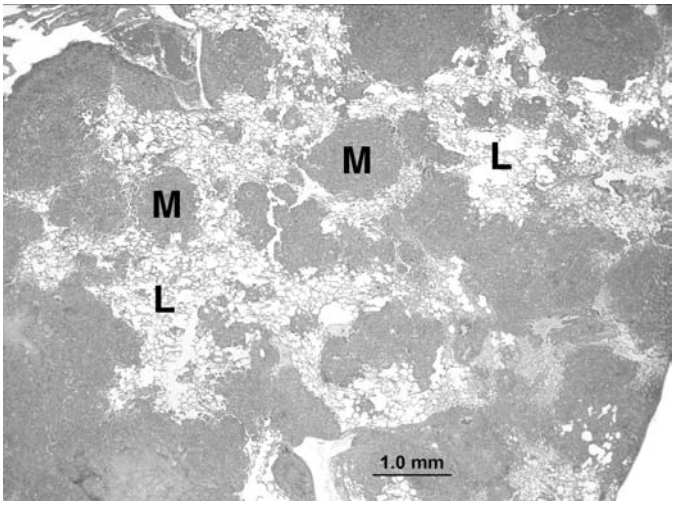


PLATE 4
Renal tubule carcinoma from Plate 3. Note the multifocal metastasis (M) to the lung (L). H&E; 2×

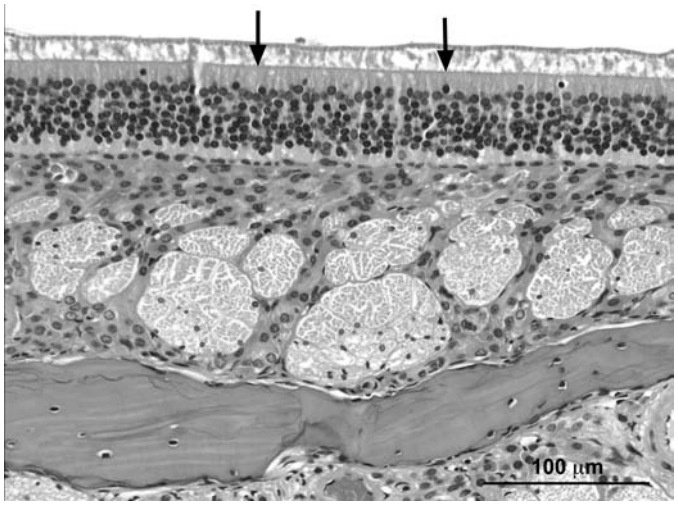


PLATE 5
Olfactory epithelium in a male chamber control F344/N rat from the 2-year study of α-methylstyrene. Note that the normal epithelium is pseudostratified columnar (arrows) consisting of sustentacular, sensory, and a single row of basal cells. H&E; 32×

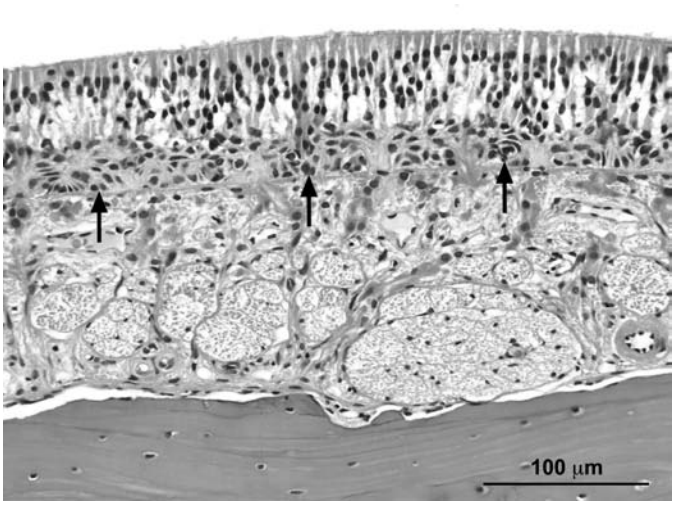


PLATE 6
Olfactory epithelium degeneration in a male F344/N rat exposed to 1,000 ppm α-methylstyrene by inhalation for 2 years. Note the basal cell hyperplasia (arrows) where there is crowding of basal cells along the basement membrane of the olfactory epithelium. Also note the olfactory epithelium with vacuolation and loss of cells within the epithelium. H&E; 32×

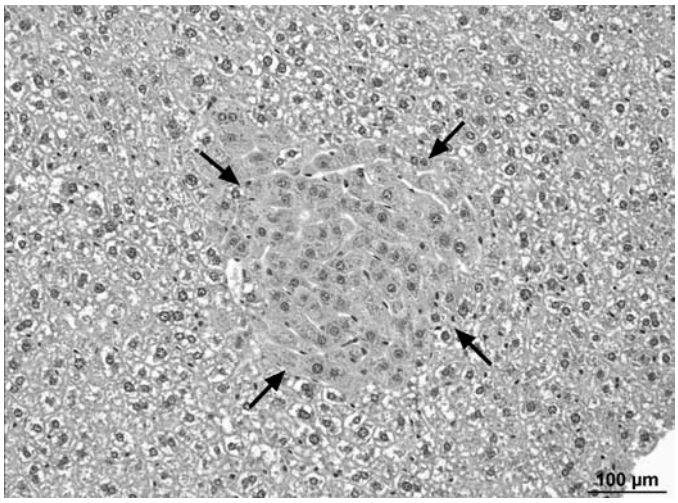


PLATE 7
Eosinophilic focus in a female $B6C3F_1$ mouse exposed to 600 ppm α-methylstyrene by inhalation for 2 years. Note the focal lesion (arrows) consists of enlarged hepatocytes with eosinophilic cytoplasm that merge with surrounding normal hepatocytes. H&E; 25×

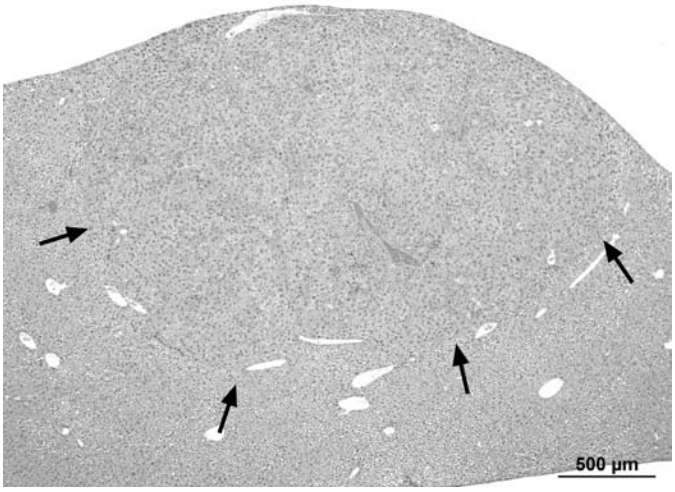


PLATE 8
Hepatocellular adenoma in a female $B6C3F_1$ mouse exposed to 600 ppm α-methylstyrene by inhalation for 2 years. The adenoma (arrows) is well circumscribed, occupies numerous hepatic lobules, and causes compression of the adjacent hepatocytes. H&E; 5×

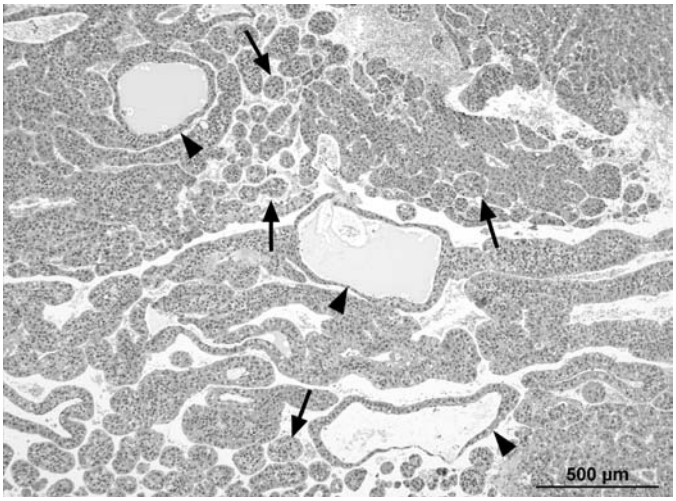


PLATE 9
Hepatocellular carcinoma in a female B6C3F$_1$ mouse exposed to 600 ppm α-methylstyrene by inhalation for 2 years. Note the normal liver is replaced by malignant hepatocytes that form trabeculae (arrows) and glandular structures (arrowheads). H&E; 6.4×

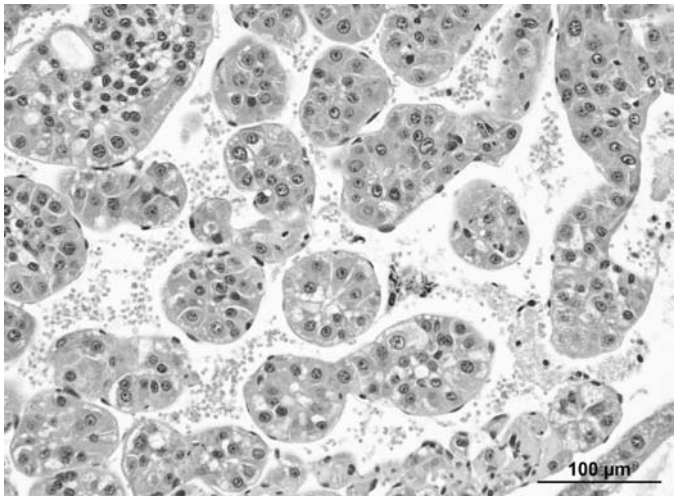


PLATE 10
Higher magnification of Plate 9 showing trabeculae of neoplastic hepatocytes that were three to eight cell layers thick. H&E; 32×

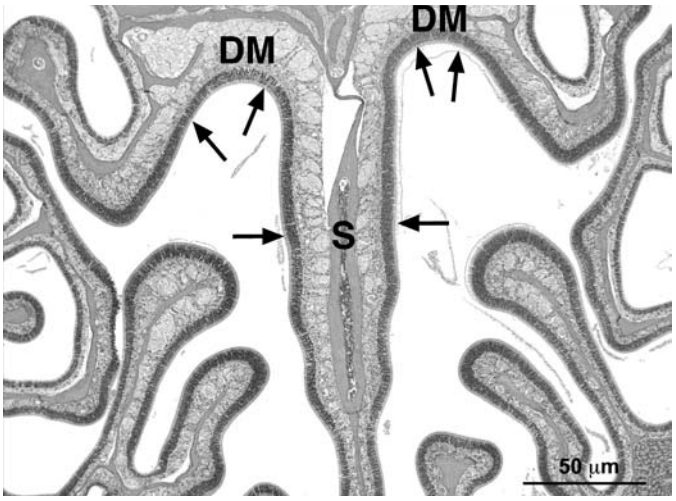


PLATE 11
Olfactory epithelium of Level III of the nose in a male chamber control B6C3F$_1$ mouse from the 2-year inhalation study of α-methylstyrene. Note that the normal olfactory epithelium (arrows) of the dorsal meatus (DM) and nasal septum (S) is pseudostratified columnar. H&E; 6.4×

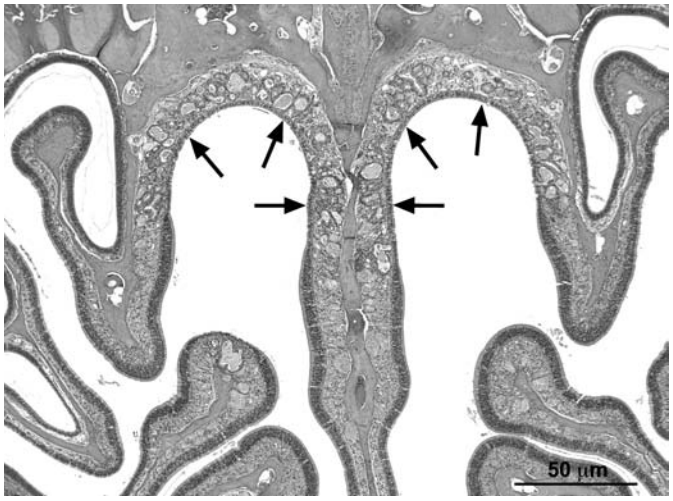


PLATE 12
Level III of the nose in a male B6C3F$_1$ mouse exposed to 100 ppm α-methylstyrene by inhalation for 2 years. Note that the olfactory epithelium is replaced by ciliated columnar epithelium (metaplasia) in the dorsal meatus (arrows) and upper part of the nasal septum (arrows). Also note the prominent hyperplastic glands in the submucosa of the dorsal meatus when compared to Plate 11. H&E; 6.4×

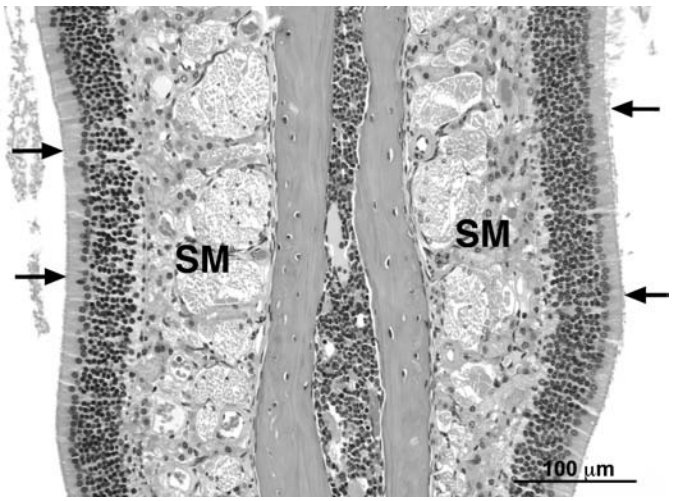


Plate 13
Higher magnification of Plate 11. Note the normal pseudostratified olfactory epithelium (arrows) and the underlying submucosa (SM). H&E; 32×

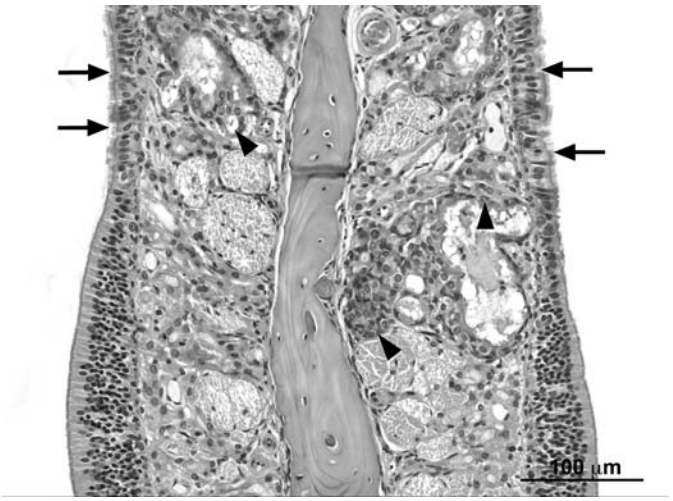


Plate 14
Higher magnification of Plate 12. Note the replacement of the olfactory epithelium by ciliated columnar epithelium (arrows). Also note the prominent hyperplastic glands in the submucosa (arrowheads). H&E; 32×

DISCUSSION AND CONCLUSIONS

α-Methylstyrene was nominated by the U.S. Environmental Protection Agency for toxicological evaluation and genotoxicity studies based on its high production volume and limited toxicological information. The effects of whole-body inhalation exposure to α-methylstyrene for 3 months or 2 years were studied in male and female F344/N rats and B6C3F$_1$ mice.

In the 3-month study, the kidney was the primary target organ in rats. Increased kidney weights were observed at 600 ppm in females and at 1,000 ppm in both sexes. In males, hyaline droplet accumulation in the renal proximal tubules occurred in chamber control and all exposed groups with larger and more irregularly shaped hyaline droplets observed at higher exposure concentrations. Increased renal cell proliferation (as determined by labeling index) and α2u-globulin concentrations were observed at 150 ppm or greater. Exposure concentration-related increases in urine markers for kidney toxicity were observed primarily in the 300 ppm or greater males and the 600 ppm or greater females. These findings are suggestive of α2u-globulin-mediated nephropathy in male rats.

Increased kidney weights and increased urine markers of kidney toxicity were also observed in female rats, which are not susceptible to developing α2u-globulin nephropathy. Therefore, mechanisms independent of α2u-globulin-mediated nephropathy may contribute to the observed effects in the kidney. In contrast to rats, renal toxicity was not observed in mice exposed to α-methylstyrene for 3 months.

In the 3-month studies, absolute and relative liver weights were increased in male and female rats and mice. In rats, an exposure concentration-related increase in serum bile acid concentrations was observed in both sexes; this increase could suggest a cholestatic or hepatocellular effect. However, serum alkaline phosphatase activity, another marker of cholestasis, was unaffected, and serum alanine aminotransferase and sorbitol dehydrogenase activities, which are typically increased with hepatocellular injury, were either unaffected or decreased. In mice, corresponding increases in the incidences of centrilobular hypertrophy were observed at 600 and 1,000 ppm. No exposure-related changes in histopathology were observed in the liver of rats. These effects on liver weights are consistent with results from a 12-day inhalation study of α-methylstyrene in B6C3F$_1$ mice and F344/N rats (Morgan *et al.*, 1999); similar results have also been observed in oral and inhalation studies of styrene, a structurally related compound to α-methylstyrene (Wolf *et al.*, 1956; ATSDR, 1992). However, these effects were not observed in more recent chronic inhalation studies in CD rats and CD-1 mice (Cruzan *et al.*, 1998, 2001). Inhalation exposure to styrene in rodents increases hepatic cytochrome P450 content (Vainio *et al.*, 1979). The current findings of increased liver weights and the incidences of centrilobular hypertrophy in mice are consistent with a similar effect of styrene on the expression of hepatic cytochrome P450. However, no information is available on the effects of α-methylstyrene on hepatic cytochrome P450 expression.

In general, mice were more sensitive than rats to the effects of α-methylstyrene in the 3-month studies. In rats, there were no treatment-related effects on survival, final mean body weights, mean body weight gain, or clinical findings. In mice, survival of the 1,000 ppm females was somewhat less than that of chamber controls, and moderate to severe sedation in males and ataxia in both sexes were observed at 1,000 ppm. Final mean body weights and mean body weight gains were decreased in males at 600 ppm or greater. In females, final mean body weights were decreased at 75, 300, and 1,000 ppm, and mean body weight gains were decreased at 300 ppm or greater. The nose was the primary target site in mice. Lesions in the nasal cavity included atrophy and metaplasia of the olfactory epithelium and atrophy and hyperplasia of the Bowman's glands. These lesions were observed in all exposed groups, with an increased severity at 300 ppm or greater. Significantly increased incidences of hyaline degeneration of the respiratory epithelium were observed in females at 150 ppm or greater. The olfactory epithelium is

sensitive to toxicants, and these types of lesions are commonly observed adaptive or protective nasal changes following chemical exposure (Buckley *et al.*, 1985; Boorman *et al.*, 1990).

The only significant reproductive effect in the 3-month studies was an exposure concentration-dependent increase in the length of the estrous cycle in female mice. Estrous cycle characterization through vaginal cytology demonstrated that the increase in cycle length was primarily due to a prolongation of the estrous phase with a slightly decreased diestrous period. These results suggest that α-methylstyrene is a potential reproductive toxicant in female mice. In male mice, decreased epididymal weights were observed, but no histopathology was seen in the reproductive tract that might support classification of α-methylstyrene as a potential reproductive toxicant in male mice. In rats, α-methylstyrene exhibited no reproductive toxicity potential in males or females. Although no reproductive toxicology information is available in the literature on α-methylstyrene, a single study in male rats administered styrene demonstrated altered testicular function (Srivastava *et al.*, 1989). However, other studies of styrene indicated that it is not a reproductive toxicant (Murray *et al.*, 1978; Beliles *et al.*, 1985; Salomaa *et al.*, 1985).

In the current 2-year rat study, age-related chronic nephropathy occurred in most chamber control and exposed rats. This is a spontaneous finding in older F344/N rats that is generally more commonly observed with a greater severity in males than in females (Montgomery and Seely, 1990). In the current study, there was also an increased incidence of papilla mineralization in males at 1,000 ppm and females at 300 and 1,000 ppm. In the standard single-section evaluation of the male rat kidney, one renal tubule adenoma and one renal tubule carcinoma were observed at 300 ppm, and two renal tubule carcinomas were observed at 1,000 ppm. In a subsequent extended evaluation of kidney step sections, additional renal tubule adenomas were identified in the chamber control and exposed groups. The final combined incidence of renal tubule adenomas and carcinomas was significantly increased in the 1,000 ppm males compared to chamber controls. There was an exposure concentration-related trend in the combined incidences of renal tubule adenoma and carcinoma, and the incidences in all exposed groups exceeded the historical ranges for chamber controls. The increased incidences of renal tubule neoplasms, combined with the effects observed in the 3-month study, suggest that the induction of renal tubule adenomas and carcinomas in male rats may be mediated through an α2u-globulin-associated mechanism.

α-Methylstyrene meets some, but not all, of the required criteria established by the International Agency for Research on Cancer (1999) and the U.S. Environmental Protection Agency (1991) for male rat kidney carcinogenicity through an α2u-globulin-associated response. α-Methylstyrene is a nongenotoxic compound that induced a male-specific kidney neoplasm response in the 2-year study and increased severity of hyaline droplet accumulation with increased concentrations of α2u-globulin in the 3-month study. The increased incidences of renal tubule neoplasms in the 2-year study did not correlate with increased incidences of renal cell proliferation, the incidence or severity of hyaline droplet accumulation, and the accumulation of α2u-globulin. A significant increase in the incidence of renal tubule adenoma or carcinoma (combined) was observed only in the 1,000 ppm males. However, similar effects were observed at 300, 600, and 1,000 ppm for renal cell proliferation (as determined by labeling index), the incidence or severity of hyaline droplet accumulation, and the accumulation of α2u-globulin. No other hallmark effects of α2u-globulin-related nephropathy were observed in male rats in either the 3-month or 2-year studies. Additionally, there is currently no information available characterizing the binding of α-methylstyrene or any of its metabolites to α2u-globulin. The current findings, which are suggestive of weak α2u-globulin-mediated effects, are consistent with the findings of the NTP studies on propylene glycol mono-*t*-butyl ether (NTP, 2004a) and Stoddard Solvent IIC (NTP, 2004b). Propylene glycol mono-*t*-butyl ether induced dose-dependent increases in the severity of nephropathy; the incidences of hyaline droplet accumulation, mineralization of the renal papilla, and renal tubule hyperplasia; and α2u-globulin accumulation but did not affect the incidences of renal tubule neoplasms (NTP, 2004a). Similarly, Stoddard Solvent IIC induced renal tubule hyperplasia, chronic nephropathy, and mineralization of the renal papilla but had no effect on the incidence of renal tubule neoplasms (NTP, 2004b). In contrast, decalin and *d*-limonene, two compounds that have been demonstrated to interact with α2u-globulin to induce nephropathy and renal neoplasms, induced characteristic renal nonneoplastic lesions that were more severe than in the current study (NTP 1990, 2005). In those studies,

increased incidences and severity of renal tubule hyperplasia, regeneration, and granular casts of the renal medulla were also observed. The incidences of renal tubule adenoma or carcinoma (combined) were greater in those studies than in the current study. Additionally, the kidney effects observed in the current studies were not limited to male rats. Urinalysis demonstrated exposure concentration-related changes indicative of nephrotoxicity in female rats similar to those observed in males. In the 2-year mouse study, there was a significant increase in the incidence of nephropathy in the 600 ppm females compared to chamber controls. These findings suggest that if the kidney neoplasms are induced by α-methylstyrene through an α2u-globulin-associated mechanism in male rats, it is a weak response compared to other compounds that induce nephropathy and renal neoplasms through an α2u-globulin-associated mechanism. The effects in female rats and mice suggest that another mechanism for nephrotoxicity may be involved.

The incidence of mononuclear cell leukemia in 1,000 ppm male rats was significantly increased compared to chamber controls. An overall exposure concentration-related trend was observed; however, only the incidence in 1,000 ppm males (76%) exceeded the historical control incidence (32% to 66%). Mononuclear cell leukemias are also common neoplasms in F344/N rats. Thus, the increased incidence of mononuclear cell leukemia was considered an equivocal finding that may have been related to α-methylstyrene exposure.

In the 2-year rat study, the incidences of adenoma of the testis were increased in all exposed groups of males compared to chamber controls. Since the incidences only slightly exceeded the range in historical controls and this lesion is one of the most common in the F344/N rat, this finding was considered incidental. Similar findings were observed in male CD rats in a chronic inhalation study with styrene (Cruzan *et al.*, 1998). In that study, an increased trend in the incidences of testicular interstitial cell adenomas was observed. However, the finding was considered incidental since there was a lack of statistical significance by pairwise comparison to control incidences, the incidences were within historical control range, and no associated nonneoplastic lesions were observed.

In the current 2-year mouse study, the incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) in females occurred with positive trends, and the incidences of these lesions were significantly increased in all exposed groups of females. The incidences of hepatocellular carcinoma in female mice were greater than that in chamber controls at all exposure concentrations and was significantly increased at 600 ppm. Exposed females had greater incidences of eosinophilic foci compared to chamber controls, with a significant increase observed at 600 ppm. These results demonstrate a neoplastic response to α-methylstyrene in the liver of female mice and are consistent with the continuum that is generally believed to exist in the progression from preneoplastic altered hepatocellular foci to benign hepatocellular adenomas to malignant carcinomas.

Male mice exposed to 100 and 600 ppm for 2 years had significant increases in the incidences of hepatocellular adenoma or carcinoma (combined). No significant increased incidences were observed for either hepatocellular adenoma or hepatocellular carcinoma when evaluated individually. The incidences in all exposed groups were greater than the respective chamber controls, with the greatest incidence of carcinoma being observed in the 600 ppm males. Therefore, the increase in the incidences of hepatocellular adenoma or carcinoma (combined) was primarily due to the incidences of heptocellular carcinoma. No eosinophilic foci were observed in males. These findings suggest that increased incidences of hepatocellular adenoma or carcinoma (combined) may be related to α-methylstyrene exposure.

In the current 2-year study, an increased incidence of nephropathy was observed in female mice at 600 ppm. There was no exposure concentration-related increase in either the incidence or severity of this lesion. However, this finding in the female mouse kidney further suggests that there may be a mechanism independent of α2u-globulin contributing to the renal effects of α-methylstyrene.

In the current 2-year studies, nonneoplastic lesions of the olfactory epithelium of the nose were noted in rats and mice. Exposed male and female rats had increased incidences of olfactory epithelium degeneration and hyperplasia of the basal cells. Exposed male and female mice had increased incidences and severity of olfactory epithelium metaplasia and hyperplasia of the olfactory epithelium glands. Exposed male mice had increased incidences of atrophy of the olfactory epithelium. As previously mentioned, these types of nasal lesions are commonly observed adaptive or protective responses following chemical exposure, especially irritants. These

lesions are consistent with the known effects of α-methylstyrene as a nasal irritant (NIOSH, 2003; ACGIH, 2005). Additionally, similar atrophic and degenerative changes of the olfactory epithelium and underlying Bowman's glands have been observed in rats and mice following exposure to styrene (Cruzan *et al.*, 1997, 1998, 2001), which is also an upper respiratory tract irritant (Stewart *et al.*, 1968; Alarie, 1973; Ohashi *et al.*, 1986).

In the disposition and metabolism studies of α-methylstyrene (De Costa *et al.*, 2001; Appendix M), F344/N rats were exposed to α-methylstyrene via intravenous or nose-only inhalation exposure. In both studies, α-methylstyrene was eliminated primarily in the urine (approximately 90%) within 72 hours, with volatile breath and feces accounting for only a small amount (1% to 3%) of elimination. In the inhalation study, the elimination half-life was calculated at 3 to 5 hours, with the highest concentrations of α-methylstyrene-derived radioactivity retained in the adipose tissue, urinary bladder, liver, kidney, and skin. Following intravenous dosing, the kidney, heart, lung, liver, urinary bladder, and spleen retained the highest concentrations of radioactivity. In both the intravenous study and the inhalation study, the major urinary metabolites of α-methylstyrene were the glucuronide conjugate of 2-phenyl-1,2-propanediol and atrolactic acid. In the inhalation study, the major metabolites in the blood were 2-phenyl-1,2-propanediol and 2-phenylpropionic acid. Based on these studies, the proposed metabolic pathway for α-methylstyrene involves an initial non-stereoselective epoxidation followed by hydrolysis to form 2-phenyl-1,2-propanediol followed by either oxidation to atrolactic acid or formation of the glucuronide conjugate, conjugation with glutathione and subsequent cleavage to the mercapturate, or rearrangement to form an aldehyde that is oxidized to yield 2-phenylpropionic acid. The dose-dependent pharmacokinetic parameters coupled with decreased excretion of 2-phenyl-1,2-propanediol glucuronide at 900 ppm indicate that glucuronide formation was saturated at this dose.

A physiologically based pharmacokinetic (PBPK) model was developed to describe the absorption, distribution, metabolism, and elimination of α-methylstyrene in male F344/N rats (Appendix L). The PBPK model has compartments representing the amounts of α-methylstyrene and metabolite in the adipose tissue, liver, kidney, and slowly- and rapidly-perfused tissues. The major assumptions of the model were that the liver is the only metabolizing tissue and that metabolism, absorption, and urine elimination are first-order processes. An alternative model with saturable metabolism was considered, but it was not statistically different from the model with linear metabolism. The majority of the data used to estimate unknown model parameters were from total radioactivity studies, making it difficult to identify any processes that may be saturable for α-methylstyrene. The model demonstrates that the absorption, distribution, metabolism, and excretion of α-methylstyrene-derived radioactivity is linear. However, the model with saturable metabolism had identical predictions as the model with linear metabolism.

Although α-methylstyrene differs from styrene only in the presence of a methyl group on the α carbon, overall, α-methylstyrene did not elicit similar toxicity or carcinogenic effects. Chronic carcinogenicity studies have demonstrated that styrene is not carcinogenic in rats (Maltoni *et al.*, 1982; Conti *et al.*, 1988; ATSDR, 1992; Cruzan *et al.*, 1998) but induces lung tumors in mice (NCI, 1979; Cruzan *et al.*, 2001). In the current study of α-methylstyrene, increased incidences of neoplasms were observed in the male rat kidney and female mouse liver. No exposure-related neoplasms of the lung were observed. Increased kidney weights, as were observed in the current α-methylstyrene study, have been reported in female rats orally administered styrene for 6 months (Wolf *et al.*, 1956) but not in a more recent chronic inhalation study in CD rats and CD-1 mice (Cruzan *et al.*, 1998, 2001). Styrene has been reported to induce minor effects on the kidney in studies involving both inhalation and oral exposures (Vainio *et al.*, 1979; Das *et al.*, 1983; Viau *et al.*, 1987). However, these findings differ from the current findings for α-methylstyrene, which demonstrate moderate renal toxicity primarily involving a weak α2u-globulin response. Additionally, changes in urinalysis indicating renal tubule toxicity observed in the current study were not observed in subchronic or chronic inhalation studies with styrene (Cruzan *et al.*, 1997, 1998). The mechanism for the differences in target organ toxicity between these two structural analogues is not clearly understood. While both the lungs and the kidneys express biotransformation enzymes, the reactive styrene epoxide likely reacts quickly with lung macromolecules to produce localized toxic effects. The methyl group may stabilize the α-methylstyrene epoxide reactive intermediate allowing it time to distribute to the kidney before reacting with renal macromolecules. Alternately, the α-methyl group may prevent significant pulmonary metabolism but may cause renal toxicity resulting from increased concentration in the urine.

CONCLUSIONS

Under the conditions of this 2-year inhalation study, there was *some evidence of carcinogenic activity** of α-methylstyrene in male F344/N rats based on increased incidences of renal tubule adenomas and carcinomas (combined). The increased incidence of mononuclear cell leukemia in 1,000 ppm male F344/N rats may have been related to α-methylstyrene exposure. There was *no evidence of carcinogenic activity* of α-methylstyrene in female F344/N rats exposed to 100, 300, or 1,000 ppm. There was *equivocal evidence of carcinogenic activity* of α-methylstyrene in male $B6C3F_1$ mice based on marginally increased incidences of hepatocellular adenoma or carcinoma (combined). There was *clear evidence of carcinogenic activity* of α-methylstyrene in female $B6C3F_1$ mice based on increased incidences of hepatocellular adenomas and carcinomas.

Exposure of rats to α-methylstyrene resulted in kidney toxicity, which in males exhibited some features of α2u-globulin nephropathy. Exposure to α-methylstyrene resulted in nonneoplastic lesions of the nose in male and female rats and mice and of the liver and kidney in female mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1992). Toxicological Profile for Styrene. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Alarie, Y. (1973). Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* **24**, 279-297.

The Aldrich Library of ^{13}C and FT-IR Spectra (1993). 1st ed. (C.J. Pouchert, Ed.), Vol. 2, p. 23B. Aldrich Chemical Company, Inc., Milwaukee, WI.

The Aldrich Library of FT-NMR Spectra (1997). 2nd ed. (C.J. Pouchert, Ed.), Vol. 2, p. 1643D. Aldrich Chemical Company, Inc., Milwaukee, WI.

The Aldrich Library of Infrared Spectra (1981). 3rd ed. (C.J. Pouchert, Ed.), Vol. 1, spectrum 569D. Aldrich Chemical Co., Milwaukee, WI.

The Aldrich Library of NMR Spectra (1983). 2nd ed. (C.J. Pouchert, Ed.), spectrum 748D. Aldrich Chemical Co., Milwaukee, WI.

American Conference of Governmental Industrial Hygienists (ACGIH) (2005). *TLVs® and BEIs®: Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices*. ACGIH Worldwide, Cincinnati, OH.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bardodej, Z., and Bardodejova, E. (1970). Biotransformation of ethyl benzene, styrene, and alpha-methylstyrene in man. *Am. Ind. Hyg. Assoc. J.* **31**, 206-209.

Beliles, R.P., Butala, J.H., Stack, C.R., and Makris, S. (1985). Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. *Fundam. Appl. Toxicol.* **5**, 855-868.

Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Boorman, G.A., Morgan, K.T., and Uriah, L.C. (1990). Nose, Larynx, and Trachea. In *Pathology of the Fischer Rat* (G.A. Boorman, S.L. Eustis, M.R. Elwell, C.A. Montgomery, Jr., and W.F. MacKenzie, Eds.), pp. 315-337. Academic Press, Inc., San Diego.

Brecher, G., and Schneiderman, M. (1950). A time-saving device for the counting of reticulocytes. *Am. J. Clin. Pathol.*, **20**, 1079-1083.

Buckley, L.A., Morgan, K.T., Swenberg, J.A., James, R.A., Hamm, T.E., Jr., and Barrow, C.S. (1985). The toxicity of dimethylamine in F-344 rats and B6C3F1 mice following a 1-year inhalation exposure. *Fundam. Appl. Toxicol.* **5**, 341-352.

Code of Federal Regulations (CFR) **21**, Part 58.

Conti, B., Maltoni, C., Perino, G., and Ciliberti, A. (1988). Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. *Ann. N. Y. Acad. Sci.* **534**, 203-234.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Cruzan, G., Cushman, J.R., Andrews, L.S., Granville, G.C., Miller, R.R., Hardy, C.J., Coombs, D.W., and Mullins, P.A. (1997). Subchronic inhalation studies of styrene in CD rats and CD-1 mice. *Fundam. Appl. Toxicol.* **35**, 152-165.

Cruzan, G., Cushman, J.R., Andrews, L.S., Granville, G.C., Johnson, K.A., Hardy, C.J., Coombs, D.W., Mullins, P.A., and Brown, W.R. (1998). Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. *Toxicol. Sci.* **46**, 266-281.

Cruzan G., Cushman, J.R., Andrews, L.S., Granville, G.C., Johnson, K.A., Bevan, C., Hardy, C.J., Coombs, D.W., Mullins, P.A., and Brown, W.R. (2001). Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. *J. Appl. Toxicol.* **21**, 185-198.

Das, M., Srivastava, S.P., and Seth, P.K. (1983). Effect of styrene on glutathione content and some xenobiotic metabolizing enzymes of rat kidney. *Acta Pharmacol. Toxicol. (Copenh.)* **52**, 47-50.

De Costa, K.S., Black, S.R., Thomas, B.F., Burgess, J.P., and Mathews, J.M. (2001). Metabolism and disposition of alpha-methylstyrene in rats. *Drug Metab. Dispos.* **29**, 166-171.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis,* 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Eustis, S.L., Hailey, J.R., Boorman, G.A., and Haseman, J.K. (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol. Pathol.* **22**, 457-472.

Gagnaire, F., and Langlais, C. (2005). Relative ototoxicity of 21 aromatic solvents. *Arch. Toxicol.* **79**, 346-354.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Halacy, D. (1983). *Kirk-Othmer Encyclopedia of Chemical Technology*. 3rd ed. (M. Grayson and D. Eckroth, Eds.), Vol. 21, p. 310. John Wiley & Sons, New York.

Hansch, C., Leo, A., and Hoekman, D. (1995). *Exploring QSAR - Hydrophobic, Electronic, and Steric Constants*, p. 56. American Chemical Society, Washington, DC.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* **123**, 61-118.

Henderson, L.M., and Speit, G. (2005). Review of the genotoxicity of styrene in humans. *Mutat. Res.* **589**, 158-191.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods,* pp. 120-123. John Wiley and Sons, New York.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Data Management and Statistical Analysis Software, Version 1.4. ILS, Inc., P.O. Box 13501, Research Triangle Park, NC 27707.

International Agency for Research on Cancer (IARC) (1999). Consensus Report. In *Species Differences in Thyroid, Kidney, and Urinary Bladder Carcinogenesis* (C.C. Capen, E. Dybing, J.M. Rice, and J.D. Wilbourn, Eds.). IARC Scientific Publications No. 147. IARC, Lyon, France.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kirk-Othmer Encyclopedia of Chemical Technology (1997). 4th ed., Vol. 22, p. 990. John Wiley and Sons, New York.

Kligerman, A.D., Allen, J.W., Erexson, G.L., and Morgan, D.L. (1993). Cytogenetic studies of rodents exposed to styrene by inhalation. *IARC Sci. Publ.* **127**, 217-224.

Leibman, K.C. (1975). Metabolism and toxicity of styrene. *Environ. Health Perspect.* **11**, 115-119.

Lewis, P., Hagopian, C., and Koch, P. (1983). Styrene. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed. (M. Grayson and D. Eckroth, Eds.), Vol. 21, pp. 770-801. John Wiley & Sons, New York.

Lewis, R.J., Sr. (1997). *Hawley's Condensed Chemical Dictionary*, 13th ed., p. 749. John Wiley & Sons, Inc., New York.

Lijinsky, W. (1986). Rat and mouse forestomach tumors induced by chronic oral administration of styrene oxide. *J. Natl. Cancer Inst.* **77**, 471-476.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Maltoni, C., Ciliberti, A., and Carretti, D. (1982). Experimental contributions in identifying brain potential carcinogens in the petrochemical industry. *Ann. N. Y. Acad. Sci.* **381**, 216-249.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Montgomery, C.A., Jr., and Seely, J.C. (1990). Kidney. In *Pathology of the Fischer Rat* (G.A. Boorman, S.L. Eustis, M.R. Elwell, C.A. Montgomery, Jr., and W.F. MacKenzie, Eds.), pp. 127-153. Academic Press, Inc., San Diego.

Morgan, D.L., Mahler, J.F., Kirkpatrick, D.T., Price, H.C., O'Connor, R.W., Wilson, R.E., and Moorman, M.P. (1999). Characterization of inhaled alpha-methylstyrene vapor toxicity for B6C3F_1 mice and F344 rats. *Toxicol. Sci.* **47**, 187-194.

Morrison, D.F. (1976). *Multivariate Statistical Methods,* 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

Murray, F.J., John, J.A., Balmer, M.F., and Schwetz, B.A. (1978). Teratologic evaluation of styrene given to rats and rabbits by inhalation or by gavage. *Toxicology* **11**, 335-343.

National Cancer Institute (NCI) (1979). Bioassay of a Solution of β-Nitrostyrene and Styrene for Possible Carcinogenicity. Technical Report Series No. 170. NIH Publication No. 79-1726. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH) (2003). *NIOSH Pocket Guide to Chemical Hazards*, p. 216. Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Cincinnati, OH.

National Toxicology Program (NTP) (1990). Toxicology and Carcinogenesis Studies of *d*-Limonene (CAS No. 5989-27-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 347. NIH Publication No. 90-2802. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2002). *10th Report on Carcinogens*, pp. III-220-III-222. National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

National Toxicology Program (NTP) (2004a). Toxicology and Carcinogenesis Studies of Propylene Glycol Mono-*t*-Butyl Ether (CAS No. 57018-52-7) in F344/N Rats and B6C3F$_1$ Mice and a Toxicology Study of Propylene Glycol Mono-*t*-Butyl Ether in Male NBR Rats (Inhalation Studies). Technical Report Series No. 515. NIH Publication No. 04-4449. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2004b). Toxicology and Carcinogenesis Studies of Stoddard Solvent IIC (CAS No. 64742-88-7) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 519. NIH Publication No. 04-4453. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2005). Toxicology and Carcinogenesis Studies of Decalin (CAS No. 91-17-8) in F344/N Rats and B6C3F$_1$ Mice and a Toxicology Study of Decalin in Male NBR Rats (Inhalation Studies). Technical Report Series No. 513. NIH Publication No. 05-4447. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

NIST/EPA/NIH Mass Spectral Database (1994). Standard Reference Database 1A. Standard Reference Data Program. National Institute of Standards and Technology. U.S. Department of Commerce, Gaithersburg, MD.

Norppa, H., and Sorsa, M. (1993). Genetic toxicity of 1,3-butadiene and styrene. *IARC Sci. Publ.* **127**, 185-193.

Norppa, H., and Vainio, H. (1983). Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. *Mutat. Res.* **116**, 379-387.

Ohashi, Y., Nakai, Y., Ikeoka, H., Koshimo, H., Nakata, J., Esaki, Y., Horiguchi, S., and Teramoto, K. (1986). Degeneration and regeneration of respiratory mucosa of rats after exposure to styrene. *J. Appl. Toxicol.* **6**, 405-412.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology,* Section 6.3.2. Chapman and Hall, London.

Ponomarkov, V., Cabral, J.R., Wahrendorf, J., and Galendo, D. (1984). A carcinogenicity study of styrene-7,8-oxide in rats. *Cancer Lett.* **24**, 95-101.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Preston, R.J., and Abernethy, D.J. (1993). Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation. *IARC Sci. Publ.* **127**, 225-233.

Rao, G.N. (1996). New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fundam. Appl. Toxicol.* **32**, 102-108.

Rao, G.N. (1997). New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J. Nutr.* **127**, 842s-846s.

Salomaa, S., Donner, M., and Norppa, H. (1985). Inactivity of styrene in the mouse sperm morphology test. *Toxicol. Lett.* **24**, 151-155.

Santodonato, J., Meylan, W.M., Davis, L.N., Howard, P.H., Orzel, D.M., and Bogyo, D.A. (1980). Investigation of Selected Potential Environmental Contaminants: Styrene, Ethylbenzene, and Related Compounds. Syracuse Research Corporation report to the U.S. Environmental Protection Agency, Office of Toxic Substances, pp. 4-228. Syracuse Research Corporation, Syracuse, NY.

Schmid, W. (1975). The micronucleus test. *Mutat. Res.* **31**, 9-15.

Scott, D., and Preston, R.J. (1994). A re-evaluation of the cytogenetic effects of styrene. *Mutat. Res.* **318**, 175-203.

Shelby, M.D. (1988). The genetic toxicity of human carcinogens and its implications. *Mutat. Res.* **204**, 3-15.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., and Zeiger, E. (1990). Activity of human carcinogens in the Salmonella and rodent bone-marrow cytogenetics tests. *Mutat. Res.* **234**, 257-261.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Sittig, M. (1991). *Handbook of Toxic and Hazardous Chemicals and Carcinogens*, 3rd ed., Vol. 2, pp. 1139-1141. Noyes Publications, Park Ridge, N.J.

Speit, G., and Henderson, L. (2005). Review of the *in vivo* genotoxicity tests performed with styrene. *Mutat. Res.* **589**, 67-79.

SRI International (SRI) (1985). *Directory of Chemical Producers, USA, 1984*, p. 723. SRI International, Menlo Park, CA.

Srivastava, S., Seth, P.K., and Srivastava, S.P. (1989). Effect of styrene administration on rat testis. *Arch. Toxicol.* **63**, 43-46.

Stewart, R.D., Dodd, H.C., Baretta, E.D., and Schaffer, A.W. (1968). Human exposure to styrene vapor. *Arch. Environ. Health* **16**, 656-662.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* **236**, 933-941.

U.S. Environmental Protection Agency (USEPA) (1985). Computer printout (CISIS): 1977 Production Statistics for Chemicals in the Nonconfidential Initial TSCA Chemical Substance Inventory. Retrieved June 10, 1985. Office of Pesticides and Toxic Substances, Washington, DC.

U.S. Environmental Protection Agency (USEPA) (1991). $Alpha_{2U}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. EPA Report No. EPA/625/3-91/019F. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

U.S. International Trade Commission (USITC) (1981a). Synthetic Organic Chemicals, United States Production and Sales, 1980, pp. 25, 49. International Trade Commission, Washington, DC.

U.S. International Trade Commission (USITC) (1981b). Imports of Benzenoid Chemicals and Products, 1980, p. 23. International Trade Commission, Washington, DC.

U.S. International Trade Commission (USITC) (1982). Synthetic Organic Chemicals, United States Production and Sales, 1981, pp. 25, 44. International Trade Commission, Washington, DC.

U.S. International Trade Commission (USITC) (1983a). Synthetic Organic Chemicals, United States Production and Sales, 1982, pp. 27, 47. International Trade Commission, Washington, DC.

U.S. International Trade Commission (USITC) (1983b). Imports of Benzenoid Chemicals and Products, 1982, p. 21. International Trade Commission, Washington, DC.

U.S. International Trade Commission (USITC) (1984). Synthetic Organic Chemicals, United States Production and Sales, 1984, pp. 27, 46. International Trade Commission, Washington, DC.

Vaghef, H., and Hellman, B. (1998). Detection of styrene and styrene oxide-induced DNA damage in various organs of mice using the comet assay. *Pharmacol. Toxicol.* **83**, 69-74.

Vainio, H., Jarvisalo, J., and Taskinen, E. (1979). Adaptive changes caused by intermittent styrene inhalation on xenobiotic biotransformation. *Toxicol. Appl. Pharmacol.* **49**, 7-14.

Verschueren, K. (1977). *Handbook of Environmental Data on Organic Chemicals*, p. 471. Van Nostrand Reinhold Company, New York.

Viau, C., Bernard, A., De Russis, R., Ouled, A., Maldague, P., and Lauwerys, R. (1987). Evaluation of the nephrotoxic potential of styrene in man and in rat. *J. Appl. Toxicol.* **7**, 313-316.

Wenker, M.A, Kezic S., Monster, A.C., and De Wolff, F.A (2001). Metabolism of styrene in the human liver in vitro: Interindividual variation and enantioselectivity. *Xenobiotica* **31**, 61-72.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F_1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36**, 163-194.

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956). Toxicological studies of certain alkylated benzenes and benzene; experiments on laboratory animals. *AMA Arch. Ind. Health* **14**, 387-398.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988). *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.* **11** (Suppl. 12), 1-158.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

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

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	1
Moribund	19	15	21	25
Natural deaths	4	3	5	2
Survivors				
Died last week of study				1
Terminal sacrifice	27	32	23	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(47)	(46)	(47)
Intestine small, duodenum	(49)	(48)	(47)	(47)
Leiomyosarcoma			1 (2%)	
Intestine small, jejunum	(46)	(47)	(44)	(47)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Mesentery	(15)	(15)	(8)	(5)
Oral mucosa	(1)			(1)
Pancreas	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Leiomyosarcoma, metastatic, spleen	1 (2%)			
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(50)	(50)	(49)	(50)
Tongue		(1)		(1)
Tooth			(2)	
Cardiovascular System				
Blood vessel		(1)	(2)	
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			2 (4%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Ganglioneuroma			1 (2%)	
Pheochromocytoma, benign	7 (14%)	8 (16%)	9 (18%)	6 (12%)
Pheochromocytoma, complex				1 (2%)
Pheochromocytoma, malignant	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Bilateral, pheochromocytoma, benign	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	5 (10%)	3 (6%)	1 (2%)
Adenoma, multiple		1 (2%)		
Carcinoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Parathyroid gland	(47)	(49)	(48)	(47)
Pituitary gland	(49)	(49)	(49)	(49)
Adenoma	34 (69%)	36 (73%)	34 (69%)	24 (49%)
Carcinoma		1 (2%)	1 (2%)	
Pars intermedia, carcinoma			1 (2%)	

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Endocrine System (continued)				
Thyroid gland	(49)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
C-cell, adenoma	3 (6%)	7 (14%)	4 (8%)	2 (4%)
C-cell, carcinoma		2 (4%)	1 (2%)	
Follicular cell, adenoma		1 (2%)	2 (4%)	1 (2%)
Follicular cell, carcinoma			1 (2%)	2 (4%)
General Body System				
Peritoneum		(2)		(3)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(1)			
Preputial gland	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	3 (6%)		
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	17 (34%)	31 (62%)	23 (46%)	38 (76%)
Interstitial cell, adenoma	16 (32%)	13 (26%)	18 (36%)	6 (12%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney				1 (2%)
Lymph node	(6)	(3)	(6)	(12)
Deep cervical, carcinoma, metastatic, kidney				1 (8%)
Lymph node, bronchial	(10)	(8)	(14)	(15)
Lymph node, mediastinal	(17)	(25)	(20)	(18)
Carcinoma, metastatic, kidney				1 (6%)
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Spleen	(50)	(50)	(49)	(50)
Leiomyosarcoma	1 (2%)			
Thymus	(42)	(49)	(47)	(47)
Integumentary System				
Mammary gland	(48)	(49)	(50)	(50)
Fibroadenoma	1 (2%)		3 (6%)	3 (6%)
Fibroadenoma, multiple				1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	3 (6%)	1 (2%)	
Basal cell carcinoma			1 (2%)	1 (2%)
Keratoacanthoma	1 (2%)			2 (4%)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma			1 (2%)	
Pinna, neural crest tumor		1 (2%)		
Sebaceous gland, adenoma			1 (2%)	
Subcutaneous tissue, fibroma	3 (6%)		4 (8%)	4 (8%)
Subcutaneous tissue, fibroma, multiple	1 (2%)			
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (2%)		
Subcutaneous tissue, lipoma	1 (2%)		1 (2%)	
Subcutaneous tissue, schwannoma, benign			1 (2%)	

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Intervertebral disc, chordoma		1 (2%)	1 (2%)	
Skeletal muscle	(2)	(1)	(1)	
Chordoma, metastatic, bone		1 (100%)		
Liposarcoma			1 (100%)	
Squamous cell carcinoma, metastatic, lung	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland		1 (2%)	1 (2%)	
Glioma malignant		1 (2%)		
Medulloblastoma malignant	1 (2%)			
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		
Carcinoma, metastatic, kidney				2 (4%)
Carcinoma, metastatic, preputial gland		1 (2%)		
Chordoma, metastatic, bone			1 (2%)	
Squamous cell carcinoma	1 (2%)			
Nose	(50)	(50)	(50)	(49)
Pleura	(6)	(5)	(6)	(5)
Squamous cell carcinoma, metastatic, lung	1 (17%)			
Special Senses System				
Eye	(49)	(49)	(50)	(49)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland		(1)	(2)	(2)
Adenoma				1 (50%)
Carcinoma		1 (100%)	1 (50%)	1 (50%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma			1 (2%)	
Renal tubule, carcinoma			1 (2%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	26 (52%)	32 (64%)	29 (58%)	38 (76%)
Mesothelioma malignant		2 (4%)		3 (6%)

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	49	48	48
Total primary neoplasms	125	155	151	140
Total animals with benign neoplasms	48	49	48	47
Total benign neoplasms	89	107	110	89
Total animals with malignant neoplasms	32	39	32	38
Total malignant neoplasms	36	47	41	51
Total animals with metastatic neoplasms	2	3	2	2
Total metastatic neoplasms	3	3	2	7
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 α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adrenal Medulla: Pheochromocytoma Benign				
Overall rate[a]	8/50 (16%)	8/50 (16%)	10/50 (20%)	6/50 (12%)
Adjusted rate[b]	18.9%	17.6%	23.5%	14.8%
Terminal rate[c]	6/27 (22%)	5/32 (16%)	6/23 (26%)	4/22 (18%)
First incidence (days)	682	603	652	646
Poly-3 test[d]	P=0.368N	P=0.544N	P=0.401	P=0.416N
Adrenal Medulla: Pheochromocytoma Benign, Complex, or Malignant				
Overall rate	10/50 (20%)	8/50 (16%)	11/50 (22%)	8/50 (16%)
Adjusted rate	23.4%	17.6%	25.8%	19.7%
Terminal rate	7/27 (26%)	5/32 (16%)	7/23 (30%)	6/22 (27%)
First incidence (days)	587	603	652	646
Poly-3 test	P=0.496N	P=0.339N	P=0.495	P=0.442N
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
Adjusted rate	2.4%	4.5%	2.4%	12.4%
Terminal rate	1/27 (4%)	1/32 (3%)	1/23 (4%)	3/22 (14%)
First incidence (days)	729 (T)	723	729 (T)	653
Poly-3 test	P=0.033	P=0.524	P=0.761	P=0.091
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted rate	2.4%	4.5%	4.8%	12.4%
Terminal rate	1/27 (4%)	1/32 (3%)	1/23 (4%)	3/22 (14%)
First incidence (days)	729 (T)	723	716	653
Poly-3 test	P=0.043	P=0.524	P=0.500	P=0.091
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	7/50 (14%)
Adjusted rate	2.4%	4.5%	7.1%	17.2%
Terminal rate	1/27 (4%)	1/32 (3%)	2/23 (9%)	3/22 (14%)
First incidence (days)	729 (T)	723	716	653
Poly-3 test	P=0.006	P=0.524	P=0.305	P=0.026
Mammary Gland: Fibroadenoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate	2.4%	0.0%	7.1%	9.9%
Terminal rate	1/27 (4%)	0/32 (0%)	2/23 (9%)	2/22 (9%)
First incidence (days)	729 (T)	—[e]	652	653
Poly-3 test	P=0.041	P=0.486N	P=0.307	P=0.167
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	6/50 (12%)	3/50 (6%)	1/50 (2%)
Adjusted rate	7.1%	13.3%	7.1%	2.5%
Terminal rate	2/27 (7%)	4/32 (13%)	2/23 (9%)	1/22 (5%)
First incidence (days)	554	700	674	729 (T)
Poly-3 test	P=0.124N	P=0.270	P=0.659	P=0.327N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	8/50 (16%)	4/50 (8%)	2/50 (4%)
Adjusted rate	9.4%	17.6%	9.5%	5.0%
Terminal rate	3/27 (11%)	5/32 (16%)	3/23 (13%)	2/22 (9%)
First incidence (days)	554	660	674	729 (T)
Poly-3 test	P=0.131N	P=0.207	P=0.639	P=0.365N

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Pituitary Gland (Unspecified Site): Adenoma				
Overall rate	34/49 (69%)	36/49 (73%)	34/49 (69%)	24/49 (49%)
Adjusted rate	75.3%	76.3%	75.6%	57.0%
Terminal rate	19/27 (70%)	25/32 (78%)	20/23 (87%)	15/21 (71%)
First incidence (days)	522	603	558	401
Poly-3 test	P=0.015N	P=0.553	P=0.585	P=0.047N
Pituitary Gland (Unspecified Site): Adenoma or Carcinoma				
Overall rate	34/49 (69%)	37/49 (76%)	35/49 (71%)	24/49 (49%)
Adjusted rate	75.3%	77.7%	77.9%	57.0%
Terminal rate	19/27 (70%)	25/32 (78%)	21/23 (91%)	15/21 (71%)
First incidence (days)	522	603	558	401
Poly-3 test	P=0.011N	P=0.485	P=0.482	P=0.047N
Preputial Gland: Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.7%	6.7%	0.0%	0.0%
Terminal rate	0/27 (0%)	2/32 (6%)	0/23 (0%)	0/22 (0%)
First incidence (days)	702	700	—	—
Poly-3 test	P=0.105N	P=0.530	P=0.238N	P=0.249N
Skin: Basal Cell Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.4%	6.6%	2.4%	0.0%
Terminal rate	1/27 (4%)	2/32 (6%)	1/23 (4%)	0/22 (0%)
First incidence (days)	729 (T)	663	729 (T)	—
Poly-3 test	P=0.186N	P=0.332	P=0.761	P=0.509N
Skin: Basal Cell Adenoma or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	2.4%	6.6%	4.8%	2.5%
Terminal rate	1/27 (4%)	2/32 (6%)	2/23 (9%)	1/22 (5%)
First incidence (days)	729 (T)	663	729 (T)	729 (T)
Poly-3 test	P=0.448N	P=0.332	P=0.500	P=0.751
Skin: Squamous Cell Papilloma, Keratocanthoma, or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.4%	0.0%	2.4%	7.4%
Terminal rate	1/27 (4%)	0/32 (0%)	0/23 (0%)	0/22 (0%)
First incidence (days)	729 (T)	—	674	646
Poly-3 test	P=0.071	P=0.486N	P=0.760N	P=0.293
Skin: Squamous Cell Papilloma, Keratocanthoma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	4/50 (8%)
Adjusted rate	4.8%	6.6%	7.1%	9.8%
Terminal rate	2/27 (7%)	2/32 (6%)	2/23 (9%)	1/22 (5%)
First incidence (days)	729 (T)	663	674	646
Poly-3 test	P=0.279	P=0.533	P=0.502	P=0.322
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	4/50 (8%)	0/50 (0%)	4/50 (8%)	4/50 (8%)
Adjusted rate	9.3%	0.0%	9.5%	9.9%
Terminal rate	1/27 (4%)	0/32 (0%)	2/23 (9%)	3/22 (14%)
First incidence (days)	590	—	705	562
Poly-3 test	P=0.261	P=0.055N	P=0.632	P=0.611

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	5/50 (10%)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rate	11.6%	2.2%	9.5%	9.9%
Terminal rate	2/27 (7%)	1/32 (3%)	2/23 (9%)	3/22 (14%)
First incidence (days)	590	729 (T)	705	562
Poly-3 test	P=0.430	P=0.091N	P=0.514N	P=0.537N
Testes: Adenoma				
Overall rate	33/50 (66%)	44/50 (88%)	41/50 (82%)	44/50 (88%)
Adjusted rate	73.4%	90.7%	87.7%	95.8%
Terminal rate	22/27 (82%)	31/32 (97%)	23/23 (100%)	22/22 (100%)
First incidence (days)	548	562	560	519
Poly-3 test	P=0.007	P=0.017	P=0.053	P<0.001
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/49 (6%)	7/50 (14%)	4/50 (8%)	2/50 (4%)
Adjusted rate	7.1%	15.4%	9.5%	5.0%
Terminal rate	0/26 (0%)	5/32 (16%)	3/23 (13%)	1/22 (5%)
First incidence (days)	587	612	688	660
Poly-3 test	P=0.212N	P=0.189	P=0.500	P=0.520N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/49 (6%)	9/50 (18%)	5/50 (10%)	2/50 (4%)
Adjusted rate	7.1%	19.8%	11.9%	5.0%
Terminal rate	0/26 (0%)	7/32 (22%)	4/23 (17%)	1/22 (5%)
First incidence (days)	587	612	688	660
Poly-3 test	P=0.141N	P=0.077	P=0.355	P=0.520N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/49 (0%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	0.0%	2.2%	7.1%	7.5%
Terminal rate	0/26 (0%)	1/32 (3%)	2/23 (9%)	1/22 (5%)
First incidence (days)	—	729 (T)	656	702
Poly-3 test	P=0.102	P=0.518	P=0.123	P=0.115
All Organs: Mononuclear Cell Leukemia				
Overall rate	26/50 (52%)	32/50 (64%)	29/50 (58%)	38/50 (76%)
Adjusted rate	58.7%	67.7%	61.9%	80.2%
Terminal rate	17/27 (63%)	23/32 (72%)	12/23 (52%)	14/22 (64%)
First incidence (days)	495	562	558	401
Poly-3 test	P=0.018	P=0.239	P=0.459	P=0.016
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	4.5%	0.0%	7.5%
Terminal rate	0/27 (0%)	2/32 (6%)	0/23 (0%)	2/22 (9%)
First incidence (days)	—	729 (T)	—[f]	714
Poly-3 test	P=0.092	P=0.252	—	P=0.110
All Organs: Benign Neoplasms				
Overall rate	48/50 (96%)	49/50 (98%)	48/50 (96%)	47/50 (94%)
Adjusted rate	99.1%	99.2%	99.0%	98.8%
Terminal rate	27/27 (100%)	32/32 (100%)	23/23 (100%)	22/22 (100%)
First incidence (days)	522	562	558	401
Poly-3 test	P=0.748N	P=0.969	P=0.948N	P=0.919N

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
All Organs: Malignant Neoplasms				
Overall rate	32/50 (64%)	39/50 (78%)	32/50 (64%)	38/50 (76%)
Adjusted rate	69.1%	80.2%	68.2%	80.2%
Terminal rate	19/27 (70%)	25/32 (78%)	14/23 (61%)	14/22 (64%)
First incidence (days)	345	562	558	401
Poly-3 test	P=0.226	P=0.148	P=0.553N	P=0.150
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	48/50 (96%)	48/50 (96%)
Adjusted rate	100.0%	99.2%	99.0%	99.7%
Terminal rate	27/27 (100%)	32/32 (100%)	23/23 (100%)	22/22 (100%)
First incidence (days)	345	562	558	401
Poly-3 test	P=0.915	P=0.945N	P=0.863N	P=1.000N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 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 Neoplasms in Control Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Chamber Controls Given NTP-2000 Diet			
Decalin	1/50	0/50	1/50
Divinylbenzene	0/50	0/50	0/50
Indium phosphide	0/50	0/50	0/50
Methyl isobutyl ketone	0/50	0/50	0/50
Naphthalene	0/49	0/49	0/49
Propylene glycol mono-*t*-butyl ether	1/50	0/50	1/50
Stoddard solvent (type IIC)	0/50	1/50	1/50
Vanadium pentoxide	1/50	0/50	1/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	3/399 (0.8%)	1/399 (0.3%)	4/399 (1.0%)
Mean ± standard deviation	0.8% ± 1.0%	0.3% ± 0.7%	1.0% ± 1.1%
Range	0%-2%	0%-2%	0%-2%
Overall Historical Incidence: All Routes			
Total (%)	6/1,448 (0.4%)	1/1,448 (0.1%)	7/1,448 (0.5%)
Mean ± standard deviation	0.5% ± 0.9%	0.1% ± 0.4%	0.5% ± 0.9%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of January 28, 2005

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

Study	Incidence in Controls
Historical Incidence in Chamber Controls Given NTP-2000 Diet	
Decalin	40/50
Divinylbenzene	38/50
Indium phosphide	40/50
Methyl isobutyl ketone	42/50
Naphthalene	38/49
Propylene glycol mono-*t*-butyl ether	41/50
Stoddard solvent (type IIC)	41/50
Vanadium pentoxide	36/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	316/399 (79.2%)
Mean ± standard deviation	79.2% ± 3.9%
Range	72%-84%
Overall Historical Incidence: All Routes	
Total (%)	1,264/1,459 (86.6%)
Mean ± standard deviation	85.8% ± 7.4%
Range	72%-98%

[a] Data as of January 28, 2005

TABLE A3c
Historical Incidence of Mononuclear Cell Leukemia in Control Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence in Chamber Controls Given NTP-2000 Diet	
Decalin	19/50
Divinylbenzene	22/50
Indium phosphide	16/50
Methyl isobutyl ketone	25/50
Naphthalene	26/49
Propylene glycol mono-*t*-butyl ether	33/50
Stoddard solvent (type IIC)	25/50
Vanadium pentoxide	22/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	188/399 (47.1%)
Mean ± standard deviation	47.1% ± 10.3%
Range	32%-66%
Overall Historical Incidence: All Routes	
Total (%)	622/1,459 (42.6%)
Mean ± standard deviation	41.4% ± 12.3%
Range	22%-68%

[a] Data as of January 28, 2005; includes data for lymphocytic, monocytic, or undifferentiated leukemia

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	1
Moribund	19	15	21	25
Natural deaths	4	3	5	2
Survivors				
Died last week of study				1
Terminal sacrifice	27	32	23	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(47)	(46)	(47)
Ulcer		1 (2%)		
Epithelium, atrophy		1 (2%)		
Intestine small, duodenum	(49)	(48)	(47)	(47)
Ulcer		1 (2%)		
Intestine small, jejunum	(46)	(47)	(44)	(47)
Inflammation, chronic active		1 (2%)		
Epithelium, ulcer		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	1 (2%)	4 (8%)
Basophilic focus	2 (4%)	8 (16%)	3 (6%)	1 (2%)
Basophilic focus, multiple		1 (2%)		
Bile stasis		1 (2%)		
Clear cell focus	8 (16%)	7 (14%)	5 (10%)	3 (6%)
Clear cell focus, multiple	1 (2%)			
Degeneration, cystic	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule		2 (4%)	5 (10%)	10 (20%)
Necrosis	2 (4%)	2 (4%)	3 (6%)	4 (8%)
Thrombosis		1 (2%)	1 (2%)	1 (2%)
Vacuolization cytoplasmic	3 (6%)	1 (2%)	4 (8%)	
Bile duct, dilatation		2 (4%)		
Bile duct, hyperplasia	30 (60%)	42 (84%)	39 (78%)	30 (60%)
Mesentery	(15)	(15)	(8)	(5)
Necrosis	15 (100%)	14 (93%)	8 (100%)	5 (100%)
Fat, hemorrhage		1 (7%)		
Oral mucosa	(1)			(1)
Gingival, hyperplasia, squamous, focal	1 (100%)			1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	2 (4%)	2 (4%)	1 (2%)	
Stomach, forestomach	(50)	(50)	(49)	(50)
Hyperplasia, squamous	1 (2%)		1 (2%)	1 (2%)
Inflammation, suppurative				1 (2%)
Necrosis	1 (2%)			
Ulcer	4 (8%)	1 (2%)	2 (4%)	
Epithelium, mineralization		1 (2%)		
Stomach, glandular	(50)	(50)	(49)	(50)
Erosion	2 (4%)		2 (4%)	3 (6%)
Ulcer	1 (2%)			
Epithelium, mineralization		1 (2%)		

[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 α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Alimentary System (continued)				
Tongue		(1)		(1)
Epithelium, hyperplasia				1 (100%)
Tooth			(2)	
Inflammation, suppurative			1 (50%)	
Peridontal tissue, inflammation			1 (50%)	
Cardiovascular System				
Blood vessel		(1)	(2)	
Pulmonary artery, infiltration cellular, polymorphonuclear			1 (50%)	
Pulmonary artery, mineralization			1 (50%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	7 (14%)	3 (6%)	4 (8%)	6 (12%)
Atrium, myocardium, hypertrophy		1 (2%)		
Atrium, necrosis				1 (2%)
Atrium, thrombosis	3 (6%)	1 (2%)	5 (10%)	5 (10%)
Myocardium, hypertrophy		1 (2%)		
Valve, thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)		
Hyperplasia	14 (28%)	10 (20%)	12 (24%)	9 (18%)
Hypertrophy	2 (4%)	2 (4%)		
Mineralization	1 (2%)			
Necrosis			1 (2%)	
Vacuolization cytoplasmic	9 (18%)	21 (42%)	10 (20%)	5 (10%)
Adrenal medulla	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Hyperplasia	15 (30%)	13 (26%)	9 (18%)	14 (28%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	3 (6%)		
Parathyroid gland	(47)	(49)	(48)	(47)
Hyperplasia	1 (2%)		1 (2%)	
Pituitary gland	(49)	(49)	(49)	(49)
Cyst		1 (2%)	1 (2%)	1 (2%)
Hemorrhage		1 (2%)		
Hyperplasia	9 (18%)	8 (16%)	6 (12%)	14 (29%)
Thyroid gland	(49)	(50)	(50)	(50)
Cyst				1 (2%)
C-cell, hyperplasia	8 (16%)	9 (18%)	9 (18%)	6 (12%)
Follicle, cyst				1 (2%)
Follicular cell, hyperplasia		3 (6%)	1 (2%)	2 (4%)
General Body System				
Peritoneum		(2)		(3)

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(1)			
Inflammation, chronic active	1 (100%)			
Preputial gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Hyperplasia	2 (4%)	4 (8%)	1 (2%)	2 (4%)
Inflammation, suppurative			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hyperplasia	6 (12%)		1 (2%)	2 (4%)
Inflammation, suppurative	34 (68%)	35 (70%)	31 (62%)	24 (48%)
Seminal vesicle	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Dilatation			1 (2%)	
Inflammation, suppurative		1 (2%)	1 (2%)	
Testes	(50)	(50)	(50)	(50)
Artery, inflammation, chronic active			1 (2%)	
Germinal epithelium, atrophy	5 (10%)	5 (10%)	8 (16%)	6 (12%)
Germinal epithelium, mineralization		1 (2%)		
Interstitial cell, hyperplasia	6 (12%)	7 (14%)	10 (20%)	7 (14%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(6)	(3)	(6)	(12)
Deep cervical, hemorrhage				1 (8%)
Lymph node, bronchial	(10)	(8)	(14)	(15)
Hyperplasia, histiocytic			1 (7%)	
Infiltration cellular, histiocyte			1 (7%)	
Lymph node, mediastinal	(17)	(25)	(20)	(18)
Angiectasis	1 (6%)		1 (5%)	
Hemorrhage		1 (4%)		
Hyperplasia, lymphoid		2 (8%)		1 (6%)
Infiltration cellular, histiocyte			1 (5%)	
Pigmentation			1 (5%)	1 (6%)
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Spleen	(50)	(50)	(49)	(50)
Accessory spleen	1 (2%)		1 (2%)	1 (2%)
Fibrosis	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)		
Hemorrhage		1 (2%)		2 (4%)
Hyperplasia, lymphoid		1 (2%)		1 (2%)
Necrosis	3 (6%)	3 (6%)	5 (10%)	8 (16%)
Thymus	(42)	(49)	(47)	(47)
Cyst		1 (2%)		
Inflammation			1 (2%)	
Thrombosis			1 (2%)	

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Integumentary System				
Mammary gland	(48)	(49)	(50)	(50)
Galactocele	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Hyperplasia		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)	3 (6%)		
Hyperkeratosis		2 (4%)	1 (2%)	2 (4%)
Hyperplasia, squamous		1 (2%)		
Inflammation, chronic		1 (2%)	1 (2%)	
Ulcer	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Subcutaneous tissue, metaplasia, osseous			1 (2%)	
Subcutaneous tissue, mineralization	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	1 (2%)			
Maxilla, fracture			1 (2%)	
Skeletal muscle	(2)	(1)	(1)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	11 (22%)	11 (22%)	8 (16%)	2 (4%)
Gliosis				1 (2%)
Hemorrhage	7 (14%)	2 (4%)	4 (8%)	2 (4%)
Cerebrum, demyelination, focal				1 (2%)
Cerebrum, necrosis, focal	1 (2%)			
Choroid plexus, hemorrhage			1 (2%)	
Meninges, hemorrhage				1 (2%)
Respiratory System				
Larynx	(50)	(49)	(50)	(50)
Foreign body	4 (8%)	5 (10%)	2 (4%)	5 (10%)
Inflammation, suppurative	6 (12%)		4 (8%)	2 (4%)
Inflammation, chronic	4 (8%)	11 (22%)	6 (12%)	10 (20%)
Epiglottis, hyperplasia				1 (2%)
Respiratory epithelium, hyperplasia		2 (4%)		
Respiratory epithelium, metaplasia, squamous	1 (2%)			
Lung	(50)	(50)	(50)	(50)
Hemorrhage	7 (14%)	3 (6%)	1 (2%)	7 (14%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic	4 (8%)	1 (2%)	3 (6%)	2 (4%)
Thrombosis	1 (2%)			
Alveolar epithelium, degeneration, mucoid, focal			1 (2%)	
Alveolar epithelium, hyperplasia	3 (6%)	6 (12%)	6 (12%)	3 (6%)
Alveolus, emphysema		2 (4%)	1 (2%)	
Alveolus, infiltration cellular, histiocyte	11 (22%)	12 (24%)	10 (20%)	4 (8%)
Interstitium, fibrosis	3 (6%)		2 (4%)	1 (2%)
Mediastinum, inflammation, suppurative			1 (2%)	

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(49)
Foreign body	6 (12%)	4 (8%)	3 (6%)	2 (4%)
Hemorrhage			1 (2%)	
Inflammation, suppurative	10 (20%)	8 (16%)	7 (14%)	5 (10%)
Inflammation, chronic		1 (2%)	2 (4%)	
Glands, dilatation	3 (6%)	7 (14%)	6 (12%)	
Goblet cell, hyperplasia	5 (10%)	5 (10%)	5 (10%)	5 (10%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	1 (2%)	1 (2%)	
Nerve, olfactory epithelium, degeneration				1 (2%)
Olfactory epithelium, degeneration	1 (2%)	3 (6%)	3 (6%)	16 (33%)
Olfactory epithelium, degeneration, hyaline	1 (2%)	1 (2%)	1 (2%)	
Olfactory epithelium, hyperplasia, basal cell		17 (34%)	18 (36%)	43 (88%)
Olfactory epithelium, metaplasia	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Respiratory epithelium, degeneration, hyaline	2 (4%)		2 (4%)	
Respiratory epithelium, hyperplasia		1 (2%)		2 (4%)
Respiratory epithelium, metaplasia, squamous				1 (2%)
Pleura	(6)	(5)	(6)	(5)
Inflammation, chronic	5 (83%)	5 (100%)	6 (100%)	5 (100%)
Mesothelium, hyperplasia		1 (20%)		
Special Senses System				
Eye	(49)	(49)	(50)	(49)
Atrophy			1 (2%)	1 (2%)
Cornea, fibrosis				1 (2%)
Cornea, hyperplasia, squamous		1 (2%)		1 (2%)
Cornea, mineralization	1 (2%)			
Lens, cataract	3 (6%)		5 (10%)	1 (2%)
Retina, atrophy	1 (2%)		1 (2%)	
Sclera, metaplasia, osseous	32 (65%)	32 (65%)	32 (64%)	25 (51%)
Harderian gland	(50)	(50)	(50)	(50)
Inflammation, chronic			2 (4%)	
Zymbal's gland		(1)	(2)	(2)
Inflammation			1 (50%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	2 (4%)	2 (4%)	2 (4%)	
Infarct	3 (6%)			5 (10%)
Nephropathy	41 (82%)	46 (92%)	46 (92%)	45 (90%)
Thrombosis				1 (2%)
Bilateral, pelvis, dilatation		1 (2%)	1 (2%)	
Bilateral, infarct				1 (2%)
Bilateral, infarct, multiple				1 (2%)
Cortex, renal tubule, accumulation, hyaline droplet			1 (2%)	1 (2%)
Glomerulus, fibrosis	1 (2%)			
Papilla, mineralization	12 (24%)	16 (32%)	10 (20%)	33 (66%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Pelvis, transitional epithelium, mineralization	1 (2%)			
Pelvis, dilatation		1 (2%)	1 (2%)	
Pelvis, hemorrhage			1 (2%)	
Renal tubule, casts		1 (2%)		
Renal tubule, mineralization		1 (2%)		

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Urinary System (continued)				
Urinary bladder	(50)	(50)	(50)	(50)
Calculus microscopic observation only	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Cyst			1 (2%)	
Hemorrhage	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Inflammation, suppurative	1 (2%)			
Inflammation, chronic		1 (2%)		
Necrosis			2 (4%)	
Transitional epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)	2 (4%)

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

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2			
Moribund	15	21	12	20
Natural deaths	6	5	2	4
Survivors				
Terminal sacrifice	27	24	36	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, rectum	(49)	(48)	(50)	(50)
Serosa, fibrosarcoma, metastatic, skin				1 (2%)
Intestine small, ileum	(45)	(46)	(48)	(47)
Intestine small, jejunum	(45)	(46)	(48)	(48)
Liver	(50)	(50)	(50)	(50)
Mesentery	(14)	(17)	(12)	(10)
Sarcoma stromal, metastatic, uterus		1 (6%)		
Oral mucosa	(1)		(1)	
Pharyngeal, squamous cell papilloma			1 (100%)	
Pancreas	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(50)
Tongue		(1)	(2)	
Cardiovascular System				
Blood vessel		(1)		(1)
Heart	(50)	(50)	(50)	(50)
Epicardium, squamous cell carcinoma, metastatic, lung				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	2 (4%)			2 (4%)
Bilateral, adenoma				1 (2%)
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)			
Pheochromocytoma malignant		1 (2%)	1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	3 (6%)		1 (2%)	
Carcinoma			1 (2%)	
Pituitary gland	(50)	(49)	(50)	(50)
Adenoma	35 (70%)	29 (59%)	37 (74%)	29 (58%)
Carcinoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	2 (4%)	6 (12%)	4 (8%)	4 (8%)
C-cell, carcinoma	1 (2%)	2 (4%)		
Follicular cell, adenoma		1 (2%)		
General Body System				
None				

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Fibrosarcoma	1 (2%)			
Fibrosarcoma, metastatic, skin				1 (2%)
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign		1 (2%)		
Granulosa cell tumor malignant	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	6 (12%)	13 (26%)	9 (18%)	10 (20%)
Polyp stromal, multiple		1 (2%)		
Sarcoma stromal		1 (2%)		
Serosa, hemangioma	1 (2%)			
Vagina		(1)		(1)
Hematopoietic System				
Lymph node	(2)	(3)	(2)	(5)
Deep cervical, carcinoma, metastatic, thyroid gland		1 (33%)		
Lymph node, bronchial	(4)	(7)	(8)	(3)
Lymph node, mandibular	(3)		(1)	(1)
Lymph node, mediastinal	(26)	(25)	(21)	(28)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(50)	(48)	(48)	(48)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Carcinoma, multiple	2 (4%)		1 (2%)	
Fibroadenoma	14 (28%)	16 (32%)	16 (32%)	18 (36%)
Fibroadenoma, multiple	8 (16%)	14 (28%)	7 (14%)	5 (10%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Basal cell carcinoma			1 (2%)	
Keratoacanthoma		2 (4%)	1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)			1 (2%)
Subcutaneous tissue, fibrosarcoma		1 (2%)		1 (2%)
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(2)	(1)		
Rhabdomyosarcoma	1 (50%)			

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)			
Carcinoma, metastatic, pituitary gland		1 (2%)		
Glioma, malignant	1 (2%)			
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma				1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Squamous cell carcinoma, metastatic, lung				1 (2%)
Mediastinum, sarcoma stromal, metastatic, uterus		1 (2%)		
Mediastinum, squamous cell carcinoma				1 (2%)
Nose	(49)	(49)	(50)	(50)
Pleura	(16)	(13)	(15)	(30)
Squamous cell carcinoma, metastatic, lung				1 (3%)
Special Senses System				
Eye	(48)	(49)	(50)	(50)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Ureter	(1)			
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, carcinoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Leukemia mononuclear	18 (36%)	21 (42%)	21 (42%)	22 (44%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	49	47	49
Total primary neoplasms	102	117	107	102
Total animals with benign neoplasms	43	44	44	41
Total benign neoplasms	74	85	76	71
Total animals with malignant neoplasms	25	29	26	30
Total malignant neoplasms	28	32	31	31
Total animals with metastatic neoplasms		3		2
Total metastatic neoplasms		5		5

[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 α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	2/50 (4%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[b]	5.0%	0.0%	0.0%	7.0%
Terminal rate[c]	1/27 (4%)	0/24 (0%)	0/36 (0%)	2/26 (8%)
First incidence (days)	687	—[e]	—	581
Poly-3 test[d]	P=0.149	P=0.229N	P=0.212N	P=0.531
Clitoral Gland: Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.5%	2.4%	6.6%	6.9%
Terminal rate	1/27 (4%)	1/24 (4%)	2/36 (6%)	1/26 (4%)
First incidence (days)	730 (T)	730 (T)	705	458
Poly-3 test	P=0.242	P=0.752N	P=0.350	P=0.334
Mammary Gland: Fibroadenoma				
Overall rate	22/50 (44%)	30/50 (60%)[f]	23/50 (46%)	23/50 (46%)
Adjusted rate	51.3%	67.1%	49.9%	52.8%
Terminal rate	11/27 (41%)	17/24 (71%)	18/36 (50%)	16/26 (62%)
First incidence (days)	460	590	647	663
Poly-3 test	P=0.345N	P=0.092	P=0.532N	P=0.533
Mammary Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted rate	7.4%	4.7%	8.9%	4.7%
Terminal rate	2/27 (7%)	1/24 (4%)	4/36 (11%)	2/26 (8%)
First incidence (days)	618	557	730 (T)	730 (T)
Poly-3 test	P=0.455N	P=0.480N	P=0.560	P=0.477N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted rate	7.4%	7.1%	8.9%	4.7%
Terminal rate	2/27 (7%)	1/24 (4%)	4/36 (11%)	2/26 (8%)
First incidence (days)	618	557	730 (T)	730 (T)
Poly-3 test	P=0.380N	P=0.642N	P=0.560	P=0.477N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	24/50 (48%)	32/50 (64%)	24/50 (48%)	24/50 (48%)
Adjusted rate	55.5%	70.7%	52.1%	55.1%
Terminal rate	12/27 (44%)	18/24 (75%)	19/36 (53%)	17/26 (65%)
First incidence (days)	460	557	647	663
Poly-3 test	P=0.280N	P=0.095	P=0.456N	P=0.572N
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.5%	0.0%	2.2%	0.0%
Terminal rate	3/27 (11%)	0/24 (0%)	1/36 (3%)	0/26 (0%)
First incidence (days)	730 (T)	—	730 (T)	—
Poly-3 test	P=0.166N	P=0.111N	P=0.263N	P=0.108N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.5%	0.0%	4.4%	0.0%
Terminal rate	3/27 (11%)	0/24 (0%)	2/36 (6%)	0/26 (0%)
First incidence (days)	730 (T)	—	730 (T)	—
Poly-3 test	P=0.173N	P=0.111N	P=0.445N	P=0.108N

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Pituitary Gland (Unspecified Site): Adenoma				
Overall rate	35/50 (70%)	29/49 (59%)	37/50 (74%)	29/50 (58%)
Adjusted rate	79.7%	64.6%	76.8%	64.2%
Terminal rate	21/27 (78%)	16/24 (67%)	27/36 (75%)	19/26 (73%)
First incidence (days)	521	435	477	569
Poly-3 test	P=0.147N	P=0.076N	P=0.464N	P=0.069N
Pituitary Gland (Unspecified Site): Adenoma or Carcinoma				
Overall rate	35/50 (70%)	30/49 (61%)	37/50 (74%)	29/50 (58%)
Adjusted rate	79.7%	66.8%	76.8%	64.2%
Terminal rate	21/27 (78%)	17/24 (71%)	27/36 (75%)	19/26 (73%)
First incidence (days)	521	435	477	569
Poly-3 test	P=0.121N	P=0.114N	P=0.464N	P=0.069N
Skin: Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	4.4%	0.0%
Terminal rate	0/27 (0%)	2/24 (8%)	1/36 (3%)	0/26 (0%)
First incidence (days)	—	649	647	—
Poly-3 test	P=0.260N	P=0.127	P=0.266	—[g]
Thyroid Gland (C-Cell): Adenoma				
Overall rate	2/50 (4%)	6/50 (12%)	4/50 (8%)	4/50 (8%)
Adjusted rate	4.9%	14.2%	8.8%	9.3%
Terminal rate	1/27 (4%)	3/24 (13%)	3/36 (8%)	1/26 (4%)
First incidence (days)	585	649	626	610
Poly-3 test	P=0.575	P=0.146	P=0.392	P=0.367
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	7/50 (14%)	4/50 (8%)	4/50 (8%)
Adjusted rate	7.4%	16.5%	8.8%	9.3%
Terminal rate	2/27 (7%)	4/24 (17%)	3/36 (8%)	1/26 (4%)
First incidence (days)	585	649	626	610
Poly-3 test	P=0.457N	P=0.174	P=0.563	P=0.535
Uterus: Stromal Polyp				
Overall rate	6/50 (12%)	14/50 (28%)[h]	9/50 (18%)	10/50 (20%)
Adjusted rate	15.0%	31.9%	19.9%	22.8%
Terminal rate	6/27 (22%)	8/24 (33%)	8/36 (22%)	5/26 (19%)
First incidence (days)	730 (T)	435	677	610
Poly-3 test	P=0.555	P=0.056	P=0.381	P=0.265
All Organs: Mononuclear Cell Leukemia				
Overall rate	18/50 (36%)	21/50 (42%)	21/50 (42%)	22/50 (44%)
Adjusted rate	43.5%	47.7%	43.8%	47.3%
Terminal rate	12/27 (44%)	9/24 (38%)	12/36 (33%)	8/26 (31%)
First incidence (days)	607	495	495	543
Poly-3 test	P=0.456	P=0.431	P=0.573	P=0.440
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	44/50 (88%)	44/50 (88%)	41/50 (82%)
Adjusted rate	94.4%	91.1%	91.0%	87.8%
Terminal rate	25/27 (93%)	22/24 (92%)	33/36 (92%)	24/26 (92%)
First incidence (days)	460	435	477	569
Poly-3 test	P=0.201N	P=0.413N	P=0.406N	P=0.204N

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

	Chamber Control	100 ppm	300 ppm	1,000 ppm
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	29/50 (58%)	26/50 (52%)	30/50 (60%)
Adjusted rate	58.0%	62.6%	53.9%	61.6%
Terminal rate	16/27 (59%)	12/24 (50%)	16/36 (44%)	10/26 (39%)
First incidence (days)	439	435	495	458
Poly-3 test	P=0.448	P=0.406	P=0.427N	P=0.445
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	49/50 (98%)	47/50 (94%)	49/50 (98%)
Adjusted rate	99.2%	98.0%	94.0%	98.0%
Terminal rate	27/27 (100%)	23/24 (96%)	33/36 (92%)	25/26 (96%)
First incidence (days)	439	435	477	458
Poly-3 test	P=0.628N	P=0.648N	P=0.190N	P=0.648N

(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, pancreatic islets, 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] One adenoma occurred in an animal that also had a fibroadenoma.
[g] Value of statistic cannot be computed
[h] One stromal sarcoma occurred in an animal that also had a stromal polyp.

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of α-Methylstyrene[a]

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2			
Moribund	15	21	12	20
Natural deaths	6	5	2	4
Survivors				
Terminal sacrifice	27	24	36	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, rectum	(49)	(48)	(50)	(50)
Intestine small, ileum	(45)	(46)	(48)	(47)
Necrosis		1 (2%)		
Intestine small, jejunum	(45)	(46)	(48)	(48)
Diverticulum		2 (4%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Basophilic focus	21 (42%)	23 (46%)	21 (42%)	21 (42%)
Basophilic focus, multiple	5 (10%)	5 (10%)	10 (20%)	8 (16%)
Clear cell focus	6 (12%)	6 (12%)	6 (12%)	2 (4%)
Clear cell focus, multiple	3 (6%)		1 (2%)	
Eosinophilic focus	1 (2%)		2 (4%)	1 (2%)
Hematopoietic cell proliferation				1 (2%)
Hepatodiaphragmatic nodule	2 (4%)	6 (12%)	6 (12%)	7 (14%)
Inflammation, suppurative			1 (2%)	
Necrosis	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Thrombosis	1 (2%)		1 (2%)	
Vacuolization cytoplasmic	8 (16%)	5 (10%)	1 (2%)	
Bile duct, bile stasis	1 (2%)			
Bile duct, hyperplasia			2 (4%)	
Hepatocyte, regeneration			1 (2%)	
Serosa, fibrosis			1 (2%)	
Mesentery	(14)	(17)	(12)	(10)
Necrosis	14 (100%)	17 (100%)	12 (100%)	10 (100%)
Oral mucosa	(1)		(1)	
Inflammation, suppurative	1 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	1 (2%)			
Duct, cyst		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)		
Hyperplasia, squamous		4 (8%)	2 (4%)	2 (4%)
Inflammation, suppurative				1 (2%)
Ulcer	5 (10%)	5 (10%)	3 (6%)	2 (4%)
Epithelium, muscularis, inflammation, suppurative		1 (2%)		
Stomach, glandular	(49)	(50)	(50)	(50)
Erosion	2 (4%)			2 (4%)
Hyperplasia				1 (2%)
Ulcer		1 (2%)	1 (2%)	
Tongue		(1)	(2)	
Epithelium, hyperplasia		1 (100%)	1 (50%)	

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Cardiovascular System				
Blood vessel		(1)		(1)
Infiltration cellular, polymorphonuclear		1 (100%)		
Inflammation				1 (100%)
Thrombosis				1 (100%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy			1 (2%)	
Atrium, thrombosis	1 (2%)	1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)			
Hyperplasia	7 (14%)	5 (10%)	10 (20%)	8 (16%)
Hypertrophy		1 (2%)		2 (4%)
Necrosis	1 (2%)	1 (2%)	1 (2%)	
Thrombosis				1 (2%)
Vacuolization cytoplasmic	18 (36%)	18 (36%)	18 (36%)	12 (24%)
Adrenal medulla	(49)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Hyperplasia			2 (4%)	3 (6%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Pituitary gland	(50)	(49)	(50)	(50)
Cyst	4 (8%)	5 (10%)	3 (6%)	2 (4%)
Hemorrhage	2 (4%)		1 (2%)	1 (2%)
Hyperplasia	7 (14%)	8 (16%)	8 (16%)	14 (28%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	8 (16%)	5 (10%)	9 (18%)	6 (12%)
Follicle, cyst				1 (2%)
Follicular cell, hyperplasia				2 (4%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)			
Hyperplasia	7 (14%)	6 (12%)	3 (6%)	4 (8%)
Inflammation, chronic	2 (4%)		1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Cyst	9 (18%)	2 (4%)	9 (18%)	8 (16%)
Uterus	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Hydrometra			1 (2%)	
Necrosis	1 (2%)			
Thrombosis			1 (2%)	1 (2%)
Cervix, myometrium, hyperplasia	2 (4%)			
Endometrium, hyperplasia	6 (12%)	3 (6%)	4 (8%)	5 (10%)
Endometrium, inflammation, suppurative			1 (2%)	
Myometrium, hyperplasia		1 (2%)		
Vagina		(1)		(1)
Infiltration cellular, mixed cell				1 (100%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Hematopoietic System				
Lymph node	(2)	(3)	(2)	(5)
Inflammation, chronic active				1 (20%)
Pancreatic, hemorrhage			1 (50%)	
Lymph node, bronchial	(4)	(7)	(8)	(3)
Angiectasis	1 (25%)			
Congestion	1 (25%)			
Hemorrhage				1 (33%)
Hyperplasia, lymphoid	1 (25%)	1 (14%)	1 (13%)	
Lymph node, mandibular	(3)		(1)	(1)
Lymph node, mediastinal	(26)	(25)	(21)	(28)
Hyperplasia, lymphoid			1 (5%)	
Pigmentation				1 (4%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Congestion	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Accessory spleen		1 (2%)		
Fibrosis	1 (2%)		2 (4%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Pigmentation				1 (2%)
Thymus	(50)	(48)	(48)	(48)
Cyst		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	2 (4%)	3 (6%)	4 (8%)	1 (2%)
Inflammation, suppurative		1 (2%)	1 (2%)	
Necrosis				1 (2%)
Duct, cyst				1 (2%)
Epithelium, hyperplasia	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Hyperkeratosis	1 (2%)	1 (2%)		
Inflammation, chronic				1 (2%)
Ulcer		1 (2%)	3 (6%)	1 (2%)
Subcutaneous tissue, hemorrhage				1 (2%)
Subcutaneous tissue, inflammation, granulomatous		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Maxilla, fracture	1 (2%)			
Skeletal muscle	(2)	(1)		
Infiltration cellular, lipocyte	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	7 (14%)	7 (14%)	11 (22%)	8 (16%)
Hemorrhage	6 (12%)	4 (8%)	5 (10%)	1 (2%)
Cerebellum, hydrocephalus		1 (2%)		
Cerebrum, infiltration cellular, mononuclear cell, focal	1 (2%)			

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Inflammation, suppurative	2 (4%)		1 (2%)	
Inflammation, chronic	1 (2%)	1 (2%)		4 (8%)
Epiglottis, metaplasia, squamous	1 (2%)			1 (2%)
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Respiratory epithelium, metaplasia, squamous			1 (2%)	
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Infiltration cellular, polymorphonuclear		1 (2%)		
Inflammation, suppurative			1 (2%)	
Inflammation, granulomatous		1 (2%)		
Inflammation, chronic	5 (10%)	1 (2%)	6 (12%)	5 (10%)
Alveolar epithelium, hyperplasia	3 (6%)	6 (12%)	2 (4%)	5 (10%)
Alveolar epithelium, metaplasia, squamous	1 (2%)			
Alveolus, infiltration cellular, histiocyte	20 (40%)	19 (38%)	22 (44%)	30 (60%)
Bronchiole, foreign body		1 (2%)		
Bronchiole, hyperplasia	1 (2%)			1 (2%)
Bronchiole, inflammation, chronic		1 (2%)		
Interstitium, fibrosis				1 (2%)
Nose	(49)	(49)	(50)	(50)
Foreign body	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Inflammation, suppurative	2 (4%)	5 (10%)	6 (12%)	6 (12%)
Inflammation, chronic				1 (2%)
Glands, dilatation			2 (4%)	
Goblet cell, hyperplasia	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Nasolacrimal duct, inflammation, suppurative	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Olfactory epithelium, degeneration	1 (2%)	1 (2%)	7 (14%)	24 (48%)
Olfactory epithelium, degeneration, hyaline	4 (8%)	8 (16%)	6 (12%)	4 (8%)
Olfactory epithelium, hyperplasia, basal cell		14 (29%)	30 (60%)	49 (98%)
Olfactory epithelium, metaplasia	1 (2%)			1 (2%)
Respiratory epithelium, degeneration, hyaline	1 (2%)	3 (6%)	2 (4%)	
Respiratory epithelium, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Respiratory epithelium, metaplasia, squamous	1 (2%)		2 (4%)	2 (4%)
Pleura	(16)	(13)	(15)	(30)
Inflammation, chronic	16 (100%)	13 (100%)	15 (100%)	27 (90%)
Mesothelium, hyperplasia				1 (3%)
Special Senses System				
Eye	(48)	(49)	(50)	(50)
Atrophy				2 (4%)
Inflammation, suppurative	1 (2%)			
Anterior chamber, hemorrhage				1 (2%)
Lens, cataract	5 (10%)	1 (2%)	4 (8%)	5 (10%)
Lens, mineralization			3 (6%)	
Retina, atrophy	2 (4%)			2 (4%)
Sclera, metaplasia, osseous		1 (2%)		

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst			1 (2%)	
Infiltration cellular, lipocyte			1 (2%)	
Nephropathy	34 (69%)	27 (54%)	35 (70%)	31 (62%)
Capsule, hemorrhage		1 (2%)		
Cortex, infarct		1 (2%)		1 (2%)
Cortex, infarct, multiple			1 (2%)	
Papilla, mineralization	1 (2%)	6 (12%)	8 (16%)	7 (14%)
Pelvis, transitional epithelium, hyperplasia	5 (10%)	3 (6%)		
Pelvis, transitional epithelium, mineralization	31 (63%)	26 (52%)	31 (62%)	16 (32%)
Pelvis, dilatation		1 (2%)		
Renal tubule, degeneration	1 (2%)			
Renal tubule, pigmentation				2 (4%)
Ureter	(1)			
Transitional epithelium, hyperplasia	1 (100%)			
Urinary bladder	(50)	(50)	(50)	(50)
Serosa, edema		1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		2 (4%)

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

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	8	7	9
Natural deaths	10	10	3	5
Survivors				
Terminal sacrifice	35	32	40	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(42)	(41)	(45)	(39)
Intestine large, cecum	(43)	(42)	(47)	(45)
Intestine large, colon	(48)	(44)	(48)	(46)
Intestine small, ileum	(43)	(41)	(47)	(45)
Intestine small, jejunum	(43)	(41)	(48)	(45)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	4 (8%)			
Hepatocellular adenoma	15 (30%)	16 (32%)	13 (26%)	12 (24%)
Hepatocellular adenoma, multiple	9 (18%)	11 (22%)	14 (28%)	13 (26%)
Hepatocellular carcinoma	8 (16%)	10 (20%)	7 (14%)	14 (28%)
Hepatocellular carcinoma, multiple	2 (4%)	2 (4%)	4 (8%)	3 (6%)
Hepatocholangiocarcinoma		1 (2%)	1 (2%)	1 (2%)
Ito cell tumor, malignant	1 (2%)			
Mesentery	(5)	(3)	(2)	(5)
Pancreas	(50)	(48)	(49)	(49)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(49)	(48)
Squamous cell papilloma		1 (2%)		2 (4%)
Stomach, glandular	(48)	(47)	(49)	(47)
Tooth		(1)	(2)	(1)
Odontoma		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Hemangiosarcoma	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(49)	(49)	(50)
Capsule, adenoma		1 (2%)	1 (2%)	
Capsule, carcinoma		1 (2%)		
Adrenal medulla	(50)	(49)	(49)	(49)
Pheochromocytoma, benign		1 (2%)		1 (2%)
Islets, pancreatic	(50)	(48)	(49)	(49)
Carcinoma	1 (2%)			
Pituitary gland	(48)	(47)	(49)	(45)
Pars intermedia, adenoma	1 (2%)		1 (2%)	

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	3 (6%)		1 (2%)	2 (4%)
Follicular cell, carcinoma		1 (2%)		
General Body System				
Peritoneum		(4)		
Hepatocellular carcinoma, metastatic, liver		1 (25%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (25%)		
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(1)	(1)		(1)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(49)	(49)	(49)
Seminal vesicle	(49)	(49)	(49)	(45)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(49)
Lymph node		(4)	(1)	
Renal, hepatocholangiocarcinoma, metastatic, liver		1 (25%)		
Lymph node, bronchial	(42)	(34)	(37)	(33)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (3%)
Hepatocholangiocarcinoma, metastatic, liver			1 (3%)	
Lymph node, mandibular	(21)	(25)	(28)	(22)
Lymph node, mediastinal	(41)	(41)	(30)	(32)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (3%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (3%)	
Lymph node, mesenteric	(48)	(47)	(48)	(47)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Spleen	(50)	(49)	(49)	(47)
Hemangiosarcoma	1 (2%)			
Thymus	(41)	(46)	(44)	(40)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (3%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, granular cell tumor, benign				1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Musculoskeletal System				
Skeletal muscle		(2)		
Hepatocholangiocarcinoma, metastatic, liver		1 (50%)		

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar, adenoma	7 (14%)	4 (8%)	7 (14%)	7 (14%)
Alveolar/bronchiolar, adenoma, multiple	1 (2%)		1 (2%)	
Alveolar/bronchiolar, carcinoma	5 (10%)	2 (4%)	1 (2%)	1 (2%)
Alveolar/bronchiolar, carcinoma, multiple				1 (2%)
Hemangiosarcoma	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	4 (8%)	6 (12%)	4 (8%)	7 (14%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	1 (2%)
Bronchiole, adenoma, multiple			1 (2%)	
Bronchus, adenoma				1 (2%)
Nose	(50)	(50)	(50)	(50)
Pleura			(1)	(1)
Alveolar/bronchiolar, carcinoma, metastatic, lung				1 (100%)
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Trachea	(49)	(50)	(50)	(50)
Special Senses System				
Eye	(48)	(44)	(47)	(46)
Harderian gland	(50)	(49)	(50)	(47)
Adenoma	10 (20%)	4 (8%)	3 (6%)	7 (15%)
Carcinoma	1 (2%)		1 (2%)	2 (4%)
Urinary System				
Kidney	(50)	(49)	(50)	(49)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Renal tubule, adenoma			1 (2%)	
Urethra		(1)		(1)
Urinary bladder	(50)	(48)	(50)	(48)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)	1 (2%)	1 (2%)
Lymphoma, malignant		1 (2%)	3 (6%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	43	40	37	44
Total primary neoplasms	72	62	61	70
Total animals with benign neoplasms	35	31	33	32
Total benign neoplasms	46	41	43	46
Total animals with malignant neoplasms	19	20	17	22
Total malignant neoplasms	26	21	18	24
Total animals with metastatic neoplasms	4	7	5	9
Total metastatic neoplasms	4	14	9	15

[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 α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Harderian Gland: Adenoma				
Overall rate[a]	10/50 (20%)	4/50 (8%)	3/50 (6%)	7/50 (14%)
Adjusted rate[b]	21.8%	9.3%	6.3%	16.3%
Terminal rate[c]	7/35 (20%)	2/32 (6%)	3/40 (8%)	7/36 (19%)
First incidence (days)	549	532	729 (T)	729 (T)
Poly-3 test[d]	P=0.362N	P=0.092N	P=0.029N	P=0.349N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	11/50 (22%)	4/50 (8%)	4/50 (8%)	8/50 (16%)
Adjusted rate	23.7%	9.3%	8.4%	18.6%
Terminal rate	7/35 (20%)	2/32 (6%)	4/40 (10%)	8/36 (22%)
First incidence (days)	549	532	729 (T)	729 (T)
Poly-3 test	P=0.426N	P=0.061N	P=0.039N	P=0.372N
Liver: Hemangiosarcoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.7%	0.0%	0.0%	0.0%
Terminal rate	1/35 (3%)	0/32 (0%)	0/40 (0%)	0/36 (0%)
First incidence (days)	456	—[e]	—	—
Poly-3 test	P=0.022N	P=0.073N	P=0.056N	P=0.069N
Liver: Hepatocellular Adenoma				
Overall rate	24/50 (48%)	27/50 (54%)	27/50 (54%)	25/50 (50%)
Adjusted rate	50.3%	59.7%	55.3%	55.3%
Terminal rate	16/35 (46%)	19/32 (59%)	23/40 (58%)	22/36 (61%)
First incidence (days)	486	453	383	429
Poly-3 test	P=0.453	P=0.238	P=0.385	P=0.389
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	12/50 (24%)	11/50 (22%)	17/50 (34%)
Adjusted rate	21.2%	27.4%	22.7%	36.3%
Terminal rate	3/35 (9%)	5/32 (16%)	7/40 (18%)	8/36 (22%)
First incidence (days)	549	537	565	429
Poly-3 test	P=0.081	P=0.329	P=0.529	P=0.082
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	28/50 (56%)	36/50 (72%)	33/50 (66%)	37/50 (74%)
Adjusted rate	57.7%	77.4%	66.7%	76.7%
Terminal rate	17/35 (49%)	23/32 (72%)	26/40 (65%)	26/36 (72%)
First incidence (days)	486	453	383	429
Poly-3 test	P=0.093	P=0.031	P=0.239	P=0.035
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	8/50 (16%)	4/50 (8%)	8/50 (16%)	7/50 (14%)
Adjusted rate	17.8%	9.6%	16.8%	16.3%
Terminal rate	7/35 (20%)	4/32 (13%)	6/40 (15%)	7/36 (19%)
First incidence (days)	715	729 (T)	726	729 (T)
Poly-3 test	P=0.466	P=0.214N	P=0.560N	P=0.539N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.9%	4.8%	2.1%	4.6%
Terminal rate	3/35 (9%)	1/32 (3%)	1/40 (3%)	1/36 (3%)
First incidence (days)	555	711	729 (T)	537
Poly-3 test	P=0.167N	P=0.257N	P=0.094N	P=0.239N

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	13/50 (26%)	6/50 (12%)	9/50 (18%)	9/50 (18%)
Adjusted rate	28.2%	14.4%	18.9%	20.6%
Terminal rate	10/35 (29%)	5/32 (16%)	7/40 (18%)	8/36 (22%)
First incidence (days)	555	711	726	537
Poly-3 test	P=0.363N	P=0.092N	P=0.206N	P=0.279N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.7%	0.0%	2.1%	4.7%
Terminal rate	3/35 (9%)	0/32 (0%)	1/40 (3%)	2/36 (6%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.587N	P=0.132N	P=0.285N	P=0.520N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.7%	2.4%	2.1%	4.7%
Terminal rate	3/35 (9%)	1/32 (3%)	1/40 (3%)	2/36 (6%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.486N	P=0.332N	P=0.285N	P=0.520N
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.7%	0.0%	0.0%	0.0%
Terminal rate	1/35 (3%)	0/32 (0%)	0/40 (0%)	0/36 (0%)
First incidence (days)	456	—	—	—
Poly-3 test	P=0.022N	P=0.073N	P=0.056N	P=0.069N
All Organs: Malignant Lymphoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	2.4%	6.2%	2.3%
Terminal rate	0/35 (0%)	0/32 (0%)	2/40 (5%)	0/36 (0%)
First incidence (days)	—	550	608	705
Poly-3 test	P=0.328	P=0.488	P=0.131	P=0.491
All Organs: Benign Neoplasms				
Overall rate	35/50 (70%)	31/50 (62%)	33/50 (66%)	32/50 (64%)
Adjusted rate	73.3%	67.5%	66.8%	70.8%
Terminal rate	27/35 (77%)	21/32 (66%)	26/40 (65%)	29/36 (81%)
First incidence (days)	486	453	383	429
Poly-3 test	P=0.484N	P=0.346N	P=0.316N	P=0.485N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	20/50 (40%)	17/50 (34%)	22/50 (44%)
Adjusted rate	39.2%	44.8%	34.7%	46.1%
Terminal rate	8/35 (23%)	9/32 (28%)	10/40 (25%)	11/36 (31%)
First incidence (days)	456	532	565	429
Poly-3 test	P=0.366	P=0.366	P=0.401N	P=0.315

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

	Chamber Control	100 ppm	300 ppm	600 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	43/50 (86%)	40/50 (80%)	37/50 (74%)	44/50 (88%)
Adjusted rate	87.1%	84.7%	74.1%	89.6%
Terminal rate	30/35 (86%)	25/32 (78%)	28/40 (70%)	31/36 (86%)
First incidence (days)	456	453	383	429
Poly-3 test	P=0.450	P=0.482N	P=0.082N	P=0.465

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 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
Historical Incidence of Hepatocellular Neoplasms in Control Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Chamber Controls Given NTP-2000 Diet			
Decalin	22/50	10/50	28/50
Divinylbenzene	22/50	13/50	30/50
Indium phosphide	17/50	11/50	26/50
Methyl isobutyl ketone	17/50	12/50	27/50
Propylene glycol mono-*t*-butyl ether	18/50	9/50	25/50
Stoddard solvent (type IIC)	23/50	16/50	34/50
Vanadium pentoxide	15/50	14/50	26/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	134/350 (38.3%)	85/350 (24.3%)	196/350 (56.0%)
Mean ± standard deviation	38.3% ± 6.3%	24.3% ± 4.8%	56.0% ± 6.2%
Range	30%-46%	18%-32%	50%-68%
Overall Historical Incidence: All Routes			
Total (%)	490/1,506 (32.5%)	344/1,506 (22.8%)	745/1,506 (49.5%)
Mean ± standard deviation	32.6% ± 12.7%	22.9% ± 10.0%	49.5% ± 17.8%
Range	12%-63%	8%-46%	20%-85%

[a] Data as of January 28, 2005

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of α-Methylstyrene[a]

	Chamber Control	100 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	8	7	9
Natural deaths	10	10	3	5
Survivors				
Terminal sacrifice	35	32	40	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(42)	(41)	(45)	(39)
Degeneration, hyaline	1 (2%)			
Infiltration cellular, polymorphonuclear	1 (2%)			1 (3%)
Intestine, large, cecum	(43)	(42)	(47)	(45)
Hemorrhage		1 (2%)	1 (2%)	
Intestine, large, colon	(48)	(44)	(48)	(46)
Intestine, small, ileum	(43)	(41)	(47)	(45)
Intestine, small, jejunum	(43)	(41)	(48)	(45)
Liver	(50)	(50)	(50)	(50)
Basophilic focus	6 (12%)	5 (10%)	7 (14%)	3 (6%)
Clear cell focus	13 (26%)	8 (16%)	13 (26%)	14 (28%)
Eosinophilic focus	9 (18%)	5 (10%)	10 (20%)	6 (12%)
Fatty change			1 (2%)	
Hematopoietic cell proliferation		1 (2%)		
Infarct	2 (4%)		1 (2%)	2 (4%)
Inflammation, granulomatous		1 (2%)		
Mineralization		1 (2%)		
Mixed cell focus	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Necrosis	3 (6%)	4 (8%)	1 (2%)	4 (8%)
Tension lipidosis	3 (6%)	3 (6%)	3 (6%)	
Vacuolization, cytoplasmic, focal	1 (2%)	1 (2%)		
Bile duct, cyst				1 (2%)
Bile duct, degeneration, hyaline			1 (2%)	
Hepatocyte, erythrophagocytosis	1 (2%)			
Portal, infiltration, cellular, mononuclear cell			1 (2%)	
Mesentery	(5)	(3)	(2)	(5)
Artery, inflammation			1 (50%)	
Fat, inflammation, chronic active			1 (50%)	
Fat, necrosis	5 (100%)	2 (67%)	1 (50%)	5 (100%)
Pancreas	(50)	(48)	(49)	(49)
Atrophy	1 (2%)		1 (2%)	
Basophilic focus			1 (2%)	
Necrosis			1 (2%)	
Duct, cyst		1 (2%)		
Salivary gland	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Stomach, forestomach	(50)	(49)	(49)	(48)
Infiltration, cellular, mast cell				1 (2%)
Inflammation		2 (4%)	1 (2%)	5 (10%)
Ulcer	3 (6%)	3 (6%)		1 (2%)
Ulcer, focal				1 (2%)
Epithelium, hyperplasia	1 (2%)	4 (8%)	7 (14%)	11 (23%)

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Alimentary System (continued)				
Stomach, glandular	(48)	(47)	(49)	(47)
Hyperplasia	1 (2%)		2 (4%)	
Metaplasia, hepatocyte		1 (2%)		
Mineralization		1 (2%)		
Necrosis	1 (2%)	1 (2%)		
Epithelium, hyperplasia, focal		1 (2%)		
Tooth		(1)	(2)	(1)
Malformation		1 (100%)	2 (100%)	1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Metaplasia, osseous	1 (2%)			
Mineralization		1 (2%)		
Thrombosis		1 (2%)	3 (6%)	
Artery, inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)
Endocrine System				
Adrenal cortex	(50)	(49)	(49)	(50)
Hyperplasia	8 (16%)	12 (24%)	4 (8%)	2 (4%)
Hypertrophy	25 (50%)	13 (27%)	14 (29%)	13 (26%)
Inflammation, chronic active			1 (2%)	
Adrenal medulla	(50)	(49)	(49)	(49)
Hyperplasia	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Islets, pancreatic	(50)	(48)	(49)	(49)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Pituitary gland	(48)	(47)	(49)	(45)
Hemorrhage			1 (2%)	
Pars distalis, hyperplasia		1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, hyperplasia	14 (28%)	11 (22%)	22 (44%)	16 (32%)
General Body System				
Peritoneum		(4)		
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Hemorrhage			1 (2%)	
Penis	(1)	(1)		(1)
Inflammation, suppurative		1 (100%)		1 (100%)
Preputial gland	(50)	(50)	(50)	(50)
Inflammation, chronic active			2 (4%)	1 (2%)
Necrosis			1 (2%)	
Prostate	(50)	(49)	(49)	(49)
Hyperplasia		1 (2%)		
Inflammation, suppurative	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Seminal vesicle	(49)	(49)	(49)	(45)
Inflammation, suppurative	1 (2%)		1 (2%)	
Inflammation, chronic	1 (2%)	1 (2%)		
Testes	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	1 (2%)	1 (2%)	2 (4%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(49)
Angiectasis	1 (2%)			
Hyperplasia, mast cell				1 (2%)
Lymph node		(4)	(1)	
Lymph node, bronchial	(42)	(34)	(37)	(33)
Lymph node, mandibular	(21)	(25)	(28)	(22)
Lymph node, mediastinal	(41)	(41)	(30)	(32)
Lymph node, mesenteric	(48)	(47)	(48)	(47)
Angiectasis		1 (2%)		
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
Spleen	(50)	(49)	(49)	(47)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)	
Thymus	(41)	(46)	(44)	(40)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst, epithelial inclusion	1 (2%)			
Inflammation, acute	1 (2%)		1 (2%)	
Inflammation, chronic active	9 (18%)	2 (4%)	4 (8%)	3 (6%)
Musculoskeletal System				
Skeletal muscle		(2)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis				1 (2%)
Necrosis			1 (2%)	
Meninges, infiltration cellular, polymorphonuclear			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion, chronic			1 (2%)	
Inflammation, chronic active			1 (2%)	
Thrombosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia	6 (12%)	5 (10%)	2 (4%)	4 (8%)
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Bronchiole, hyperplasia	1 (2%)		1 (2%)	2 (4%)
Perivascular, infiltration cellular, mononuclear cell			1 (2%)	
Perivascular, inflammation, suppurative		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Glands, olfactory epithelium, hyperplasia	4 (8%)	50 (100%)	50 (100%)	50 (100%)
Olfactory epithelium, atrophy		2 (4%)	8 (16%)	12 (24%)
Olfactory epithelium, degeneration, hyaline		1 (2%)	1 (2%)	
Olfactory epithelium, metaplasia	6 (12%)	47 (94%)	49 (98%)	49 (98%)
Olfactory epithelium, necrosis		1 (2%)	1 (2%)	
Respiratory epithelium, degeneration, hyaline		1 (2%)		
Respiratory epithelium, hyperplasia				1 (2%)
Pleura			(1)	(1)
Trachea	(49)	(50)	(50)	(50)
Necrosis			1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Special Senses System				
Eye	(48)	(44)	(47)	(46)
Degeneration	1 (2%)			
Cornea, hyperplasia, squamous	1 (2%)			
Cornea, inflammation, chronic active	1 (2%)			1 (2%)
Harderian gland	(50)	(49)	(50)	(47)
Hyperplasia	3 (6%)	6 (12%)	2 (4%)	2 (4%)
Urinary System				
Kidney	(50)	(49)	(50)	(49)
Cyst	3 (6%)	1 (2%)		
Infarct	2 (4%)	3 (6%)	5 (10%)	7 (14%)
Inflammation, suppurative			4 (8%)	
Metaplasia, osseous	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Nephropathy	43 (86%)	37 (76%)	40 (80%)	38 (78%)
Artery, inflammation, chronic active			1 (2%)	
Capsule, fibrosis		2 (4%)		2 (4%)
Capsule, hemorrhage				1 (2%)
Papilla, inflammation, suppurative				1 (2%)
Pelvis, dilatation	3 (6%)		3 (6%)	2 (4%)
Renal tubule, accumulation, hyaline droplet				1 (2%)
Renal tubule, necrosis	1 (2%)			
Urethra		(1)		(1)
Bulbourethral gland, hyperplasia		1 (100%)		
Bulbourethral gland, inflammation, suppurative		1 (100%)		1 (100%)
Urinary bladder	(50)	(48)	(50)	(48)
Infiltration cellular, polymorphonuclear				1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	
Transitional epithelium, hyperplasia		1 (2%)	2 (4%)	

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

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	8	9	8	11
Natural deaths	3	3	4	2
Survivors				
Terminal sacrifice	39	38	37	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(47)	(43)	(41)	(43)
Intestine large, colon	(49)	(48)	(48)	(50)
Intestine large, rectum	(48)	(48)	(48)	(50)
Leiomyosarcoma			1 (2%)	
Intestine small, duodenum	(48)	(48)	(46)	(49)
Intestine small, ileum	(48)	(48)	(46)	(48)
Carcinoma		1 (2%)		
Intestine small, jejunum	(49)	(47)	(46)	(48)
Carcinoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)	1 (2%)		
Hemangiosarcoma	1 (2%)			
Hepatoblastoma				1 (2%)
Hepatocellular adenoma	6 (12%)	16 (32%)	9 (18%)	7 (14%)
Hepatocellular adenoma, multiple	4 (8%)	4 (8%)	12 (24%)	16 (32%)
Hepatocellular carcinoma	3 (6%)	7 (14%)	6 (12%)	17 (34%)
Hepatocellular carcinoma, multiple		2 (4%)		1 (2%)
Hepatocholangiocarcinoma			1 (2%)	
Mesentery	(8)	(5)	(5)	(6)
Pancreas	(49)	(50)	(49)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)	1 (2%)
Stomach, glandular	(49)	(48)	(49)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Sarcoma, metastatic, skin		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Capsule, adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(49)	(49)
Pheochromocytoma, benign	2 (4%)	1 (2%)	2 (4%)	
Islets, pancreatic	(49)	(50)	(50)	(50)

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Endocrine System (continued)				
Pituitary gland	(50)	(48)	(50)	(47)
Pars distalis, adenoma	4 (8%)	6 (13%)	4 (8%)	2 (4%)
Pars intermedia, adenoma			2 (4%)	
Thyroid gland	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Follicular cell, adenoma	3 (6%)		1 (2%)	2 (4%)
Follicular cell, carcinoma		1 (2%)		
General Body System				
None				
Genital System				
Clitoral gland	(43)	(41)	(42)	(41)
Squamous cell carcinoma	1 (2%)			
Ovary	(48)	(50)	(49)	(50)
Cystadenoma	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Luteoma		1 (2%)		
Tubulostromal adenoma				1 (2%)
Oviduct				(1)
Uterus	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)			
Leiomyoma	1 (2%)			
Polyp, stromal	3 (6%)		3 (6%)	
Endometrium, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(49)	(48)	(50)
Lymph node	(4)	(5)	(2)	(1)
Lymph node, bronchial	(41)	(41)	(39)	(37)
Hepatocellular carcinoma, metastatic, liver				1 (3%)
Hepatocholangiocarcinoma, metastatic, liver			1 (3%)	
Sarcoma, metastatic, skin		1 (2%)		
Lymph node, mandibular	(40)	(39)	(37)	(36)
Lymph node, mediastinal	(34)	(40)	(34)	(36)
Fibrosarcoma, metastatic, skin				1 (3%)
Hepatocholangiocarcinoma, metastatic, liver			1 (3%)	
Lymph node, mesenteric	(48)	(48)	(47)	(50)
Spleen	(50)	(50)	(49)	(50)
Thymus	(50)	(45)	(49)	(46)
Sarcoma, metastatic, skin		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma		1 (2%)		1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, liposarcoma	1 (2%)			
Subcutaneous tissue, liposarcoma, multiple				1 (2%)
Subcutaneous tissue, sarcoma	6 (12%)	2 (4%)		2 (4%)
Subcutaneous tissue, sarcoma, multiple				1 (2%)

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle			(1)	
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Larynx	(49)	(50)	(49)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar, adenoma	1 (2%)	2 (4%)	4 (8%)	4 (8%)
Alveolar/bronchiolar, carcinoma	1 (2%)	5 (10%)	1 (2%)	2 (4%)
Alveolar/bronchiolar, carcinoma, multiple			1 (2%)	
Fibrosarcoma, metastatic, skin				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	5 (10%)	3 (6%)	13 (26%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Sarcoma, metastatic, skin		1 (2%)		2 (4%)
Nose	(49)	(49)	(50)	(50)
Trachea	(49)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(47)	(46)	(49)
Harderian gland	(50)	(48)	(48)	(50)
Adenoma	3 (6%)	3 (6%)	3 (6%)	1 (2%)
Carcinoma	1 (2%)			1 (2%)
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Urinary bladder	(49)	(48)	(48)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Lymphoma, malignant	15 (30%)	6 (12%)	11 (22%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	36	42	41
Total primary neoplasms	67	61	69	68
Total animals with benign neoplasms	26	29	35	27
Total benign neoplasms	33	35	44	36
Total animals with malignant neoplasms	29	21	22	25
Total malignant neoplasms	34	26	25	32
Total animals with metastatic neoplasms	1	6	4	16
Total metastatic neoplasms	1	11	7	20

[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 α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate[b]	6.5%	6.7%	6.8%	2.2%
Terminal rate[c]	3/39 (8%)	2/38 (5%)	3/37 (8%)	1/37 (3%)
First incidence (days)	731 (T)	705	731 (T)	731 (T)
Poly-3 test[d]	P=0.217N	P=0.653	P=0.644	P=0.305N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	8.7%	6.7%	6.8%	4.3%
Terminal rate	4/39 (10%)	2/38 (5%)	3/37 (8%)	2/37 (5%)
First incidence (days)	731 (T)	705	731 (T)	731 (T)
Poly-3 test	P=0.278N	P=0.511N	P=0.521N	P=0.336N
Liver: Hepatocellular Adenoma				
Overall rate	10/50 (20%)	20/50 (40%)	21/50 (42%)	23/50 (46%)
Adjusted rate	21.7%	43.9%	47.5%	48.7%
Terminal rate	9/39 (23%)	16/38 (42%)	21/37 (57%)	19/37 (51%)
First incidence (days)	725	640	731 (T)	464
Poly-3 test	P=0.014	P=0.018	P=0.007	P=0.005
Liver: Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	9/50 (18%)	6/50 (12%)	18/50 (36%)
Adjusted rate	6.5%	19.6%	13.1%	37.8%
Terminal rate	1/39 (3%)	6/38 (16%)	4/37 (11%)	11/37 (30%)
First incidence (days)	634	537	416	612
Poly-3 test	P<0.001	P=0.056	P=0.234	P<0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	3/50 (6%)	9/50 (18%)	6/50 (12%)	19/50 (38%)
Adjusted rate	6.5%	19.6%	13.1%	39.9%
Terminal rate	1/39 (3%)	6/38 (16%)	4/37 (11%)	12/37 (32%)
First incidence (days)	634	537	416	612
Poly-3 test	P<0.001	P=0.056	P=0.234	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	13/50 (26%)	26/50 (52%)	24/50 (48%)	33/50 (66%)[e]
Adjusted rate	27.9%	56.0%	52.5%	68.0%
Terminal rate	10/39 (26%)	20/38 (53%)	22/37 (60%)	24/37 (65%)
First incidence (days)	634	537	416	464
Poly-3 test	P<0.001	P=0.004	P=0.012	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	4/50 (8%)
Adjusted rate	2.2%	4.5%	9.0%	8.7%
Terminal rate	1/39 (3%)	2/38 (5%)	3/37 (8%)	4/37 (11%)
First incidence (days)	731 (T)	731 (T)	663	731 (T)
Poly-3 test	P=0.114	P=0.492	P=0.169	P=0.179
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.2%	11.1%	4.5%	4.3%
Terminal rate	1/39 (3%)	4/38 (11%)	1/37 (3%)	1/37 (3%)
First incidence (days)	731 (T)	725	663	677
Poly-3 test	P=0.472N	P=0.096	P=0.488	P=0.502

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	7/50 (14%)	5/50 (10%)	6/50 (12%)
Adjusted rate	4.4%	15.6%	11.2%	13.0%
Terminal rate	2/39 (5%)	6/38 (16%)	4/37 (11%)	5/37 (14%)
First incidence (days)	731 (T)	725	663	677
Poly-3 test	P=0.255	P=0.073	P=0.203	P=0.135
Ovary: Cystadenoma				
Overall rate	2/48 (4%)	1/50 (2%)	3/49 (6%)	2/50 (4%)
Adjusted rate	4.5%	2.2%	6.9%	4.3%
Terminal rate	1/37 (3%)	0/38 (0%)	3/37 (8%)	2/37 (5%)
First incidence (days)	634	670	731 (T)	731 (T)
Poly-3 test	P=0.484	P=0.493N	P=0.490	P=0.680N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	6/48 (13%)	4/50 (8%)	2/47 (4%)
Adjusted rate	8.7%	13.5%	8.9%	4.5%
Terminal rate	3/39 (8%)	4/38 (11%)	2/37 (5%)	1/36 (3%)
First incidence (days)	670	556	649	527
Poly-3 test	P=0.176N	P=0.345	P=0.625	P=0.356N
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	6/50 (12%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	13.0%	4.4%	0.0%	6.5%
Terminal rate	3/39 (8%)	1/38 (3%)	0/37 (0%)	1/37 (3%)
First incidence (days)	670	640	—[1]	645
Poly-3 test	P=0.205N	P=0.140N	P=0.018N	P=0.241N
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	6/50 (12%)	3/50 (6%)	0/50 (0%)	4/50 (8%)
Adjusted rate	13.0%	6.6%	0.0%	8.6%
Terminal rate	3/39 (8%)	1/38 (3%)	0/37 (0%)	1/37 (3%)
First incidence (days)	670	640	—	645
Poly-3 test	P=0.308N	P=0.251N	P=0.018N	P=0.366N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.5%	0.0%	2.3%	4.3%
Terminal rate	2/39 (5%)	0/38 (0%)	1/37 (3%)	1/37 (3%)
First incidence (days)	705	—	731 (T)	686
Poly-3 test	P=0.602N	P=0.123N	P=0.320N	P=0.498N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.5%	2.2%	2.3%	4.3%
Terminal rate	2/39 (5%)	1/38 (3%)	1/37 (3%)	1/37 (3%)
First incidence (days)	705	731 (T)	731 (T)	686
Poly-3 test	P=0.497N	P=0.314N	P=0.320N	P=0.498N
Uterus: Stromal Polyp				
Overall rate	3/50 (6%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	6.5%	0.0%	6.8%	0.0%
Terminal rate	3/39 (8%)	0/38 (0%)	2/37 (5%)	0/37 (0%)
First incidence (days)	731 (T)	—	719	—
Poly-3 test	P=0.200N	P=0.123N	P=0.645	P=0.118N

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

	Chamber Control	100 ppm	300 ppm	600 ppm
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.7%	0.0%	0.0%	0.0%
Terminal rate	3/39 (8%)	0/38 (0%)	0/37 (0%)	0/37 (0%)
First incidence (days)	705	—	—	—
Poly-3 test	P=0.027N	P=0.063N	P=0.066N	P=0.060N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/50 (10%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	10.9%	2.2%	0.0%	0.0%
Terminal rate	4/39 (10%)	0/38 (0%)	0/37 (0%)	0/37 (0%)
First incidence (days)	705	509	—	—
Poly-3 test	P=0.011N	P=0.104N	P=0.034N	P=0.031N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	2.2%	6.7%	2.2%
Terminal rate	0/39 (0%)	0/38 (0%)	0/37 (0%)	1/37 (3%)
First incidence (days)	621	705	702	731 (T)
Poly-3 test	P=0.539	P=0.754	P=0.291	P=0.759
All Organs: Malignant Lymphoma				
Overall rate	15/50 (30%)	6/50 (12%)	11/50 (22%)	4/50 (8%)
Adjusted rate	31.3%	13.3%	24.7%	8.7%
Terminal rate	11/39 (28%)	5/38 (13%)	9/37 (24%)	4/37 (11%)
First incidence (days)	149	640	656	731 (T)
Poly-3 test	P=0.019N	P=0.031N	P=0.320N	P=0.005N
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	29/50 (58%)	35/50 (70%)	27/50 (54%)
Adjusted rate	55.5%	61.9%	77.5%	56.4%
Terminal rate	21/39 (54%)	22/38 (58%)	30/37 (81%)	22/37 (60%)
First incidence (days)	634	509	649	464
Poly-3 test	P=0.500	P=0.338	P=0.019	P=0.546
All Organs: Malignant Neoplasms				
Overall rate	29/50 (58%)	21/50 (42%)	22/50 (44%)	25/50 (50%)
Adjusted rate	58.4%	45.2%	47.0%	52.1%
Terminal rate	19/39 (49%)	14/38 (37%)	13/37 (35%)	15/37 (41%)
First incidence (days)	149	537	416	612
Poly-3 test	P=0.421N	P=0.136N	P=0.179N	P=0.336N

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

	Chamber Control	100 ppm	300 ppm	600 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	36/50 (72%)	42/50 (84%)	41/50 (82%)
Adjusted rate	80.6%	75.4%	89.2%	83.1%
Terminal rate	30/39 (77%)	27/38 (71%)	32/37 (87%)	29/37 (78%)
First incidence (days)	149	509	416	464
Poly-3 test	P=0.247	P=0.356N	P=0.183	P=0.476

(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 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] One animal with an adenoma also had a hepatoblastoma.
[f] Not applicable; no neoplasms in animal group

TABLE D3
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Chamber Controls Given NTP-2000 Diet			
Decalin	7/49	4/49	11/49
Divinylbenzene	17/49	5/49	19/49
Indium phosphide	12/50	6/50	18/50
Methyl isobutyl ketone	13/50	6/50	17/50
Propylene glycol mono-*t*-butyl ether	14/49	4/49	18/49
Stoddard solvent (type IIC)	9/50	6/50	13/50
Vanadium pentoxide	6/50	6/50	12/50
Overall Historical Incidence: Inhalation Studies			
Total (%)	78/347 (22.5%)	37/347 (10.7%)	108/347 (31.1%)
Mean ± standard deviation	22.5% ± 8.1%	10.7% ± 1.8%	31.1% ± 6.8%
Range	12%-35%	8%-12%	22%-39%
Overall Historical Incidence: All Routes			
Total (%)	312/1,549 (20.1%)	128/1,549 (8.3%)	408/1,549 (26.4%)
Mean ± standard deviation	21.2% ± 13.4%	8.7% ± 15.5%	27.7% ± 15.5%
Range	6%-61%	0%-26%	8%-63%

[a] Data as of January 28, 2005

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	8	9	8	11
Natural deaths	3	3	4	2
Survivors				
Terminal sacrifice	39	38	37	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, suppurative				1 (2%)
Gallbladder	(47)	(43)	(41)	(43)
Necrosis		1 (2%)		
Intestine large, colon	(49)	(48)	(48)	(50)
Intestine large, rectum	(48)	(48)	(48)	(50)
Intestine small, duodenum	(48)	(48)	(46)	(49)
Intestine small, ileum	(48)	(48)	(46)	(48)
Intestine small, jejunum	(49)	(47)	(46)	(48)
Peyer's patch, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	5 (10%)	2 (4%)	3 (6%)	3 (6%)
Clear cell focus	5 (10%)	1 (2%)	3 (6%)	5 (10%)
Eosinophilic focus	2 (4%)	5 (10%)	7 (14%)	12 (24%)
Hematopoietic cell proliferation			1 (2%)	
Infarct		1 (2%)	1 (2%)	
Mitotic alteration				1 (2%)
Mixed cell focus			1 (2%)	
Necrosis	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Tension lipidosis	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Mesentery	(8)	(5)	(5)	(6)
Fat, necrosis	7 (88%)	5 (100%)	5 (100%)	6 (100%)
Pancreas	(49)	(50)	(49)	(50)
Atrophy			1 (2%)	
Basophilic focus		1 (2%)		
Artery, inflammation, chronic active		1 (2%)		
Duct, cyst	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Inflammation, suppurative	1 (2%)			
Necrosis			1 (2%)	
Stomach, forestomach	(50)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Inflammation	1 (2%)			1 (2%)
Mineralization			1 (2%)	
Ulcer				1 (2%)
Epithelium, hyperplasia	3 (6%)		2 (4%)	1 (2%)
Epithelium, hyperplasia, basal cell				1 (2%)
Stomach, glandular	(49)	(48)	(49)	(50)
Metaplasia, hepatocyte	1 (2%)		1 (2%)	
Necrosis	2 (4%)			
Artery, inflammation, chronic active		1 (2%)		

[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 α-Methylstyrene

	Chamber Control	100 ppm	300 ppm	600 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy				1 (2%)
Hemorrhage		1 (2%)		
Inflammation, suppurative	1 (2%)			
Mineralization	2 (4%)		1 (2%)	
Necrosis				1 (2%)
Thrombosis	1 (2%)	1 (2%)		2 (4%)
Artery, inflammation, chronic active	1 (2%)	1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Hypertrophy	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Necrosis			1 (2%)	
Adrenal medulla	(50)	(50)	(49)	(49)
Hyperplasia	4 (8%)	3 (6%)	5 (10%)	4 (8%)
Necrosis			1 (2%)	
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia		1 (2%)	1 (2%)	
Pituitary gland	(50)	(48)	(50)	(47)
Pars distalis, angiectasis			2 (4%)	
Pars distalis, hyperplasia	9 (18%)	8 (17%)	4 (8%)	7 (15%)
Pars intermedia, hyperplasia		1 (2%)		
Pars intermedia, hypertrophy	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)	1 (2%)	
C-cell, hyperplasia		1 (2%)	1 (2%)	
Follicular cell, hyperplasia	15 (30%)	11 (22%)	10 (20%)	15 (30%)
General Body System				
None				
Genital System				
Clitoral gland	(43)	(41)	(42)	(41)
Ovary	(48)	(50)	(49)	(50)
Angiectasis		3 (6%)	1 (2%)	
Cyst	15 (31%)	8 (16%)	12 (24%)	8 (16%)
Thrombosis		2 (4%)		
Oviduct				(1)
Hyperplasia				1 (100%)
Uterus	(50)	(50)	(49)	(50)
Angiectasis	2 (4%)	1 (2%)		2 (4%)
Cyst		1 (2%)		
Fibrosis	1 (2%)			
Inflammation, suppurative	1 (2%)			
Necrosis				1 (2%)
Thrombosis				1 (2%)
Endometrium, hyperplasia, cystic	5 (10%)	8 (16%)	9 (18%)	9 (18%)
Lymphatic, cyst	1 (2%)			

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Hematopoietic System				
Bone marrow	(50)	(49)	(48)	(50)
Hyperplasia, reticulum cell		1 (2%)		
Inflammation, granulomatous			1 (2%)	
Lymph node	(4)	(5)	(2)	(1)
Lumbar, angiectasis	1 (25%)			
Renal, hyperplasia, lymphoid		1 (20%)		
Lymph node, bronchial	(41)	(41)	(39)	(37)
Hyperplasia, lymphoid		1 (2%)		
Lymph node, mandibular	(40)	(39)	(37)	(36)
Hyperplasia, lymphoid				1 (3%)
Lymph node, mediastinal	(34)	(40)	(34)	(36)
Hyperplasia, lymphoid	1 (3%)			
Lymph node, mesenteric	(48)	(48)	(47)	(50)
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, plasma cell		1 (2%)	1 (2%)	
Spleen	(50)	(50)	(49)	(50)
Hematopoietic cell proliferation	6 (12%)	5 (10%)	3 (6%)	5 (10%)
Hyperplasia, lymphoid		4 (8%)	1 (2%)	
Necrosis	1 (2%)			
Thymus	(50)	(45)	(49)	(46)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Infiltration cellular, mixed cell			1 (2%)	
Inflammation, acute		1 (2%)		
Necrosis			1 (2%)	
Epidermis, hyperplasia		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Maxilla, necrosis			1 (2%)	
Skeletal muscle			(1)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis			1 (2%)	
Infiltration cellular, mononuclear cell				1 (2%)
Inflammation, suppurative	1 (2%)			
Necrosis	2 (4%)			
Artery, meninges, inflammation, chronic active		1 (2%)		

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

	Chamber Control	100 ppm	300 ppm	600 ppm
Respiratory System				
Larynx	(49)	(50)	(49)	(50)
Inflammation, suppurative				1 (2%)
Artery, inflammation, chronic active		1 (2%)		
Lung	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation, granulomatous				1 (2%)
Thrombosis		1 (2%)		
Alveolar epithelium, hyperplasia	6 (12%)	2 (4%)	2 (4%)	5 (10%)
Alveolus, infiltration cellular, histiocyte	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Bronchiole, hyperplasia			2 (4%)	2 (4%)
Perivascular, inflammation, chronic active		1 (2%)		
Nose	(49)	(49)	(50)	(50)
Inflammation, suppurative				1 (2%)
Thrombosis				1 (2%)
Glands, olfactory epithelium, hyperplasia	3 (6%)	49 (100%)	50 (100%)	50 (100%)
Glands, inflammation, acute			1 (2%)	
Olfactory epithelium, atrophy	1 (2%)	6 (12%)	4 (8%)	3 (6%)
Olfactory epithelium, degeneration, hyaline	3 (6%)	2 (4%)		1 (2%)
Olfactory epithelium, metaplasia	2 (4%)	49 (100%)	47 (94%)	50 (100%)
Respiratory epithelium, necrosis	1 (2%)			
Trachea	(49)	(50)	(50)	(50)
Inflammation, suppurative				1 (2%)
Special Senses System				
Eye	(50)	(47)	(46)	(49)
Cataract				1 (2%)
Cornea, inflammation, chronic active			1 (2%)	
Cornea, necrosis	1 (2%)	1 (2%)		
Harderian gland	(50)	(48)	(48)	(50)
Hyperplasia	1 (2%)	2 (4%)	3 (6%)	5 (10%)
Necrosis			1 (2%)	
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Amyloid deposition		1 (2%)		
Infarct	2 (4%)	3 (6%)	6 (12%)	2 (4%)
Inflammation, suppurative	2 (4%)			
Metaplasia, osseous	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Nephropathy	16 (32%)	21 (43%)	12 (24%)	26 (52%)
Urinary bladder	(49)	(48)	(48)	(50)
Inflammation, chronic active		1 (2%)		
Artery, inflammation, chronic active		1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992). α-Methylstyrene was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of α-methylstyrene. The high dose was limited by toxicity. All trials were repeated at the same or a higher S9 fraction.

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

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

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

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 25.5 hours with α-methylstyrene in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 25.5 hours, the medium containing α-methylstyrene was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with α-methylstyrene, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no α-methylstyrene. Incubation proceeded for 25.2 to 25.5 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind, and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs per cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.,* 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more

doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any response reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with α-methylstyrene for 8 hours; Colcemid was added, and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with α-methylstyrene and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test.

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

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

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990) and Witt *et al.* (2000). At the end of the 3-month toxicity study of α-methylstyrene, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) in each of up to 10 animals per exposure group and in 1,000 polychromatic erythrocytes (PCEs) in up to 10 chamber control and 1,000 ppm mice. PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs or NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure 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 was considered positive if the trend test P value was less than or equal to 0.025 or if the P value for any single exposed group was less than or equal to 0.025/N where N equals the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call was determined by the scientific staff after considering the results of statistical analyses, 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

α-Methylstyrene tested over a concentration range of 1 to 3,333 µg/plate was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, in either the presence or the absence of 10% or 30% rat or hamster liver S9 metabolic activation enzymes (Table E1; Zeiger *et al.*, 1992). In cytogenetic tests with cultured Chinese hamster ovary cells, α-methylstyrene significantly increased the frequency of SCEs in cells exposed to concentrations of 50 to 149.9 µg/mL in the presence of S9 (Table E2); without S9, no significant increases in SCEs were observed. α-Methylstyrene in concentrations up to 251.3 µg/mL did not induce chromosomal aberrations (Table E3), with or without S9 activation. *In vivo*, no significant increases in the frequencies of micronucleated erythrocytes were seen in blood samples of male mice obtained at the conclusion of the 3-month study. However, in female mice from the 3-month study, a significant increase in micronucleated NCEs was observed at the highest exposure concentration of 1,000 ppm (Table E4), resulting in a negative call for male mice and a positive call in this assay for female mice. Reticulocytes (polychromatic immature erythrocytes; PCEs) were also scored for frequency of micronucleated cells in male and female mice. No increase in micronucleated PCEs was observed in either sex at the highest exposure concentration of 1,000 ppm, indicating that the damage observed in the mature erythrocyte population in 1,000 ppm females was reflective of long-term accumulation of damage and was not detectable immediately after exposure by analyzing recently-formed (within 48 hours) reticulocytes.

TABLE E1
Mutagenicity of α-Methylstyrene 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	119 ± 5.5	119 ± 4.4	127 ± 7.6	130 ± 6.2	127 ± 7.8	119 ± 3.1
	1		115 ± 5.8				
	3	114 ± 1.2	113 ± 10.0				
	10	106 ± 11.4	123 ± 11.5	133 ± 12.3		117 ± 5.5	
	33	115 ± 10.9	129 ± 0.3	151 ± 9.8	128 ± 5.5	122 ± 7.5	134 ± 12.9
	100	121 ± 4.8	128 ± 3.6	128 ± 11.9	100 ± 10.6	134 ± 3.8	143 ± 4.0
	333	0 ± 0.0[c]		111 ± 15.0	127 ± 7.5	117 ± 10.8	125 ± 4.4
	1,000			68 ± 9.1[c]	118 ± 9.3	84 ± 7.9[c]	118 ± 5.8
	3,333				16 ± 8.1[c]		16 ± 8.5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		422 ± 14.1	433 ± 6.7	514 ± 22.9	590 ± 15.9	419 ± 13.7	490 ± 16.5
TA1535	0	15 ± 1.3	17 ± 0.7	14 ± 0.7	12 ± 1.2	15 ± 0.7	14 ± 0.7
	1	17 ± 1.8	13 ± 0.3				
	3	11 ± 1.2	17 ± 0.3				
	10	10 ± 1.8	17 ± 0.7	12 ± 1.5	13 ± 2.3	9 ± 0.3	15 ± 2.7
	33	15 ± 2.3	19 ± 4.7	13 ± 1.5	8 ± 0.7	10 ± 1.2	11 ± 0.6
	100	10 ± 1.9	10 ± 2.3	11 ± 2.5	7 ± 0.7	6 ± 2.0	10 ± 1.2
	333			12 ± 0.6	7 ± 1.2	9 ± 0.9	12 ± 4.5
	1,000			1 ± 0.7[c]	6 ± 0.9	5 ± 2.0[c]	10 ± 2.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		269 ± 8.7	479 ± 32.9	196 ± 12.5	411 ± 12.3	146 ± 12.2	89 ± 14.2
TA97	0	163 ± 4.4	156 ± 9.9	141 ± 5.2	173 ± 13.4	122 ± 6.0	187 ± 13.3
	1	163 ± 6.1	170 ± 7.1				
	3	156 ± 7.7	176 ± 3.8				
	10	149 ± 0.9	168 ± 6.4	146 ± 9.6	179 ± 13.9	157 ± 9.5	204 ± 3.8
	33	156 ± 5.2	186 ± 6.5	169 ± 4.4	175 ± 13.1	130 ± 8.5	209 ± 1.5
	100	128 ± 8.1	136 ± 6.8	114 ± 8.7	176 ± 3.0	113 ± 7.8	192 ± 15.7
	333			119 ± 5.5	129 ± 9.0	103 ± 11.0	196 ± 11.9
	1,000			79 ± 11.6[c]	100 ± 11.5	122 ± 18.8[c]	132 ± 23.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		331 ± 13.6	501 ± 48.5	412 ± 27.3	451 ± 5.0	385 ± 18.6	283 ± 13.4

TABLE E1
Mutagenicity of α-Methylstyrene in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA98	0	17 ± 3.5	18 ± 0.9	32 ± 6.7	26 ± 3.7	29 ± 1.2	21 ± 1.5
	1		18 ± 2.0				
	3	19 ± 3.2	16 ± 2.5				
	10	18 ± 1.9	15 ± 0.3	29 ± 6.0		26 ± 3.8	
	33	19 ± 2.5	15 ± 0.0	27 ± 1.8	19 ± 0.7	27 ± 2.0	21 ± 2.1
	100	12 ± 0.9	20 ± 2.6	25 ± 2.9	21 ± 3.0	30 ± 2.4	23 ± 2.3
	333	0 ± 0.0[c]		25 ± 2.2	29 ± 3.7	24 ± 1.7	20 ± 1.5
	1,000			16 ± 0.6[c]	17 ± 0.6	10 ± 3.6[c]	25 ± 3.5
	3,333				0 ± 0.0[c]		8 ± 0.5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		667 ± 85.0	785 ± 24.7	346 ± 3.5	274 ± 32.1	275 ± 24.2	87 ± 12.4

[a] Study performed at SRI International. The detailed protocol and these data are 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.

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

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
–S9								
Summary: Negative								
Dimethylsulfoxide[c]		50	1,050	351	0.33	7.02	25.5	
α-Methylstyrene	5.0	50	1,049	340	0.32	6.80	25.5	–3.04
	16.7	50	1,049	336	0.32	6.72	25.5	–4.18
	50.0	50	1,050	359	0.34	7.18	25.5	2.28
	166.7	Toxic					0.0	
					P=0.411[d]			
Mitomycin-C[e]	0.001	50	1,049	578	0.55	11.56	25.5	64.83
	0.010	5	104	200	1.92	40.00	25.5	475.28
+S9								
Trial 1								
Summary: Weakly Positive								
Dimethylsulfoxide		50	1,050	380	0.36	7.60	25.5	
α-Methylstyrene	5.0	50	1,048	399	0.38	7.98	25.5	5.20
	16.7	50	1,050	373	0.36	7.46	25.5	–1.84
	50.0	50	1,050	488	0.46	9.76	25.5	28.42*
	166.7	Toxic					0.0	
					P≤0.001			
Cyclophosphamide[e]	0.4	50	1,050	682	0.65	13.64	25.5	79.47*
	2.0	5	104	153	1.47	30.60	25.5	306.50*

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by α-Methylstyrene

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9 (continued)								
Trial 2								
Summary: Positive								
Dimethylsulfoxide		25	525	178	0.34	7.12	25.2	
α-Methylstyrene	50.0	25	524	248	0.47	9.92	25.2	39.59*
	124.4	25	524	265	0.51	10.60	25.2	49.16*
	149.9	25	518	321	0.62	12.84	25.2	82.77*
					P≤0.001			
Cyclophosphamide	0.4	25	522	344	0.66	13.76	25.2	94.37
	2.0	5	104	180	1.73	36.00	25.5	410.48

* Positive response (≥20% increase over the solvent control)
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al.* (1987).
[b] SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[c] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[d] Solvent control
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
Positive control

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by α-Methylstyrene[a]

Compound	Dose (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
–S9					
Trial 1					
Harvest time: 10.0 hours					
Summary: Negative					
Dimethylsulfoxide[b]		200	6	0.03	3.0
α-Methylstyrene	100.5	200	1	0.01	0.5
	150.0	200	2	0.01	1.0
	200.0	200	5	0.03	2.5
					P=0.616[c]
Mitomycin-C[d]	0.25	200	15	0.08	7.0
	0.75	25	19	0.76	56.0
Trial 2					
Harvest time: 10.0 hours					
Summary: Negative					
Dimethylsulfoxide		200	2	0.01	1.0
α-Methylstyrene	37.7	200	9	0.05	3.5
	50.3	200	5	0.03	2.5
	125.7	200	3	0.02	1.5
	251.3	Toxic			
					P=0.480
Mitomycin-C	0.25	200	21	0.11	9.5
	0.75	25	17	0.68	48.0

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by α-Methylstyrene

Compound	Dose (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
+S9					
Trial 1					
Harvest time: 12.0 hours					
Summary: Negative					
Dimethylsulfoxide		200	3	0.02	1.5
α-Methylstyrene	100.5	200	7	0.04	3.5
	150.0	200	8	0.04	4.0
	200.0	200	6	0.03	3.0
					P=0.166
Cyclophosphamide[d]	7.5	200	27	0.14	12.5
	37.5	25	19	0.76	56.0
Trial 2					
Harvest time: 12.0 hours					
Summary: Negative					
Dimethylsulfoxide		200	5	0.03	2.0
α-Methylstyrene	37.7	200	8	0.04	3.5
	50.3	200	5	0.03	2.0
	125.7	200	10	0.05	5.0
	251.3	Toxic			
					P=0.052
Cyclophosphamide	7.5	200	25	0.13	8.0
	37.5	25	16	0.64	56.0

[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al.* (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with α-Methylstyrene by Inhalation for 3 Months[a]

Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated Cells/1,000 Cells				PCEs (%)[b]
		PCEs[b]	P Value[c]	NCEs[b]	P Value[c]	
Male						
Chamber control	10	3.90 ± 0.66		5.30 ± 0.50		3.70 ± 0.17
75	10	—[d]		5.80 ± 0.44	0.3171	3.36 ± 0.09
150	10	—		5.80 ± 0.63	0.3171	3.12 ± 0.10
300	10	—		5.00 ± 0.65	0.6165	3.20 ± 0.15
600	10	—		4.60 ± 0.45	0.7597	3.18 ± 0.17
1,000	10	5.00 ± 0.58	0.1213	6.30 ± 1.02	0.1759	3.27 ± 0.17
				P=0.346[e]		
Female						
Chamber control	10	4.10 ± 0.59		5.10 ± 0.46		3.76 ± 0.19
75	10	—		2.40 ± 0.43	0.9991	3.19 ± 0.10
150	10	—		2.90 ± 0.90	0.9931	3.42 ± 0.15
300	10	—		3.60 ± 0.48	0.9465	3.45 ± 0.14
600	10	—		5.30 ± 0.42	0.4221	3.27 ± 0.14
1,000	8	4.75 ± 0.59	0.2561	9.13 ± 0.77	0.0006	3.53 ± 0.29
				P≤0.001		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al*. (1990) and Witt *et al.* (2000).
NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the chamber controls, significant at P≤0.025 (PCEs) or P≤0.005 (NCEs) (ILS, 1990)
[d] Not tested
[e] Significance of micronucleated cells/1,000 cells tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 3	44.0 ± 0.6	44.0 ± 0.7	44.1 ± 0.8	43.8 ± 0.8	44.2 ± 0.6	43.8 ± 0.8[b]
Day 23	48.5 ± 0.5	48.2 ± 0.4	48.6 ± 0.5	48.2 ± 0.4	48.2 ± 0.6	49.4 ± 0.5
Week 14	44.7 ± 0.4	44.5 ± 0.5	42.9 ± 0.3**	43.0 ± 0.4**	42.3 ± 0.3**	42.3 ± 0.3**
Packed cell volume (mL/dL)						
Day 3	42.0 ± 0.6	41.7 ± 0.7	41.3 ± 0.7	41.5 ± 0.9	42.2 ± 0.6	42.2 ± 0.7
Day 23	48.0 ± 0.7	47.8 ± 0.5	47.8 ± 0.6	47.4 ± 0.3	47.7 ± 0.6	49.5 ± 0.7
Week 14	44.6 ± 0.4	44.4 ± 0.5	44.0 ± 0.6	43.0 ± 0.4*	42.3 ± 0.3**	42.4 ± 0.5**
Hemoglobin (g/dL)						
Day 3	14.1 ± 0.2	13.9 ± 0.2	13.8 ± 0.3	13.7 ± 0.2	13.8 ± 0.2	14.0 ± 0.2
Day 23	15.3 ± 0.3	15.3 ± 0.2	15.2 ± 0.2	15.1 ± 0.1	15.1 ± 0.2	15.5 ± 0.2
Week 14	15.0 ± 0.1	14.8 ± 0.2	14.6 ± 0.2	14.7 ± 0.1	14.4 ± 0.1**	14.3 ± 0.1**
Erythrocytes (10^6/μL)						
Day 3	6.57 ± 0.10	6.54 ± 0.11	6.51 ± 0.11	6.56 ± 0.14	6.64 ± 0.10	6.70 ± 0.14
Day 23	8.00 ± 0.14	7.84 ± 0.11	7.97 ± 0.16	7.73 ± 0.08	7.80 ± 0.13	8.10 ± 0.11
Week 14	8.31 ± 0.08	8.27 ± 0.09	8.18 ± 0.11	8.00 ± 0.07*	7.87 ± 0.06**	7.88 ± 0.09**
Reticulocytes (10^6/μL)						
Day 3	0.44 ± 0.02	0.45 ± 0.04	0.41 ± 0.03	0.43 ± 0.04	0.45 ± 0.04	0.39 ± 0.03
Day 23	0.21 ± 0.02	0.19 ± 0.01	0.19 ± 0.02	0.20 ± 0.02	0.24 ± 0.03	0.21 ± 0.02
Week 14	0.17 ± 0.01	0.17 ± 0.01	0.20 ± 0.09	0.17 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
Mean cell volume (fL)						
Day 3	63.8 ± 0.4	64.0 ± 0.3	63.4 ± 0.5	63.2 ± 0.3	63.5 ± 0.3	63.0 ± 0.3
Day 23	60.0 ± 0.5	61.0 ± 0.4	60.2 ± 0.7	61.3 ± 0.5	61.2 ± 0.4	61.2 ± 0.4
Week 14	53.6 ± 0.2	53.7 ± 0.2	53.8 ± 0.2	53.7 ± 0.2	53.9 ± 0.1	53.8 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	21.4 ± 0.2	21.3 ± 0.2	21.2 ± 0.3	20.8 ± 0.2	20.7 ± 0.1	20.9 ± 0.2
Day 23	19.1 ± 0.2	19.5 ± 0.2	19.1 ± 0.2	19.6 ± 0.2	19.4 ± 0.2	19.1 ± 0.2
Week 14	18.1 ± 0.1	17.9 ± 0.1	17.9 ± 0.1	18.4 ± 0.1	18.3 ± 0.1	18.2 ± 0.2
Mean cell hemoglobin concentration (g/dL)						
Day 3	33.5 ± 0.4	33.3 ± 0.4	33.4 ± 0.2	32.9 ± 0.3	32.6 ± 0.2	33.2 ± 0.3
Day 23	31.8 ± 0.3	31.9 ± 0.2	31.8 ± 0.2	31.9 ± 0.1	31.6 ± 0.3	31.3 ± 0.1
Week 14	33.7 ± 0.2	33.5 ± 0.1	33.3 ± 0.2	34.2 ± 0.2	34.1 ± 0.2	33.9 ± 0.4
Platelets (10^3/μL)						
Day 3	876.1 ± 35.5	887.3 ± 32.8	897.2 ± 23.0	948.0 ± 29.3	938.5 ± 35.9	935.9 ± 34.6
Day 23	777.5 ± 15.0	773.6 ± 19.9	784.5 ± 12.4	820.7 ± 15.5*	856.2 ± 17.4**	840.5 ± 28.3**
Week 14	632.4 ± 12.1	669.6 ± 12.6	618.5 ± 28.4	638.9 ± 10.6	667.0 ± 6.1	672.1 ± 13.7
Leukocytes (10^3/μL)						
Day 3	8.95 ± 0.48	9.56 ± 0.76	9.08 ± 0.61	8.39 ± 0.62	7.70 ± 0.68	6.32 ± 0.76*
Day 23	8.73 ± 0.60	9.36 ± 0.71	8.99 ± 0.60	9.97 ± 0.75	9.28 ± 0.57	8.27 ± 0.26
Week 14	8.85 ± 0.51	8.18 ± 0.50	6.70 ± 0.86	7.11 ± 0.85	7.95 ± 0.81	7.25 ± 0.70
Segmented neutrophils (10^3/μL)						
Day 3	0.96 ± 0.10	0.92 ± 0.05	1.05 ± 0.08	1.03 ± 0.13	1.42 ± 0.14	0.97 ± 0.11
Day 23	1.15 ± 0.12	1.18 ± 0.11	1.20 ± 0.12	1.25 ± 0.13	1.12 ± 0.12	1.29 ± 0.08
Week 14	1.28 ± 0.09	1.43 ± 0.12	1.14 ± 0.14	1.20 ± 0.16	1.23 ± 0.15	1.31 ± 0.17
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
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Lymphocytes (10^3/μL)						
Day 3	7.83 ± 0.41	8.40 ± 0.78	7.76 ± 0.59	7.13 ± 0.60	6.03 ± 0.53*	5.25 ± 0.72**
Day 23	7.37 ± 0.61	7.89 ± 0.76	7.62 ± 0.55	8.37 ± 0.68	7.85 ± 0.52	6.81 ± 0.25
Week 14	7.45 ± 0.53	6.61 ± 0.39	5.51 ± 0.73	5.78 ± 0.73	6.56 ± 0.68	5.81 ± 0.54
Monocytes (10^3/μL)						
Day 3	0.15 ± 0.04	0.21 ± 0.04	0.24 ± 0.04	0.19 ± 0.06	0.18 ± 0.05	0.09 ± 0.03
Day 23	0.18 ± 0.05	0.25 ± 0.07	0.13 ± 0.06	0.29 ± 0.07	0.24 ± 0.06	0.10 ± 0.02
Week 14	0.09 ± 0.03	0.11 ± 0.03	0.04 ± 0.02	0.10 ± 0.03	0.09 ± 0.04	0.11 ± 0.04
Basophils (10^3/μL)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.008 ± 0.008	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.024 ± 0.016	0.011 ± 0.011	0.000 ± 0.000	0.017 ± 0.012	0.018 ± 0.012
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 3	0.01 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.07 ± 0.03	0.00 ± 0.00
Day 23	0.04 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01
Week 14	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.08 ± 0.04	0.02 ± 0.01
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	7.7 ± 0.5	6.5 ± 0.2	7.1 ± 0.3	7.2 ± 0.6	9.3 ± 0.7	12.4 ± 0.4**
Day 23	9.1 ± 0.5	9.3 ± 0.5	10.0 ± 0.5	9.7 ± 0.4	11.1 ± 1.0	14.6 ± 0.5**
Week 14	13.7 ± 0.3	14.4 ± 0.2	15.3 ± 0.4*	14.6 ± 0.4	13.8 ± 0.6	14.2 ± 0.4
Creatinine (mg/dL)						
Day 3	0.68 ± 0.01	0.68 ± 0.02	0.67 ± 0.02	0.70 ± 0.00	0.68 ± 0.01	0.67 ± 0.02
Day 23	0.84 ± 0.02	0.85 ± 0.02	0.84 ± 0.03	0.84 ± 0.02	0.84 ± 0.02	0.86 ± 0.02
Week 14	0.97 ± 0.03	0.98 ± 0.03	0.97 ± 0.02	0.98 ± 0.03	0.96 ± 0.02	0.96 ± 0.02
Total protein (g/dL)						
Day 3	5.7 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
Day 23	5.9 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1*	6.2 ± 0.1*
Week 14	7.0 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	7.1 ± 0.1	7.1 ± 0.1
Albumin (g/dL)						
Day 3	3.8 ± 0.1	3.7 ± 0.1	3.6 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1
Day 23	3.6 ± 0.1	3.6 ± 0.1	3.7 ± 0.0	3.7 ± 0.0	3.8 ± 0.1*	3.8 ± 0.1*
Week 14	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.3 ± 0.1
Globulin (g/dL)						
Day 3	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1**
Day 23	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Week 14	2.8 ± 0.0	2.9 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.9 ± 0.1	2.8 ± 0.1
Albumin/globulin ratio						
Day 3	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.0	2.2 ± 0.1	2.2 ± 0.1	2.6 ± 0.1**
Day 23	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Week 14	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.0	1.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	64 ± 2	60 ± 3	59 ± 2	60 ± 2	54 ± 1**	51 ± 2**
Day 23	40 ± 1	38 ± 1	38 ± 1	37 ± 1*	33 ± 1**	33 ± 1**
Week 14	97 ± 9	86 ± 7	67 ± 2**	72 ± 6**	49 ± 2**	40 ± 1**

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male (continued)						
n	10	10	10	10	10	10
Clinical Chemistry (continued)						
Alkaline phosphatase (IU/L)						
Day 3	764 ± 16	780 ± 15	788 ± 33	783 ± 22	759 ± 23	667 ± 18*
Day 23	490 ± 12	466 ± 8	475 ± 15	486 ± 12	482 ± 16	463 ± 19
Week 14	275 ± 5	284 ± 12	294 ± 9	264 ± 12	264 ± 11	265 ± 7
Creatine kinase (IU/L)						
Day 3	388 ± 41	386 ± 37	366 ± 62	444 ± 68	384 ± 16	417 ± 46
Day 23	252 ± 18	225 ± 17	260 ± 16	264 ± 22	238 ± 11	240 ± 14
Week 14	125 ± 23	113 ± 16	107 ± 16	108 ± 9	84 ± 8	118 ± 14
Sorbitol dehydrogenase (IU/L)						
Day 3	11 ± 0	10 ± 0	10 ± 0	9 ± 0**	9 ± 0*	10 ± 0*
Day 23	13 ± 1	12 ± 0	12 ± 1	12 ± 1	11 ± 0	11 ± 0
Week 14	19 ± 1	17 ± 1	15 ± 0**	17 ± 1*	13 ± 0**	13 ± 0**
Bile acids (μmol/L)						
Day 3	32.8 ± 1.9	38.8 ± 3.5	34.3 ± 1.9	37.8 ± 1.6	43.6 ± 1.5**	44.0 ± 2.0**
Day 23	24.2 ± 1.7	26.9 ± 0.6	29.7 ± 1.0**	35.7 ± 1.0**	39.8 ± 1.5**	41.1 ± 1.2**
Week 14	31.1 ± 2.3	32.4 ± 2.3	29.7 ± 1.1	35.8 ± 1.7	35.1 ± 1.8	42.2 ± 1.7**
Urinalysis						
Creatinine (mg/dL)						
Week 12	74.00 ± 12.21	40.90 ± 4.30	58.70 ± 11.45	47.60 ± 5.44	34.60 ± 4.10**	33.60 ± 3.41**
Glucose (mg/dL)						
Week 12	8 ± 2	4 ± 1	6 ± 1	6 ± 1	4 ± 1	4 ± 0
Glucose/creatinine) ratio						
Week 12	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Protein (mg/dL)						
Week 12	46 ± 7	28 ± 3	39 ± 6	37 ± 4	32 ± 4	39 ± 4
Protein/creatinine ratio						
Week 12	0.65 ± 0.04	0.68 ± 0.02	0.72 ± 0.03	0.77 ± 0.03*	0.94 ± 0.04**	1.17 ± 0.03**
Alkaline phosphatase (IU/L)						
Week 12	204 ± 23	121 ± 13*	173 ± 29	173 ± 20	129 ± 14*	127 ± 10*
Alkaline phosphatase/creatinine ratio						
Week 12	3.05 ± 0.28	2.99 ± 0.14	3.15 ± 0.22	3.67 ± 0.20*	3.78 ± 0.11**	3.88 ± 0.18**
Aspartate aminotransferase (IU/L)						
Week 12	7 ± 2	3 ± 1	8 ± 2	13 ± 2	13 ± 2*	16 ± 1**
Aspartate aminotransferase/creatinine ratio						
Week 12	0.09 ± 0.01	0.08 ± 0.02	0.12 ± 0.01	0.26 ± 0.02**	0.39 ± 0.05**	0.48 ± 0.02**
Lactate dehydrogenase (IU/L)						
Week 12	39 ± 6	24 ± 3	39 ± 7	45 ± 6	47 ± 6	52 ± 4
Lactate dehydrogenase/creatinine ratio						
Week 12	0.54 ± 0.02	0.60 ± 0.02*	0.70 ± 0.04**	0.95 ± 0.02**	1.38 ± 0.09**	1.59 ± 0.05**
γ-Glutamyltransferase (IU/L)						
Week 12	1370 ± 193	877 ± 93	1172 ± 239	1032 ± 128	697 ± 67**	720 ± 55**
γ-Glutamyltransferase/creatinine ratio						
Week 12	19.10 ± 0.84	21.56 ± 0.67	20.09 ± 0.72	21.66 ± 0.65	20.57 ± 0.63	22.09 ± 0.95
N-acetyl-β-D-glucosaminidase (IU/L)						
Week 12	12 ± 2	6 ± 1*	9 ± 2	10 ± 1	8 ± 1	9 ± 1

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male (continued)						
n	10	10	10	10	10	10
Urinalysis (continued)						
N-acetyl-β-D-glucosaminidase/creatinine ratio						
Week 12	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.21 ± 0.01**	0.22 ± 0.01**	0.26 ± 0.01**
Volume (mL/16 hours)						
Week 12	12.0 ± 1.9	22.5 ± 2.5	17.9 ± 3.5	18.5 ± 2.5	24.7 ± 3.0**	25.0 ± 2.6**
Specific gravity						
Week 12	1.018 ± 0.003	1.011 ± 0.001	1.015 ± 0.002	1.014 ± 0.002	1.013 ± 0.001	1.015 ± 0.001
Female						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 3	46.1 ± 0.9	46.2 ± 0.7	46.9 ± 0.7	46.3 ± 0.4	45.8 ± 0.6	45.1 ± 0.7
Day 23	48.5 ± 0.4	48.0 ± 0.5	48.9 ± 0.6	48.3 ± 0.3	48.8 ± 0.7	49.4 ± 0.4
Week 14	43.4 ± 0.4	43.9 ± 0.5	43.7 ± 0.2	44.3 ± 0.3	43.5 ± 0.4	42.9 ± 0.4
Packed cell volume (mL/dL)						
Day 3	43.5 ± 1.1	43.9 ± 0.6	44.5 ± 0.7	44.7 ± 0.6	43.6 ± 0.9	43.1 ± 0.7
Day 23	46.0 ± 0.4	46.1 ± 0.5	46.4 ± 0.6	46.0 ± 0.4	46.4 ± 0.8	47.1 ± 0.5
Week 14	42.9 ± 0.3	43.7 ± 0.5	43.6 ± 0.3	43.2 ± 0.5	43.0 ± 0.5	43.0 ± 0.5
Hemoglobin (g/dL)						
Day 3	14.2 ± 0.4	14.4 ± 0.2	14.4 ± 0.3	14.6 ± 0.2	14.3 ± 0.2	14.4 ± 0.2
Day 23	15.3 ± 0.2	15.6 ± 0.2	15.7 ± 0.2	15.5 ± 0.2	15.5 ± 0.2	15.7 ± 0.2
Week 14	14.6 ± 0.1	14.8 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	14.7 ± 0.1	14.6 ± 0.1
Erythrocytes (10^6/μL)						
Day 3	6.75 ± 0.16	6.86 ± 0.12	6.99 ± 0.14	7.08 ± 0.12	6.88 ± 0.14	6.82 ± 0.12
Day 23	7.51 ± 0.08	7.49 ± 0.12	7.64 ± 0.12	7.53 ± 0.12	7.61 ± 0.12	7.65 ± 0.10
Week 14	7.39 ± 0.06	7.57 ± 0.09	7.57 ± 0.05	7.47 ± 0.10	7.44 ± 0.08	7.39 ± 0.08
Reticulocytes (10^6/μL)						
Day 3	0.36 ± 0.04	0.34 ± 0.03	0.39 ± 0.03	0.36 ± 0.04	0.36 ± 0.02	0.40 ± 0.04
Day 23	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.02
Week 14	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.02	0.11 ± 0.01
Mean cell volume (fL)						
Day 3	64.4 ± 0.2	63.8 ± 0.4	63.8 ± 0.5	63.2 ± 0.3*	63.4 ± 0.2*	63.1 ± 0.3**
Day 23	61.2 ± 0.3	61.6 ± 0.5	60.9 ± 0.4	61.2 ± 0.5	61.1 ± 0.4	61.8 ± 0.6
Week 14	58.1 ± 0.1	57.7 ± 0.2	57.6 ± 0.3	57.9 ± 0.2	57.9 ± 0.2	58.2 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	20.9 ± 0.1	20.9 ± 0.1	20.6 ± 0.1	20.6 ± 0.2	20.8 ± 0.2	21.0 ± 0.1
Day 23	20.4 ± 0.3	20.8 ± 0.2	20.5 ± 0.2	20.7 ± 0.2	20.4 ± 0.2	20.5 ± 0.3
Week 14	19.8 ± 0.1	19.6 ± 0.2	19.6 ± 0.1	20.0 ± 0.2	19.8 ± 0.1	19.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.5 ± 0.2	32.7 ± 0.2	32.4 ± 0.2	32.5 ± 0.2	32.9 ± 0.3	33.3 ± 0.1*
Day 23	33.4 ± 0.3	33.7 ± 0.2	33.8 ± 0.2	33.8 ± 0.2	33.5 ± 0.2	33.3 ± 0.2
Week 14	34.1 ± 0.2	33.9 ± 0.3	34.1 ± 0.3	34.5 ± 0.2	34.2 ± 0.3	34.0 ± 0.2

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematology (continued)						
Platelets (10^3/μL)						
Day 3	926.1 ± 33.2	845.2 ± 22.4	947.0 ± 44.4	847.8 ± 30.1	824.5 ± 20.7	898.6 ± 26.6
Day 23	738.1 ± 7.8	759.9 ± 17.6	735.3 ± 18.6	746.3 ± 12.5	717.9 ± 17.3	738.8 ± 12.0
Week 14	661.7 ± 8.1	672.5 ± 15.4	650.2 ± 14.0	641.1 ± 8.5	644.8 ± 11.3	650.5 ± 10.9
Leukocytes (10^3/μL)						
Day 3	10.64 ± 0.82	10.57 ± 0.58	10.77 ± 0.69	11.14 ± 0.45	9.12 ± 0.46	6.72 ± 0.42**
Day 23	8.60 ± 0.38	9.47 ± 0.51	9.42 ± 0.68	9.30 ± 0.75	9.20 ± 0.57	7.89 ± 0.27
Week 14	7.32 ± 0.48	7.43 ± 0.37	7.22 ± 0.54	7.12 ± 0.58	7.14 ± 0.62	6.42 ± 0.38
Segmented neutrophils (10^3/μL)						
Day 3	1.33 ± 0.17	1.16 ± 0.09	1.18 ± 0.18	1.08 ± 0.11	1.02 ± 0.13	1.15 ± 0.14
Day 23	1.08 ± 0.16	1.02 ± 0.11	1.48 ± 0.24	1.32 ± 0.20	1.21 ± 0.09	1.08 ± 0.13
Week 14	0.95 ± 0.11	1.36 ± 0.22	0.97 ± 0.11	0.95 ± 0.11	1.03 ± 0.12	1.00 ± 0.19
Bands (10^3/μL)						
Day 3	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 3	9.03 ± 0.65	9.25 ± 0.57	9.41 ± 0.66	9.85 ± 0.51	7.92 ± 0.42	5.48 ± 0.36**
Day 23	7.43 ± 0.30	8.32 ± 0.49	7.83 ± 0.50	7.90 ± 0.63	7.82 ± 0.51	6.69 ± 0.27
Week 14	6.27 ± 0.49	5.94 ± 0.24	6.15 ± 0.53	6.00 ± 0.53	6.04 ± 0.56	5.29 ± 0.19
Monocytes (10^3/μL)						
Day 3	0.15 ± 0.03	0.14 ± 0.03	0.11 ± 0.03	0.16 ± 0.05	0.12 ± 0.03	0.07 ± 0.02
Day 23	0.08 ± 0.03	0.10 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.11 ± 0.04	0.06 ± 0.03
Week 14	0.03 ± 0.01	0.09 ± 0.03	0.08 ± 0.03	0.12 ± 0.03	0.04 ± 0.02	0.06 ± 0.02
Basophils (10^3/μL)						
Day 3	0.014 ± 0.014	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.005 ± 0.005
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 3	0.09 ± 0.03	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	0.02 ± 0.01
Day 23	0.02 ± 0.01	0.03 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.05 ± 0.02
Week 14	0.07 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.08 ± 0.02

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Female (continued)						
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	7.7 ± 0.7	7.7 ± 0.6	7.5 ± 0.4	8.1 ± 0.5	10.5 ± 0.7**	13.0 ± 0.8**
Day 23	12.0 ± 0.5	11.6 ± 0.5	11.7 ± 0.6	11.6 ± 0.4	10.3 ± 0.6	11.7 ± 0.5
Week 14	17.2 ± 1.1	16.6 ± 0.3	16.3 ± 0.3	16.0 ± 0.3	15.5 ± 0.4*	14.8 ± 0.5**
Creatinine (mg/dL)						
Day 3	0.66 ± 0.02	0.65 ± 0.02	0.68 ± 0.01	0.65 ± 0.02	0.68 ± 0.01	0.65 ± 0.02
Day 23	0.83 ± 0.02	0.80 ± 0.02	0.83 ± 0.02	0.81 ± 0.02	0.77 ± 0.02	0.86 ± 0.02
Week 14	0.86 ± 0.02	0.88 ± 0.01	0.88 ± 0.03	0.90 ± 0.03	0.87 ± 0.02	0.92 ± 0.01
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Day 23	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Week 14	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
Albumin (g/dL)						
Day 3	3.6 ± 0.1	3.6 ± 0.0	3.7 ± 0.0	3.6 ± 0.0	3.8 ± 0.1	3.9 ± 0.1*
Day 23	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.0
Week 14	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1
Globulin (g/dL)						
Day 3	1.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.0
Day 23	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1
Week 14	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
Albumin/globulin ratio						
Day 3	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.3 ± 0.1*
Day 23	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
Week 14	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	51 ± 2	47 ± 1	47 ± 2	48 ± 2	45 ± 2*	42 ± 1**
Day 23	33 ± 1	34 ± 1	32 ± 1	31 ± 1	31 ± 1	31 ± 1
Week 14	70 ± 6	70 ± 3	65 ± 3	56 ± 5	49 ± 3**	35 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	617 ± 16	606 ± 19	581 ± 19	596 ± 20	567 ± 18	523 ± 10**
Day 23	360 ± 8	369 ± 11	375 ± 9	381 ± 14	376 ± 7	356 ± 11
Week 14	257 ± 13	258 ± 8	273 ± 6	277 ± 10	268 ± 12	264 ± 8
Creatine kinase (IU/L)						
Day 3	346 ± 24	349 ± 28	315 ± 33	407 ± 81	368 ± 33	374 ± 48
Day 23	209 ± 18	228 ± 37	246 ± 35	221 ± 19	308 ± 54	276 ± 53
Week 14	119 ± 18	123 ± 14	121 ± 11	139 ± 9	124 ± 23	106 ± 10
Sorbitol dehydrogenase (IU/L)						
Day 3	12 ± 0	11 ± 0	13 ± 0	11 ± 1	11 ± 0	10 ± 1
Day 23	12 ± 1	12 ± 0	13 ± 1	12 ± 1	13 ± 1	13 ± 1
Week 14	13 ± 1	15 ± 1	14 ± 0	14 ± 1	13 ± 0	13 ± 0
Bile acids (μmol/L)						
Day 3	25.7 ± 1.0	25.8 ± 2.0	27.1 ± 1.5	29.6 ± 1.4	32.5 ± 1.3**	36.1 ± 3.5**
Day 23	25.9 ± 1.6	24.4 ± 0.7	26.0 ± 0.9	28.5 ± 1.2	30.3 ± 1.5*	31.7 ± 1.9**
Week 14	25.7 ± 1.1	30.7 ± 3.8	30.5 ± 2.9	28.1 ± 1.3	25.2 ± 0.8	33.5 ± 1.8**

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of α-Methylstyrene

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Female (continued)						
Urinalysis						
n	10	10	10	10	10	10
Creatinine (mg/dL)						
Week 12	37.70 ± 3.01	48.10 ± 7.05	41.50 ± 6.75	30.60 ± 2.70	29.70 ± 3.37	41.70 ± 2.77
Glucose (mg/dL)						
Week 12	4 ± 0	5 ± 1	4 ± 1	2 ± 0	3 ± 0	4 ± 1
Glucose/creatinine ratio						
Week 12	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Protein (mg/dL)						
Week 12	3 ± 0	4 ± 0[b]	4 ± 1	3 ± 0	3 ± 0	4 ± 1
Protein/creatinine ratio						
Week 12	0.08 ± 0.01	0.09 ± 0.01[b]	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.01**	0.10 ± 0.01*
Alkaline phosphatase (IU/L)						
Week 12	79 ± 9	92 ± 11	81 ± 12	70 ± 7	87 ± 12	132 ± 8**
Alkaline phosphatase/creatinine ratio						
Week 12	2.05 ± 0.10	1.97 ± 0.07	1.97 ± 0.13	2.29 ± 0.14	2.87 ± 0.10**	3.22 ± 0.15**
Aspartate aminotransferase (IU/L)						
Week 12	0 ± 0	1 ± 0	1 ± 0	1 ± 0	0 ± 0	1 ± 0
Aspartate aminotransferase/creatinine ratio						
Week 12	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Lactate dehydrogenase (IU/L)						
Week 12	12 ± 2	15 ± 2[b]	16 ± 3	14 ± 2	18 ± 2	28 ± 2**
Lactate dehydrogenase/creatinine ratio						
Week 12	0.31 ± 0.02	0.34 ± 0.03[b]	0.38 ± 0.03*	0.46 ± 0.04**	0.60 ± 0.04**	0.69 ± 0.02**
γ-Glutamyltransferase (IU/L)						
Week 12	312 ± 34	356 ± 44	427 ± 71	295 ± 28	382 ± 35	569 ± 41**
γ-Glutamyltransferase/creatinine ratio						
Week 12	8.24 ± 0.51	7.70 ± 0.47	10.34 ± 0.77	9.65 ± 0.45	13.63 ± 1.36**	13.89 ± 0.91**
N-acetyl-β-D-glucosaminidase (IU/L)						
Week 12	5 ± 1	7 ± 1	6 ± 1	4 ± 1	4 ± 1	7 ± 0
N-acetyl-β-D-glucosaminidase/creatinine ratio						
Week 12	0.12 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01*	0.16 ± 0.01**
Volume (mL/16 hours)						
Week 12	13.5 ± 1.4	11.3 ± 1.5	12.0 ± 1.4	15.8 ± 1.9	16.8 ± 1.8	10.5 ± 0.7
Specific gravity						
Week 12	1.011 ± 0.001	1.014 ± 0.002	1.013 ± 0.002	1.011 ± 0.001	1.012 ± 0.001	1.020 ± 0.001**

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

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

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Hematocrit (%)	48.1 ± 0.6	49.1 ± 0.3	48.7 ± 0.2	48.5 ± 0.4	48.8 ± 0.4	48.6 ± 0.3
Packed cell volume (mL/dL)	48.0 ± 0.5	49.7 ± 0.2*	48.4 ± 0.4	49.1 ± 0.4	49.4 ± 0.3	48.3 ± 0.3
Hemoglobin (g/dL)	15.6 ± 0.1	15.9 ± 0.1	15.8 ± 0.1	15.7 ± 0.2	15.8 ± 0.1	15.7 ± 0.1
Erythrocytes (10^6/μL)	9.83 ± 0.10	10.13 ± 0.07	9.82 ± 0.05	9.94 ± 0.06	10.02 ± 0.06	9.80 ± 0.06
Reticulocytes (10^3/μL)	201.3 ± 44.0	184.0 ± 27.0	162.9 ± 43.0	159.5 ± 50.0	164.2 ± 28.0	164.6 ± 41.0
Howell-Jolly bodies (% erythrocytes)	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	48.9 ± 0.2	49.3 ± 0.3	49.2 ± 0.2	49.2 ± 0.3	49.2 ± 0.1	49.2 ± 0.2
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.7 ± 0.1	16.0 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	16.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.5 ± 0.2	31.9 ± 0.2	32.6 ± 0.2	32.0 ± 0.2	31.9 ± 0.1*	32.5 ± 0.1
Platelets (10^3/μL)	829.7 ± 10.6	838.7 ± 17.1	785.7 ± 10.4	820.5 ± 16.4	854.5 ± 17.2	917.5 ± 22.7
Leukocytes (10^3/μL)	2.65 ± 0.20	2.80 ± 0.29	3.41 ± 0.35	2.92 ± 0.26	3.38 ± 0.30	2.97 ± 0.23
Segmented neutrophils (10^3/μL)	0.34 ± 0.04	0.37 ± 0.07	0.46 ± 0.07	0.37 ± 0.05	0.41 ± 0.07	0.40 ± 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)	2.25 ± 0.15	2.39 ± 0.23	2.92 ± 0.30	2.49 ± 0.22	2.93 ± 0.28	2.47 ± 0.19
Monocytes (10^3/μL)	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.02	0.05 ± 0.03	0.04 ± 0.02	0.09 ± 0.03
Female						
n	10	10	10	10	10	8
Hematocrit (%)	50.8 ± 0.6	53.3 ± 0.7	51.8 ± 0.4	50.5 ± 0.6	49.1 ± 0.6	49.2 ± 0.5
Packed cell volume (mL/dL)	50.8 ± 0.6	52.5 ± 0.8	51.8 ± 0.4	50.3 ± 0.5	49.7 ± 0.6	49.2 ± 0.5
Hemoglobin (g/dL)	16.4 ± 0.2	16.9 ± 0.2	16.6 ± 0.1	16.1 ± 0.2	15.8 ± 0.2	15.7 ± 0.1*
Erythrocytes (10^6/μL)	10.18 ± 0.13	10.50 ± 0.12	10.33 ± 0.09	10.04 ± 0.10	9.83 ± 0.10	9.76 ± 0.11*
Reticulocytes (10^3/μL)	225.3 ± 88.0	203.2 ± 47.0	177.8 ± 51.0	187.2 ± 58.0	176.2 ± 27.0	154.3 ± 56.0*
Howell-Jolly bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	49.9 ± 0.2	50.0 ± 0.3	50.3 ± 0.2	50.1 ± 0.1	50.7 ± 0.2	50.5 ± 0.3
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.3 ± 0.2	32.3 ± 0.2	32.1 ± 0.1	32.1 ± 0.1	31.9 ± 0.2	31.9 ± 0.1
Platelets (10^3/μL)	716.4 ± 42.4	646.0 ± 25.2	693.3 ± 26.6	729.0 ± 21.1	763.5 ± 23.5	827.9 ± 29.9*
Leukocytes (10^3/μL)	3.16 ± 0.52	3.78 ± 0.27	3.62 ± 0.48	3.28 ± 0.38	2.78 ± 0.26	2.19 ± 0.28
Segmented neutrophils (10^3/μL)	0.44 ± 0.09	0.57 ± 0.10	0.55 ± 0.10	0.37 ± 0.04	0.29 ± 0.04	0.26 ± 0.03
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)	2.63 ± 0.41	3.10 ± 0.24	2.94 ± 0.38	2.80 ± 0.32	2.41 ± 0.22	1.86 ± 0.25
Monocytes (10^3/μL)	0.07 ± 0.03	0.06 ± 0.02	0.11 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 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)	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	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
[a] Data are given as mean ± standard error. Ratios were calculated and statistical tests were performed on unrounded data.

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	330 ± 7	338 ± 10	334 ± 6	329 ± 6	327 ± 5	313 ± 5
Heart						
Absolute	0.901 ± 0.014	0.950 ± 0.029	0.933 ± 0.023	0.912 ± 0.016	0.928 ± 0.019	0.916 ± 0.012
Relative	2.736 ± 0.038	2.811 ± 0.026	2.793 ± 0.034	2.776 ± 0.031	2.837 ± 0.039*	2.928 ± 0.029**
R. Kidney						
Absolute	1.002 ± 0.025	1.071 ± 0.038	1.064 ± 0.019	1.070 ± 0.024	1.088 ± 0.024	1.104 ± 0.021*
Relative	3.037 ± 0.035	3.166 ± 0.047*	3.188 ± 0.027**	3.254 ± 0.034**	3.324 ± 0.035**	3.526 ± 0.040**
Liver						
Absolute	10.24 ± 0.25	10.75 ± 0.36	11.40 ± 0.22*	11.28 ± 0.36*	12.65 ± 0.39**	13.56 ± 0.38**
Relative	31.031 ± 0.235	31.775 ± 0.388	34.159 ± 0.332**	34.266 ± 0.673**	38.612 ± 0.740**	43.269 ± 0.852**
Lung						
Absolute	1.541 ± 0.045	1.519 ± 0.047[b]	1.524 ± 0.035	1.475 ± 0.037	1.520 ± 0.054	1.491 ± 0.033
Relative	4.674 ± 0.109	4.504 ± 0.067[b]	4.566 ± 0.069	4.489 ± 0.093	4.636 ± 0.116	4.771 ± 0.124
R. Testis						
Absolute	1.441 ± 0.023[b]	1.432 ± 0.032	1.379 ± 0.028	1.417 ± 0.024	1.392 ± 0.048	1.372 ± 0.021
Relative	4.337 ± 0.084[b]	4.254 ± 0.085	4.131 ± 0.050	4.313 ± 0.034	4.259 ± 0.143	4.392 ± 0.090
Thymus						
Absolute	0.346 ± 0.018	0.353 ± 0.019	0.352 ± 0.015	0.327 ± 0.018	0.364 ± 0.011	0.322 ± 0.016
Relative	1.051 ± 0.059	1.040 ± 0.039	1.054 ± 0.038	0.995 ± 0.055	1.116 ± 0.040	1.029 ± 0.053
Female						
Necropsy body wt	201 ± 5	203 ± 5	203 ± 5	198 ± 4	202 ± 4	192 ± 4
Heart						
Absolute	0.662 ± 0.021	0.663 ± 0.014	0.662 ± 0.007	0.663 ± 0.017	0.672 ± 0.015	0.649 ± 0.015
Relative	3.296 ± 0.081	3.270 ± 0.046	3.283 ± 0.069	3.359 ± 0.088	3.327 ± 0.055	3.375 ± 0.050
R. Kidney						
Absolute	0.665 ± 0.015	0.673 ± 0.014	0.679 ± 0.013	0.673 ± 0.012	0.713 ± 0.017*	0.717 ± 0.014*
Relative	3.313 ± 0.055	3.319 ± 0.038	3.362 ± 0.060	3.408 ± 0.058	3.526 ± 0.027**	3.729 ± 0.036**
Liver						
Absolute	5.856 ± 0.178	5.929 ± 0.167	6.074 ± 0.126	6.001 ± 0.168	6.572 ± 0.208**	7.129 ± 0.183**
Relative	29.108 ± 0.414	29.190 ± 0.379	30.135 ± 0.907	30.376 ± 0.783	32.459 ± 0.464**	37.061 ± 0.561**
Lung						
Absolute	1.118 ± 0.032	1.182 ± 0.034	1.133 ± 0.043	1.159 ± 0.031	1.165 ± 0.030	1.120 ± 0.033
Relative	5.574 ± 0.153	5.836 ± 0.174	5.599 ± 0.178	5.859 ± 0.101	5.762 ± 0.070	5.819 ± 0.103
Thymus						
Absolute	0.289 ± 0.013	0.291 ± 0.007	0.299 ± 0.017	0.302 ± 0.013	0.297 ± 0.014	0.282 ± 0.011
Relative	1.438 ± 0.058	1.437 ± 0.031	1.474 ± 0.064	1.524 ± 0.051	1.472 ± 0.068	1.466 ± 0.047

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
** P≤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).
[b] n=9

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	75 ppm	150 ppm	300 ppm	600 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	38.7 ± 0.9	37.8 ± 0.8	38.5 ± 1.2	36.8 ± 0.7	33.7 ± 0.6**	32.3 ± 0.6**
Heart						
Absolute	0.159 ± 0.004	0.163 ± 0.004	0.168 ± 0.008	0.157 ± 0.006	0.149 ± 0.006	0.141 ± 0.004*
Relative	4.116 ± 0.100	4.321 ± 0.116	4.356 ± 0.104	4.265 ± 0.091	4.422 ± 0.142	4.371 ± 0.112
R. Kidney						
Absolute	0.325 ± 0.012	0.316 ± 0.007	0.332 ± 0.013	0.306 ± 0.008	0.275 ± 0.008**	0.261 ± 0.003**
Relative	8.383 ± 0.168	8.374 ± 0.172	8.626 ± 0.187	8.334 ± 0.185	8.169 ± 0.202	8.105 ± 0.160
Liver						
Absolute	1.484 ± 0.048	1.496 ± 0.034	1.582 ± 0.070	1.572 ± 0.038	1.551 ± 0.044	1.633 ± 0.031
Relative	38.368 ± 1.024	39.618 ± 0.741	40.954 ± 0.688*	42.822 ± 0.878**	46.039 ± 0.865**	50.622 ± 0.676**
Lung						
Absolute	0.233 ± 0.006	0.256 ± 0.007	0.238 ± 0.008	0.242 ± 0.006	0.249 ± 0.018	0.221 ± 0.005
Relative	6.023 ± 0.098	6.797 ± 0.239	6.199 ± 0.176	6.594 ± 0.153	7.377 ± 0.472**	6.868 ± 0.208**
R. Testis						
Absolute	0.118 ± 0.007	0.127 ± 0.002	0.129 ± 0.002	0.127 ± 0.003	0.123 ± 0.002	0.114 ± 0.003
Relative	3.059 ± 0.199	3.372 ± 0.076*	3.359 ± 0.087	3.455 ± 0.055*	3.673 ± 0.068**	3.542 ± 0.095**
Thymus						
Absolute	0.043 ± 0.001	0.048 ± 0.009	0.045 ± 0.003	0.039 ± 0.002	0.035 ± 0.002	0.036 ± 0.002
Relative	1.113 ± 0.039	1.275 ± 0.257	1.158 ± 0.064	1.049 ± 0.049	1.032 ± 0.039	1.124 ± 0.065
Female						
n	10	10	10	10	10	8
Necropsy body wt	31.0 ± 0.8	28.3 ± 0.6*	30.7 ± 0.7	28.3 ± 0.5*	29.7 ± 0.5	27.7 ± 0.7**
Heart						
Absolute	0.135 ± 0.002	0.136 ± 0.004	0.134 ± 0.002	0.128 ± 0.002	0.131 ± 0.001	0.129 ± 0.005
Relative	4.377 ± 0.107	4.804 ± 0.109*	4.385 ± 0.107	4.537 ± 0.098	4.419 ± 0.080	4.670 ± 0.208
R. Kidney						
Absolute	0.218 ± 0.004	0.207 ± 0.005	0.210 ± 0.003	0.206 ± 0.003	0.216 ± 0.004	0.210 ± 0.005
Relative	7.080 ± 0.232	7.331 ± 0.181	6.874 ± 0.171	7.297 ± 0.102	7.281 ± 0.138	7.602 ± 0.132
Liver						
Absolute	1.357 ± 0.034	1.277 ± 0.031	1.373 ± 0.031	1.332 ± 0.033	1.568 ± 0.025**	1.596 ± 0.055**
Relative	43.807 ± 0.543	45.130 ± 0.600	44.804 ± 0.680	47.132 ± 0.899**	52.813 ± 0.660**	57.645 ± 1.035**
Lung						
Absolute	0.262 ± 0.013	0.286 ± 0.013	0.295 ± 0.013	0.255 ± 0.008	0.247 ± 0.012	0.254 ± 0.014
Relative	8.520 ± 0.505	10.136 ± 0.478*	9.635 ± 0.400	9.030 ± 0.280	8.326 ± 0.400	9.206 ± 0.521
Thymus						
Absolute	0.055 ± 0.002	0.050 ± 0.001	0.050 ± 0.002	0.049 ± 0.004	0.053 ± 0.003	0.045 ± 0.003
Relative	1.764 ± 0.041	1.759 ± 0.058	1.631 ± 0.068	1.739 ± 0.116	1.779 ± 0.080	1.624 ± 0.098

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	330 ± 7	329 ± 6	327 ± 5	313 ± 5
L. Cauda epididymis	0.1749 ± 0.0055	0.1859 ± 0.0062	0.1854 ± 0.0049	0.1771 ± 0.0035
L. Epididymis	0.2821 ± 0.0121	0.2731 ± 0.0042	0.2798 ± 0.0087	0.2652 ± 0.0069
L. Testis	1.4083 ± 0.0562	1.4660 ± 0.0223	1.4692 ± 0.0256	1.4304 ± 0.0224
Spermatid measurements				
Spermatid heads (10^3/mg testis)	132.0 ± 6.6	141.2 ± 3.8	134.5 ± 5.8	129.7 ± 2.8
Spermatid heads (10^6/testis)	176.6 ± 12.5	195.6 ± 2.8	186.5 ± 8.8	174.0 ± 4.4
Spermatid count (10^6/cauda epididymis)	100.67 ± 7.40	103.90 ± 2.74	105.02 ± 3.97	96.16 ± 6.10
Epididymal spermatozoal measurements				
Motility (%)	77.36 ± 2.76	77.74 ± 3.03	84.33 ± 2.44	83.50 ± 1.52
Concentration (10^3/mg cauda epididymal tissue)	575.4 ± 37.8	563.2 ± 18.9	568.1 ± 23.0	542.4 ± 32.9

[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 by Dunn's test (spermatid measurements and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10
Necropsy body wt (g)	201 ± 5	198 ± 4	202 ± 4	192 ± 4
Estrous cycle length (days)	5.00 ± 0.13	4.90 ± 0.31	4.75 ± 0.17	5.00 ± 0.14[b]
Estrous stages (% of cycle)				
Diestrus	38.3	45.8	35.8	37.5
Proestrus	15.0	13.3	14.2	11.7
Estrus	27.5	22.5	31.7	33.3
Metestrus	19.2	18.3	18.3	17.5

[a] Necropsy body weight 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 weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages.

[b] Estrous cycle was longer than 12 days or unclear in one animal.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	300 ppm	600 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.7 ± 0.9	36.8 ± 0.7	33.7 ± 0.6**	32.3 ± 0.6**
L. Cauda epididymis	0.0207 ± 0.0008	0.0203 ± 0.0007	0.0185 ± 0.0005*	0.0180 ± 0.0008*
L. Epididymis	0.0299 ± 0.0006	0.0282 ± 0.0012	0.0279 ± 0.0010	0.0236 ± 0.0010**
L. Testis	0.1177 ± 0.0029	0.1201 ± 0.0023	0.1178 ± 0.0014	0.1081 ± 0.0026**
Spermatid measurements				
Spermatid heads (10^3/mg testis)	197.6 ± 10.7	194.1 ± 5.7	190.7 ± 7.8	195.2 ± 8.2
Spermatid heads (10^6/testis)	20.04 ± 1.22	19.98 ± 0.65	19.13 ± 0.86	17.69 ± 0.68
Spermatid count (10^6/cauda epididymis)	16.20 ± 0.86	17.34 ± 0.76	14.75 ± 0.39	15.52 ± 0.51
Epididymal spermatozoal measurements				
Motility (%)	80.55 ± 2.25	83.19 ± 2.01	79.20 ± 1.63	82.42 ± 1.53
Concentration (10^3/mg cauda epididymal tissue)	795.5 ± 55.5	862.4 ± 48.9	795.7 ± 15.8	876.8 ± 48.4

* Significantly different ($P \le 0.05$) from the chamber control group by Williams' test
** $P \le 0.01$
[a] Data are presented as mean ± standard error. Differences in spermatid and epididymal spermatozoal measurements between exposed groups and the chamber control group are not significant by Dunn's test.

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Inhalation Study of α-Methylstyrene[a]

	Chamber Control	300 ppm	600 ppm	1,000 ppm
n	10	10	10	8
Necropsy body wt (g)	31.0 ± 0.8	28.3 ± 0.5*	29.7 ± 0.5	27.7 ± 0.7**
Estrous cycle length (days)	3.94 ± 0.04	4.16 ± 0.14[b]	4.80 ± 0.56*[b]	5.21 ± 0.66**[b]
Estrous stages (% of cycle)				
Diestrus	33.3	30.8	25.0	17.7
Proestrus	0.0	1.7	5.8	3.1
Estrus	47.5	45.8	52.5	63.5
Metestrus	19.2	21.7	16.7	15.6

* Significantly different ($P \le 0.05$) from the chamber control group by Dunnett's test (body weight) or by Dunn's test (estrous cycle length).
** $P \le 0.01$
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in one animal.

APPENDIX I
CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF α-METHYLSTYRENE

α-Methylstyrene, stabilized with 4-*tert*-butyl catechol to inhibit oxidation and polymerization during storage, was obtained in one lot from Acros Organics (Fair Lawn, NJ) by the analytical chemistry laboratory, Research Triangle Institute (RTI) (Research Triangle Park, NC), and shipped to the study laboratory, Battelle Toxicology Northwest (Richland, WA), in two shipments that were reassigned lot numbers BNW 13871-4 and BNW 13871-54. Lot BNW 13871-4 was used in the 3-month and 2-year studies; lot BNW 13871-54 was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory (BNW 13871-4 only), the study laboratory, and Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO); stability analyses were also conducted by the study laboratory. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN), and Oneida Research Services (Whitesboro, NY; data not used). Reports on analyses performed in support of the α-methylstyrene studies are on file at the National Institute of Environmental Health Sciences.

Both shipments of the chemical, a colorless liquid with a sharp, sweet, aromatic odor, were identified as α-methylstyrene by the analytical chemistry and study laboratories using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the reference spectra of α-methylstyrene (*Aldrich* 1981, 1983, 1993, and 1997). Representative IR and NMR spectra are presented in Figures I1 and I2.

The purity of lots BNW 13871-4 and BNW 13871-54 was determined by Galbraith Laboratories, Inc., using elemental analyses, by Chemir/Polytech Laboratories using moisture analysis, by RTI using gas chromatography (GC) by system A (Table I1) (lot BNW 13871-4 only) and by the study laboratory using elemental analysis and GC by system B. The study laboratory monitored the concentration of 4-*tert*-butyl catechol by high-performance liquid chromatography (HPLC). HPLC analysis of 4-*tert*-butyl catechol included a Hewlett-Packard instrument (Hewlett-Packard, Palo Alto, CA) with fluorescence detection, a Waters Nova-Pac C-18 column (3.9 mm × 300 mm, 4 or 5 µm) (Waters, Milford, MA), and a mobile phase of 1% acetic acid in methanol (A) and 1% acetic acid in water (B). The mobile phase gradient was 0%A:100%B for 2 minutes, changed to 100%A:0%B over the next 11 minutes, held for 8 minutes, and then rapidly reversed to 0%A:100%B in 0.1 minute. The flow rate was 0.75 mL/minute, and detection was at 274 and 298 nm. Polymer concentration was monitored by the study laboratory with a turbidity assay using ultraviolet/visible (UV/Vis) spectroscopy (Beckman Instruments, Fullerton, CA).

For lot BNW 13871-4, Karl Fischer titration indicated 514 ppm water. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for α-methylstyrene. GC by system A indicated one major peak and several minor peaks, one of which had a relative peak area greater than 0.05% (0.066%); the purity was determined to be greater than 99%. GC by system B, relative to an independent standard purchased from Aldrich Chemical Co. (Milwaukee, WI), indicated a relative purity of 100.8%, consistent for samples taken from the top, middle, and bottom of the drum. GC by system C indicated one major peak and seven impurities, one of which had an area greater than 0.1% (0.21%) of the total peak area, indicating a purity of 99.48% for α-methylstyrene. Chromatograms of low concentrations of possible impurities or degradation products were obtained to show method resolution and sensitivity; the 0.21% impurity matched the retention time of *sec*-butylbenzene. Analysis by GC/mass spectrometry by system D matched the spectrum of the 0.21% impurity to library reference spectra of *sec*-butylbenzene (*NIST/EPA/NIH*, 1994) and the spectrum of a *sec*-butylbenzene standard. Concentrations of 4-*tert*-butyl catechol were well above the 8 ppm action criteria as a polymerization inhibitor set by the study laboratory. Polymer concentration by UV/Vis spectroscopy was less than 10 ppm. The overall purity of lot BNW 13871-4 was estimated at 99.5%.

For lot BNW 13871-54, Karl Fischer titration indicated 141 ppm water. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for α-methylstyrene. GC by a system similar to system B indicated a purity of 100.5% relative to an independent α-methylstyrene standard. GC by system C indicated one major peak and seven impurities, one of which had an area greater than 0.1% (0.21%) of the total peak area, indicating a purity of approximately 99.5% for α-methylstyrene. The 0.21% impurity peak had previously been identified as *sec*-butylbenzene. HPLC indicated the concentration of 4-*tert*-butyl catechol was well above 8 ppm, and UV/Vis spectroscopy indicated the polymer concentration was less than 10 ppm. The overall purity of lot BNW 13871-54 was estimated at 99.5%.

Periodic purity reanalyses of the bulk chemical relative to a reference standard of the same lot were performed by the study laboratory using GC by systems B and C, HPLC to determine 4-*tert*-butyl catechol concentration, and UV/Vis spectroscopy for polymer concentration. The purity reanalyses of the bulk chemical were performed at the beginning and end of each study and every 26 weeks during the 2-year studies. To ensure stability, the bulk chemical was stored at controlled room temperature in the original containers (55 gallon metal drums). No degradation of the chemical was detected, and 4-*tert*-butyl catechol and polymer concentrations remained within the study laboratory criteria (greater than 8 ppm and less than 10 ppm, respectively).

Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the studies is shown in Figure I3. The design of the system was influenced by the relatively high boiling point for α-methylstyrene (approximately 165° C) and the need to reach relatively high concentrations. Therefore, with the exception of individual chamber inlet flows, the vapor transport lines and all dilution air were heated.

α-Methylstyrene was held in an 8-gallon stainless steel chemical reservoir. α-Methylstyrene was pumped through a preheater and into the top of a heated glass column filled with glass beads to increase the surface area for evaporation. Heated nitrogen entering the column from below vaporized the chemical as it conveyed it 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 distribution manifold cabinet, the vapor was mixed with additional heated air before it entered a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution air flow rate, all of which were monitored by the exposure operator. The pressure in the distribution manifold was fixed to ensure constant flow through the manifold and into the chambers as the flow of vapor to each chamber was adjusted.

Electronically actuated metering valves controlled the flow to each chamber. In addition, an exposure-shutoff valve, mounted in series with each chamber-metering valve, controlled vapor delivery to each chamber. Vapor was diverted to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. To start the exposure, the valves were opened to allow the flow of vapor to reach the chamber-metering valves and move into individual temperature-controlled delivery lines to each chamber. The vapor was then injected into the chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) 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 (Model 3022A, TSI, Inc., St. Paul, MN) was used to count the particles in all chambers before and during generation. No particle counts greater than 200 particles/cm^3 were detected.

VAPOR CONCENTRATION MONITORING

Summaries of the chamber vapor concentrations are given in Tables I2 and I3. The α-methylstyrene concentrations in the exposure chambers were monitored by an online GC by system E (Table I1). Samples were drawn from each exposure chamber approximately every 20 (3-month studies) or 24 (2-year studies) minutes during each 6-hour exposure period. A 16-port 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 150° C. 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 GC allowed digital measurement of sample flow. The online GC was checked throughout the day for instrument drift against an online standard of α-methylstyrene 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 graphitized carbon black sampling tubes (ORBO™-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 butylbenzene as an internal standard and analyzed by an offline GC by system F (Table I1). The offline GC was calibrated with gravimetrically prepared standard solutions of α-methylstyrene containing butylbenzene as an internal standard in toluene.

CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 9 to 12 minutes without animals and 12 to 13 minutes with animals; T_{10} values ranged from 9 to 10 minutes without animals and 12 to 13 minutes with animals. For rats and mice in the 2-year studies, T_{90} values ranged from 9 to 13 minutes without animals and 11 to 14 minutes with animals; T_{10} values ranged from 9 to 10 minutes without animals and 11 to 13 minutes with animals. A T_{90} value of 12 minutes was selected for all studies.

The uniformity of α-methylstyrene vapor concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; concentration uniformity with animals present in the chambers was also measured once during the 3-month studies and every 3 months during the 2-year studies. The vapor concentration was measured using the online GC (system E, Table I1) 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 α-methylstyrene in the chamber after vapor delivery ended was determined by monitoring the concentration with animals present in the 1,000 ppm chambers (mice and rats) in the 3-month studies and in the 600 ppm chamber (mice) and the 1,000 ppm chamber (rats) in the 2-year studies. In the 3-month studies, the concentration decreased to 1% of the target concentration within approximately 46 minutes in both 1,000 ppm chambers. In the 2-year studies, the concentration decreased to 1% of the target concentration within approximately 38 minutes in the 600 ppm chamber (mice) and 41 minutes in the 1,000 ppm chamber (rats).

In the 3-month studies, stability studies of α-methylstyrene in the generation and delivery system were performed. α-Methylstyrene in the distribution line, 75 and 1,000 ppm exposure chambers (rats and mice), generator reservoir, and vapor trap was monitored once without animals present prior to the start of the 3-month studies and once during the 3-month studies with animals present. Samples from the distribution line and exposure chambers were

collected in sorbent tubes (ORBO™-101) during the first 2 and last 2 hours of generation with animals present, extracted with methylene chloride, and analyzed by GC using a system similar to system C (Table I1). HPLC by the method previously described was used to determine the concentration of 4-*tert*-butyl catechol in exposure chambers; none was detected. Samples taken from the generator reservoir and vapor trap were prepared in toluene with *n*-propylbenzene as an internal standard and analyzed by GC using system B. To assess whether impurities or degradation products co-eluted with the test chemical or the solvent, a second analysis was performed with GC by system G using a polar column that permits resolution of compounds with similar boiling points but small differences in polarity. Polymer concentration was determined using UV/Vis spectroscopy. Results were compared to a reference standard from the same lot. No evidence of degradation was detected, and no impurities were detected that were not present in the bulk material. Polymer concentration was less than 10 ppm. These results indicated that α-methylstyrene was stable for up to 7 weeks in the generator reservoir.

For stability studies during the 2-year studies, samples were collected as described above from the distribution line, 100 and 600 ppm exposure chambers (mice), and 100 and 1,000 ppm exposure chambers (rats); extracted as described above; and analyzed by GC using systems G and H. The 1,000 ppm rat exposure chamber sample was analyzed for 4-*tert*-butyl catechol (HPLC); none was detected. Samples from the generator reservoir and vapor trap were collected at 26 weeks, prepared as described above, and analyzed by GC using system B; polymer concentration was determined by UV/Vis spectroscopy. Results were compared to a reference standard of the same lot. No evidence of degradation was detected, and no impurities were detected that were not present in the bulk material. Polymer concentration was less than 10 ppm. These results indicated that α-methylstyrene was stable for up to 26 weeks in the generator reservoir.

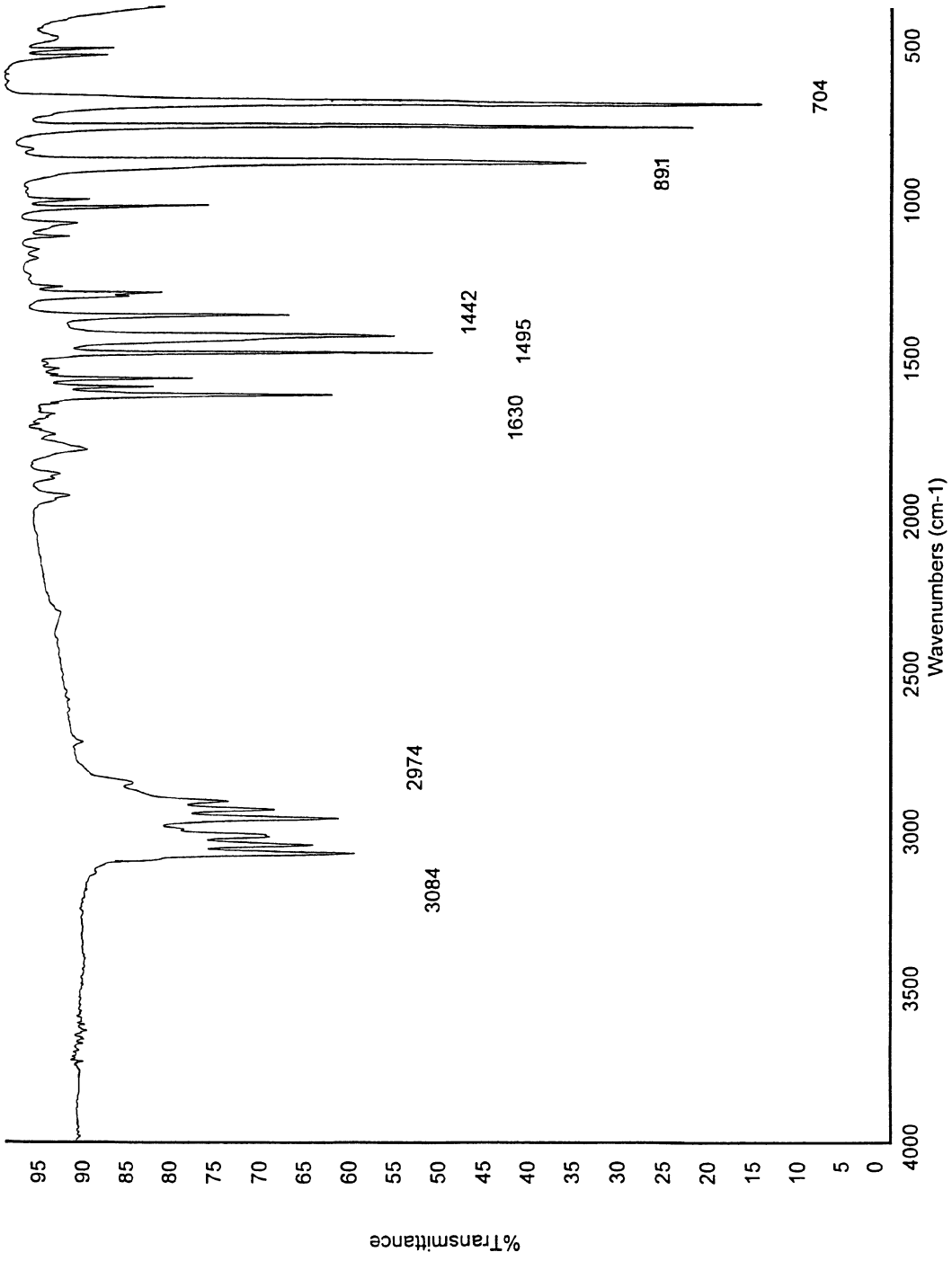


FIGURE I1
Infrared Absorption Spectrum of α-Methylstyrene, Lot BNW 13871-4

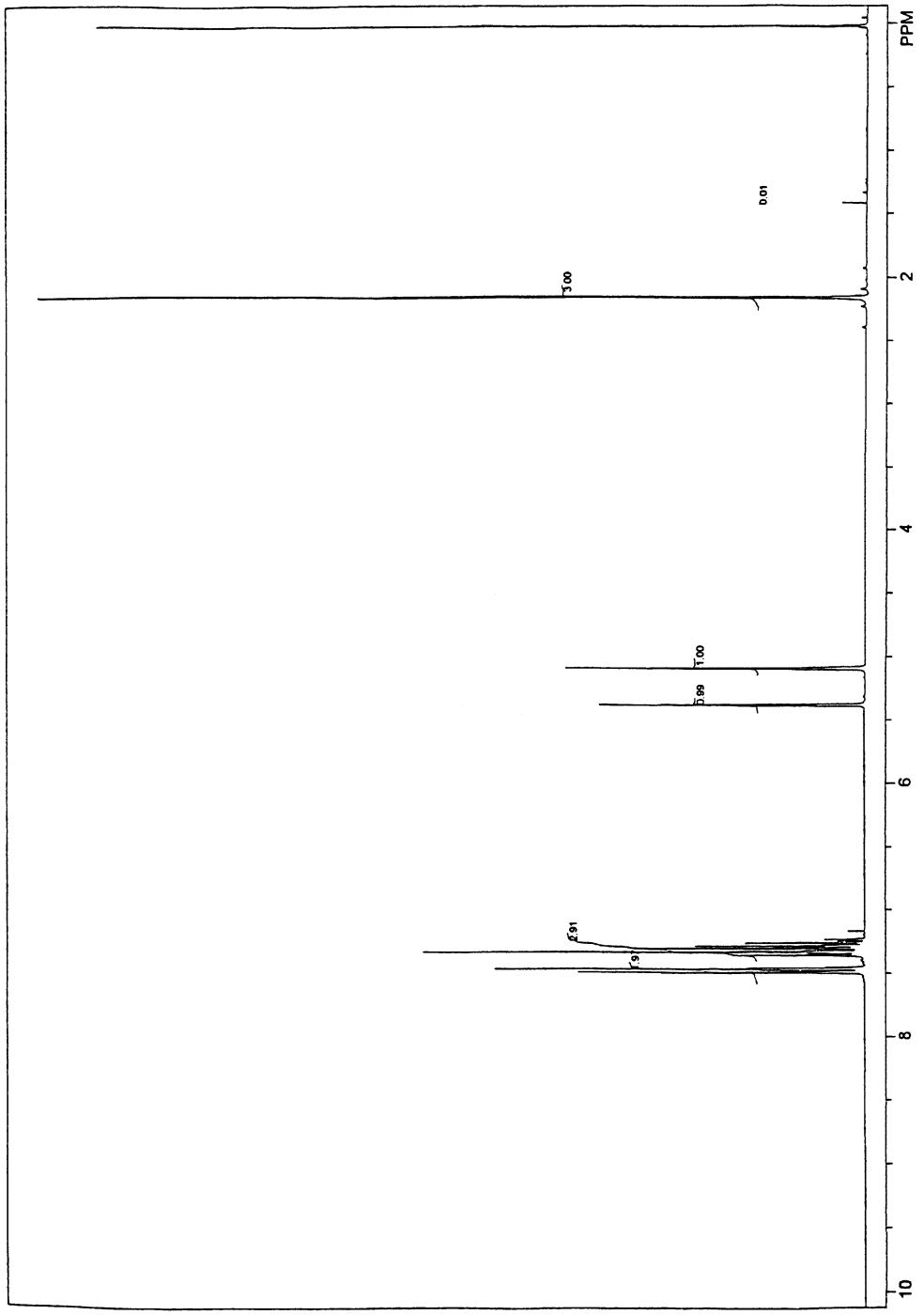


FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of α-Methylstyrene, Lot BNW 13871-4

TABLE I1
Gas Chromatography Systems Used in the Inhalation Studies of α-Methylstyrene[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-17, 30 m × 0.25 mm, 0.25-µm film (J&W Scientific, Folsom, CA)	Helium at 1 mL/minute	50° C for 5 minutes, then 10° C/minute to 250° C, held 15 minutes
System B			
Flame ionization	Rtx-5, 30 m × 0.25 mm, 1.0-µm film, (Restek, Bellefonte, PA)	Helium at 24 psi head pressure	60° C for 1 minute, then 10° C/minute to 200° C
System C			
Flame ionization	DB-5 (J&W) or Rtx-5 (Restek) 30 m × 0.25 mm, 1.0-µm film (J&W Scientific)	Helium at 24 psi head pressure	45° C for 1 minute, then 5° C/minute to 250° C, then 10° C/minute to 340° C, held 9 minutes
System D			
Mass spectrometry	DB-5 (J&W), 60 m × 0.25 mm, 1.0-µm film (J&W Scientific)	Helium at 12 psi head pressure	45° C for 1 minute, then 5° C/minute to 160° C
System E			
Flame ionization	DB-5, 15 m × 0.53 mm, 1.5-µm film (J&W Scientific)	Nitrogen at 20 mL/minute	Valve oven 150° C Column oven 105° C
System F			
Flame ionization	DB-5 (J&W Scientific) or Rtx-5 (Restek), 30 m × 0.53 mm, 1.5-µm film	Helium at 6 psi head pressure	60° C for 1 minute, then 10° C/minute to 150° C
System G			
Flame ionization	DBWax, 30 m × 0.25mm, 0.5 µm film (J&W Scientific)	Helium at 24 psi head pressure	45° C for 1 minute, then 5° C/minute to 250° C, held 8 minutes (3-month study) or 5 minutes (2-year study)
System H			
Flame ionization	Rtx-5, 30 m × 0.25 mm, 1.0-µm film (Restek)	Helium at 50 psi head pressure for 0.2 minutes, then held at 24 psi head pressure	35° C, for 1 minute, then 5° C/minute to 250° C, held 5 minutes

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

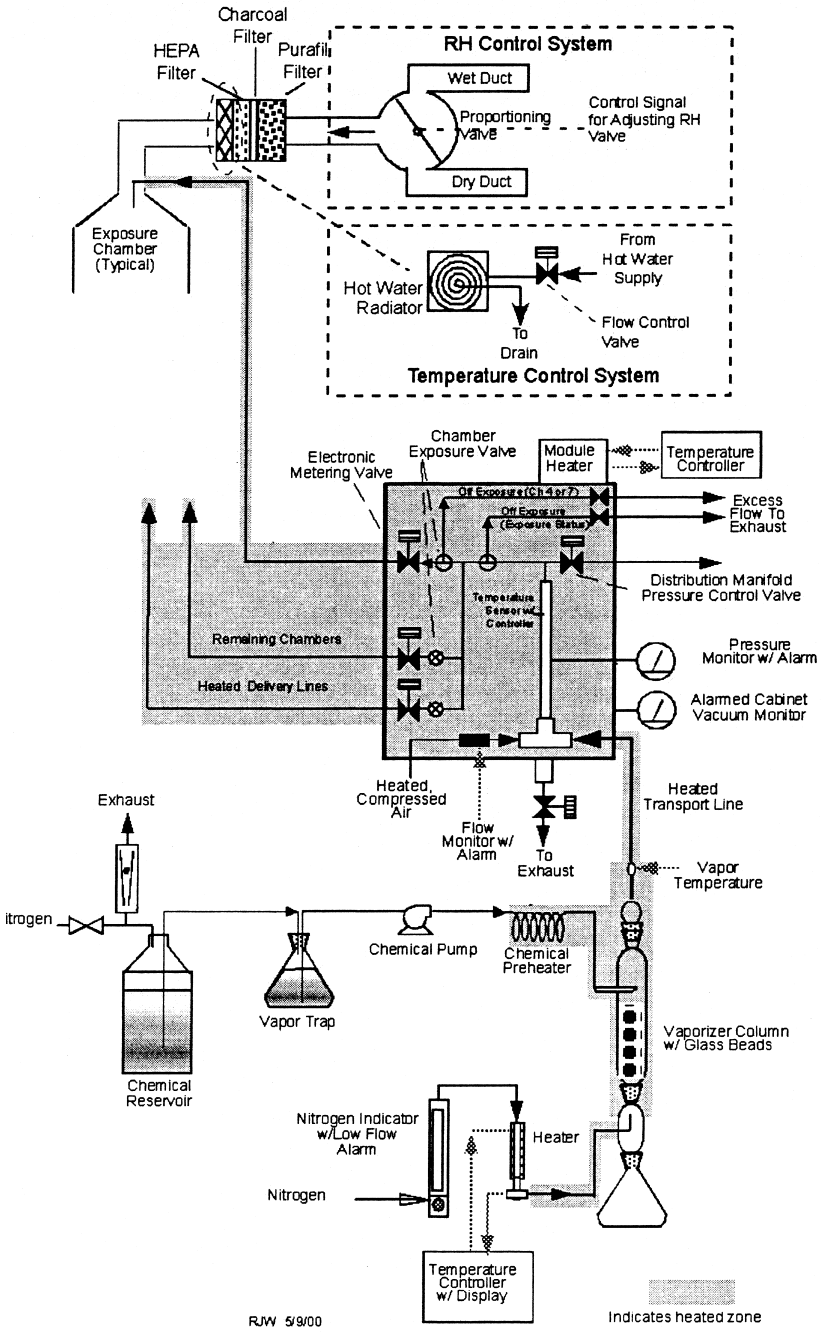


FIGURE I3
Vapor Generation and Delivery System Used in the Inhalation Studies of α-Methylstyrene

TABLE I2
Summary of Chamber Concentrations in the 3-Month Inhalation Studies of α-Methylstyrene

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
75	1,245	75 ± 2
150	1,254	150 ± 3
300	1,263	300 ± 7
600	1,263	603 ± 11
1,000	1,266	1,002 ± 20
Mouse Chambers		
75	1,284	75 ± 2
150	1,294	150 ± 3
300	1,303	300 ± 7
600	1,303	603 ± 11
1,000	1,306	1,002 ± 20

[a] Mean ± standard deviation

TABLE I3
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of α-Methylstyrene

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
100	7,863	101 ± 2
300	7,920	302 ± 6
1,000	8,144	1,007 ± 22
Mouse Chambers		
100	7,955	101 ± 3
300	7,892	302 ± 6
600	8,008	604 ± 11

[a] Mean ± standard deviation

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

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

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

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
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 J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.59	13.7 – 15.7	24
Crude fat (% by weight)	8.1 ± 0.26	7.6 – 8.6	24
Crude fiber (% by weight)	9.0 ± 0.44	8.0 – 9.9	24
Ash (% by weight)	5.2 ± 0.26	4.8 – 5.8	24
Amino Acids (% of total diet)			
Arginine	0.748 ± 0.049	0.670 – 0.850	14
Cystine	0.224 ± 0.025	0.150 – 0.250	14
Glycine	0.702 ± 0.040	0.620 – 0.750	14
Histidine	0.368 ± 0.093	0.310 – 0.680	14
Isoleucine	0.534 ± 0.039	0.430 – 0.590	14
Leucine	1.079 ± 0.061	0.960 – 1.150	14
Lysine	0.704 ± 0.013	0.310 – 0.830	14
Methionine	0.400 ± 0.051	0.260 – 0.460	14
Phenylalanine	0.613 ± 0.036	0.540 – 0.660	14
Threonine	0.491 ± 0.041	0.430 – 0.590	14
Tryptophan	0.134 ± 0.018	0.110 – 0.160	14
Tyrosine	0.377 ± 0.050	0.280 – 0.460	14
Valine	0.658 ± 0.045	0.550 – 0.710	14
Essential Fatty Acids (% of total diet)			
Linoleic	3.89 ± 0.262	3.49 – 4.54	14
Linolenic	0.30 ± 0.036	0.21 – 0.35	14
Vitamins			
Vitamin A (IU/kg)	4,849 ± 839	3,060 – 6,920	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	83.6 ± 17.06	52.0 – 110.0	14
Thiamine (ppm)[b]	7.6 ± 1.11	5.9 – 9.2	24
Riboflavin (ppm)	6.7 ± 2.17	4.20 – 11.20	14
Niacin (ppm)	79.3 ± 10.85	66.4 – 98.2	14
Pantothenic acid (ppm)	23.5 ± 3.49	17.4 – 29.1	14
Pyridoxine (ppm)[b]	9.24 ± 2.28	6.4 – 13.7	14
Folic acid (ppm)	1.76 ± 0.55	1.20 – 3.27	14
Biotin (ppm)	0.333 ± 0.12	0.225 – 0.704	14
Vitamin B_{12} (ppb)	62.8 ± 47.3	18.3 – 174.0	14
Choline (ppm)[b]	3,066 ± 280	2,700 – 3,790	14
Minerals			
Calcium (%)	1.015 ± 0.057	0.873 – 1.140	24
Phosphorus (%)	0.611 ± 0.036	0.555 – 0.701	24
Potassium (%)	0.667 ± 0.021	0.627 – 0.694	14
Chloride (%)	0.377 ± 0.042	0.300 – 0.474	14
Sodium (%)	0.192 ± 0.017	0.160 – 0.222	14
Magnesium (%)	0.202 ± 0.009	0.185 – 0.217	14
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	176 ± 42.9	135 – 311	14
Manganese (ppm)	54.6 ± 8.02	42.1 – 73.1	14
Zinc (ppm)	54.3 ± 9.45	43.3 – 78.5	14
Copper (ppm)	6.37 ± 1.492	3.21 – 9.92	14
Iodine (ppm)	0.516 ± 0.229	0.233 – 0.972	14
Chromium (ppm)	0.544 ± 0.124	0.330 – 0.751	13
Cobalt (ppm)	0.25 ± 0.076	0.20 – 0.47	13

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

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

Nutrient	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.35 ± 0.154	0.17 – 0.50	24
Cadmium (ppm)	0.05 ± 0.015	0.04 – 0.09	24
Lead (ppm)	0.07 ± 0.026	0.05 – 0.17	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.22 ± 0.054	0.14 – 0.36	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	14.3 ± 4.10	6.85 – 23.2	24
Nitrite nitrogen (ppm)[c]	<0.61		24
BHA (ppm)[d]	<1.0		24
BHT (ppm)[d]	<1.0		24
Aerobic plate count (CFU/g)	15 ± 14	10 – 70	24
Coliform (MPN/g)	3.0 ± 0.1	3.0 – 3.6	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	4.5 ± 1.47	2.4 – 8.4	24
N-Nitrosodimethylamine (ppb)[e]	2.9 ± 1.36	1.2 – 6.9	24
N-Nitrosopyrrolidine (ppb)[e]	1.7 ± 0.68	0.9 – 3.1	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.113 ± 0.066	0.020 – 0.259	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.346 ± 0.478	0.020 – 1.850	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

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

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

During the 3-month studies, serologic analyses were performed by the study laboratory on five male and five female rats and mice 3 weeks after arrival at the study laboratory. At terminal sacrifice, serum was collected from five male and five female chamber control rats and mice. During the 2-year studies, serologic analyses were performed by the study laboratory on 10 male and 10 female sentinel rats and mice 3 weeks after arrival at the study laboratory. In addition, serum samples were collected from randomly selected sentinel rats and mice at 6, 12, and 18 months and 1,000 ppm rats and 600 ppm mice at study termination. Blood from each animal was collected and allowed to clot, and the serum was separated. Samples were processed appropriately and, except for the 3-week serology samples, sent to BioReliance Corporation, Rockville, MD, for determination of antibody titers. Fecal samples from mice were tested for *Helicobacter* at 18 months. The laboratory methods and agents for which testing was performed and the times at which samples were collected during the studies are tabulated below.

Method and Test	Time of Analysis
RATS	
3-Month Study	
ELISA	
H-1 (Toolan's H-1 virus)	3 weeks after arrival
KRV (Kilham rat virus)	3 weeks after arrival
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	3 weeks after arrival, study termination
PVM (pneumonia virus of mice)	3 weeks after arrival, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	3 weeks after arrival, study termination
Sendai	3 weeks after arrival, study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
H-1	3 weeks after arrival
KRV	3 weeks after arrival
M. arthritidis	Study termination
M. pulmonis	3 weeks after arrival, study termination
PVM	3 weeks after arrival, 6, 12, and 18 months, study termination
RCV/SDA	3 weeks after arrival, 6, 12, and 18 months, study termination
Sendai	3 weeks after arrival, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	3 weeks after arrival, study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
mouse adenoma virus-FL	Study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV (mouse hepatitis virus)	3 weeks after arrival, study termination
MVM (minute virus of mice)	3 weeks after arrival
M. arthritidis	Study termination
M. pulmonis	3 weeks after arrival, study termination
PVM	3 weeks after arrival, study termination
Reovirus 3	Study termination
Sendai	3 weeks after arrival, study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	3 weeks after arrival, 6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus	6, 12, and 18 months, study termination
MCMV	Study termination
MHV	3 weeks after arrival, 6, 12, and 18 months, study termination
MVM	3 weeks after arrival
M. arthritidis	Study termination
M. pulmonis	3 weeks after arrival, study termination
PVM	3 weeks after arrival, 6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	3 weeks after arrival, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination

RESULTS

For the 3-month and 2-year studies in rats and mice, all tests were negative.

APPENDIX L
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model was developed to describe the absorption, distribution, metabolism, and elimination of α-methylstyrene in male F344/N rats. This PBPK model is based on published models developed for styrene (Ramsey and Andersen, 1984; Csanady *et al.*, 1994, 2003) due to the similarity in the chemical structures of styrene (C_8H_8) and α-methylstyrene (C_9H_{10}) and the presumed likeness of their metabolic pathways. The model is specific to male F344/N rats.

MODEL DEVELOPMENT

The PBPK model (Figure L1) has compartments representing the amounts of α-methylstyrene and α-methylstyrene metabolite in the liver, kidney, adipose tissue, and slowly perfused and rapidly perfused tissues. The model assumes that the liver is the only metabolizing tissue and that metabolism of α-methylstyrene is first order. The gastrointestinal (GI) tract is modeled as a lumen that receives no blood flow. Uptake from the GI tract to the liver is linear in the model. An instantaneous equilibrium between venous blood and inhaled air is assumed to occur within the lung compartment. Urinary excretion is modeled as a first order process in the kidney compartment with different rates for α-methylstyrene and α-methylstyrene metabolite. The model was used to study intravenous injection, oral gavage, and inhalation exposures to α-methylstyrene.

The physiological parameters for rats, shown in Table L1, were taken from Brown *et al.* (1997). The partition coefficients for α-methylstyrene are expected to be similar to those for styrene. Most models for styrene have a fat:blood partition coefficient close to 40. Beliveau *et al.* (2003) described a method that develops quantitative structure-property relationships to estimate PBPK parameters for a series of volatile organic compounds, and this method predicts a fat:blood partition coefficient less than 1. Because reasonable estimates or experimental values could not be found, the partition coefficients for styrene, shown in Table L2, were used for α-methylstyrene. The use of styrene partition coefficients is also supported by the similar log octanol:water partition coefficients of the two chemicals: 2.9 for styrene, 3.4 for α-methylstyrene. Several metabolites were identified in the urine and blood; however, only a single generalized metabolite is considered in this model, and the metabolite compartment in the model represents all possible α-methylstyrene metabolites. Published partition coefficients for styrene oxide were used for the α-methylstyrene metabolite (Table L2). Parameters for uptake from the GI tract, metabolism, and urinary excretion have not been measured experimentally but were estimated by fitting the model predictions to the data (Table L3). The data sets were for male F344/N rats dosed by intravenous injection (10 mg α-methylstyrene/kg body weight), oral gavage (1,000 mg/kg), or exposed by inhalation (300 or 900 ppm). Most of the data are for radiolabeled α-methylstyrene. The radiolabeled data include the percent of dose excreted in urine, exhaled in air, and remaining in the tissues. The model also was fit to the log concentration of radiolabeled chemicals in the liver, adipose tissue, and kidney. The nonradiolabeled data sets used in the optimization consisted of the concentrations of α-methylstyrene and α-methylstyrene metabolite in blood and the concentration of α-methylstyrene metabolite in urine as determined by high-performance liquid chromatography.

MODEL EQUATIONS

The following differential equation describes the rate of change in the amount of substance in the tissue for adipose tissue, slowly perfused tissue, and rapidly perfused tissue compartments:

$$\frac{dA_{i,j}}{dt} = Q_i \bullet \left(C_{arterial,j} - \frac{C_{i,j}}{P_i} \right)$$

where i indexes the tissue, j indexes parent α-methylstyrene or α-methylstyrene metabolite, $A_{i,j}$ (ng) is the amount of substance in the tissue, Q_i (L/hour) is the blood flow to the tissue, $C_{i,j}$ (ng/L) is the tissue concentration, $C_{arterial}$ (ng/L) is the arterial blood concentration, and P_i is the tissue:blood partition coefficient.

Tissue concentration, in turn, is given by the following equation:

$$C_{i,j} = \frac{A_{i,j}}{V_i}$$

where V_i (L) is the volume of tissue i.

The venous blood concentration of α-methylstyrene (AMS) is expressed as:

$$C_{venous,AMS} = \frac{\left(dose_{IV,AMS} + \sum Q_i \bullet \frac{C_{i,AMS}}{P_{i,AMS}} \right)}{Q_{cardiac}}$$

where $dose_{IV,AMS}$ is the dose of α-methylstyrene administered by intravenous injection. Similarly, the venous blood concentration of α-methylstyrene metabolite (MET) is given by:

$$C_{venous,MET} = \frac{\sum Q_i \bullet \frac{C_{i,MET}}{P_{i,MET}}}{Q_{cardiac}}$$

The concentrations of α-methylstyrene and α-methylstyrene metabolite in arterial blood are determined according to the following equations which were derived assuming rapid equilibrium between concentrations in inhaled air and blood:

$$C_{arterial,AMS} = \frac{Q_{alveolar} \bullet C_{inhaled,AMS} + Q_{cardiac} \bullet C_{venous,AMS}}{Q_{cardiac} + \frac{Q_{alveolar}}{P_{blood,AMS}}}$$

$$C_{arterial,MET} = \frac{Q_{cardiac} \bullet C_{venous,MET}}{Q_{cardiac} + \frac{Q_{alveolar}}{P_{blood,MET}}}$$

The differential equation describing the rate of change of the amount of α-methylstyrene or α-methylstyrene metabolite in the kidney includes a term representing the first order elimination of compound into the urine:

$$\frac{dA_{kidney,j}}{dt} = Q_{kidney,j} \bullet \left(C_{arterial,j} - \frac{C_{kidney,j}}{P_{kidney,j}} \right) - \frac{dA_{urine,j}}{dt}$$

$$\frac{dA_{urine,j}}{dt} = k_{urine,j} \bullet \frac{A_{kidney,j}}{P_{kidney,j}}$$

where $k_{urine,\,j}$ (hour^{-1}) is the first order rate constant describing renal elimination of α-methylstyrene or α-methylstyrene metabolite.

The oral uptake of α-methylstyrene from the GI tract is also a first order process:

$$\frac{dA_{uptake,AMS}}{dt} = k_{uptake} \bullet A_{gut,AMS}$$

$$\frac{dA_{gut,AMS}}{dt} = -\frac{dA_{uptake,AMS}}{dt}$$

where $A_{gut,\,AMS}(0) = dose_{oral,\,AMS}$ and k_{uptake} (hour^{-1}) is the first order rate constant describing absorption of α-methylstyrene from the GI tract to the liver.

The differential equations describing the amount of α-methylstyrene or α-methylstyrene metabolite in the liver include terms representing the first order metabolic conversion of α-methylstyrene into α-methylstyrene metabolite:

$$\frac{dA_{liver,AMS}}{dt} = Q_{liver} \bullet \left(C_{arterial,AMS} - \frac{C_{liver,AMS}}{P_{liver,AMS}} \right) - \frac{dA_{metab,AMS}}{dt} + \frac{dA_{uptake,AMS}}{dt}$$

$$\frac{dA_{liver,MET}}{dt} = Q_{liver} \bullet \left(C_{arterial,MET} - \frac{C_{liver,MET}}{P_{liver,MET}} \right) + \frac{dA_{metab}}{dt}$$

$$\frac{dA_{metab}}{dt} = k_{metab} \bullet \frac{A_{liver,AMS}}{P_{liver,AMS}}$$

where k_{metab} (hour^{-1}) is the first order rate constant describing metabolism (*metab*) of α-methylstyrene.

RESULTS

The results of simulations performed with the PBPK model for α-methylstyrene compared to the experimental data from intravenous injection, oral gavage, and inhalation studies are shown in Figures L2 to L5. The α-methylstyrene model, based largely on values for styrene, explains the data very well. This is not too surprising due to the close structural similarities of the compounds. The model also indicates that none of the uptake, elimination, or metabolism processes are saturated at the doses evaluated experimentally. A non-linear model, incorporating saturable metabolic kinetics, was tested, but the results did not show any improvement in fit to the data. While the model can be fit with linear metabolism and linear elimination, this does not contradict experimental data showing saturation of glucuronidation. The difference is due to the PBPK model being developed using total radioactivity data. Clearance of total radioactivity is described with linear rates. This data alone can not determine if any one process of absorption, distribution, metabolism, or excretion is saturable.

REFERENCES

Beliveau, M., Tardif, R., and Krishnan, K. (2003). Quantitative structure-property relationships for physiologically based pharmacokinetic modeling of volatile organic chemicals in rats. *Toxicol. Appl. Pharmacol.* **189**, 221-232.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., and Beliles, R.P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* **13**, 407-484.

Csanady, G.A., Mendrala, A.L., Nolan, R.J., and Filser, J.G. (1994). A physiologic pharmacokinetic model for styrene and styrene-7,8-oxide in mouse, rat, and man. *Arch. Toxicol.* **68**, 143-157.

Csanady, G.A., Kessler, W., Hoffmann, H.D., and Filser, J.G. (2003). A toxicokinetic model for styrene and its metabolite styrene-7,8-oxide in mouse, rat and human with special emphasis on the lung. *Toxicol. Lett.* **138**, 75-102.

Ramsey, J.C., and Andersen, M.E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* **73**, 159-175.

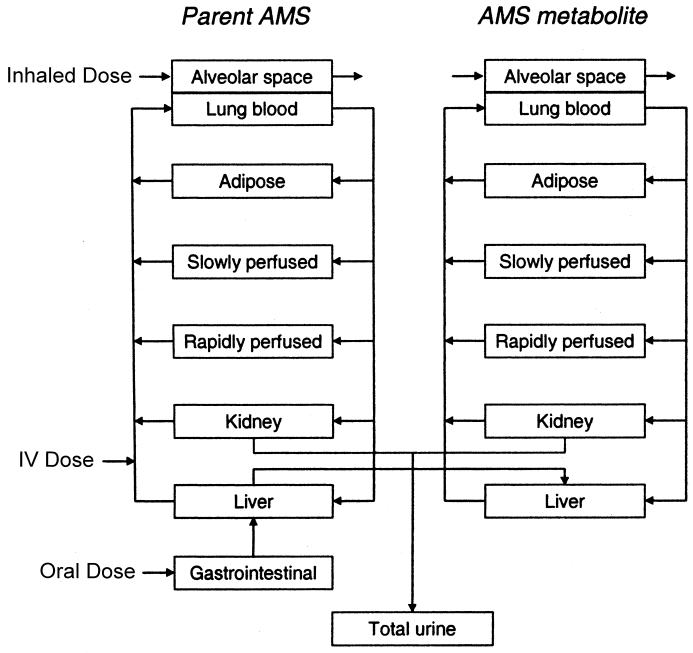


FIGURE L1
Physiologically Based Pharmacokinetic Model for Male F344/N Rats Exposed to α-Methylstyrene by Single-Dose Intravenous Injection, Oral Gavage, or Inhalation

TABLE L1
Physiological Parameters for Male F344/N Rats for the Physiologically Based Pharmacokinetic Model of α-Methylstyrene[a]

Parameter	Value
Parameter	
Body weight (kg)	0.248[b]
Cardiac output (L/hour per kg body weight$^{0.74}$)	14.1
Alveolar ventilation (L/hour per kg body weight$^{0.74}$)	13.2[c]
Tissue Volume as Fraction of Body Weight	
Adipose tissue	0.09
Kidney	0.0073
Liver	0.0366
Rapidly perfused tissue	0.1561
Slowly perfused tissue	0.53
Tissue Blood Flow as Fraction of Cardiac Output	
Adipose tissue	0.07
Kidney	0.141
Liver	0.183
Rapidly perfused tissue	0.266
Slowly perfused tissue	0.34

[a] Values taken from Brown *et al.* (1997), unless noted otherwise
[b] Mean body weight of study animals
[c] Published ventilation rate scaled by 60% to reflect diminished uptake in rodent inhalation studies (Csanady *et al.*, 1994)

TABLE L2
Partition Coefficients for α-Methylstyrene and α-Methylstyrene Metabolite for the Physiologically Based Pharmacokinetic Model of α-Methylstyrene[a]

Tissue	α-Methylstyrene	α-Methylstyrene Metabolite
Air	109.4	10,000[b]
Adipose tissue	40.9	6.1
Kidney	1.14	2.6
Liver	1.18	2.6
Rapidly perfused tissue	1.14	2.6
Slowly perfused tissue	0.86	1.5

[a] All coefficients are expressed as tissue:blood ratios with the exception of air which is blood:air. Values were taken from Csanady *et al.* (2003), unless noted otherwise.
[b] This value was assigned because a negligible amount of α-methylstyrene metabolite is exhaled.

TABLE L3
Parameter Estimates for Male F344/N Rats from the Physiologically Based Pharmacokinetic Model of α-Methylstyrene

Parameter	Optimized Value
k_{uptake} (hour^{-1})	0.07
k_{metab} (hour^{-1})	105.9
$k_{urine,\ \alpha\text{-methylstyrene}}$ (hour^{-1})	0.04
$k_{urine,\ \alpha\text{-methylstyrene metabolite}}$ (hour^{-1})	22.87

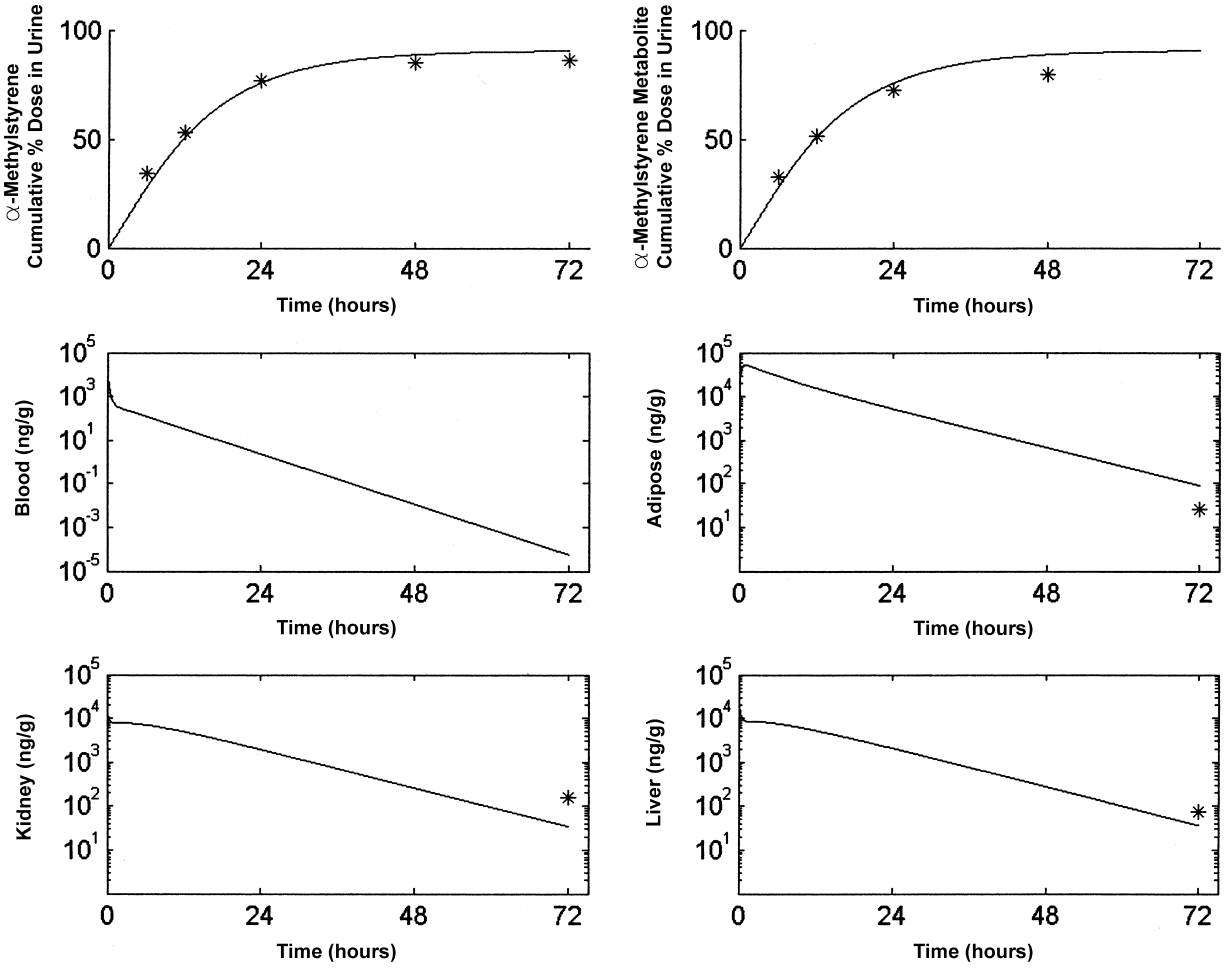


FIGURE L2
Urinary Excretion of α-Methylstyrene, Urinary Excretion of α-Methylstyrene Metabolite, and Tissue Concentrations of α-Methylstyrene in Male F344/N Rats after a Single Intravenous Injection of 10 mg/kg α-Methylstyrene
Solid lines represent the predicted best-fit curves from the PBPK model plotted through the observed data points (*).

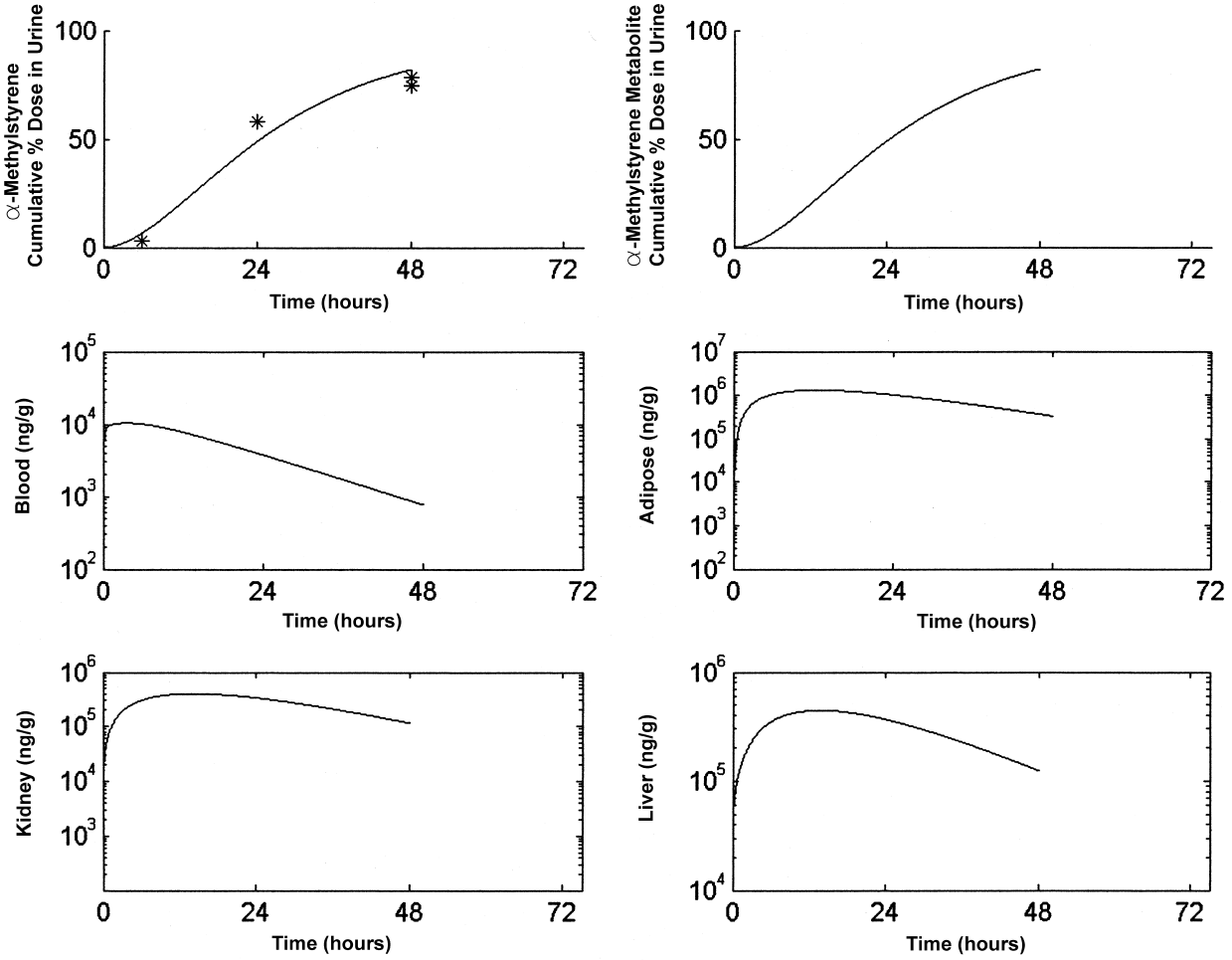


FIGURE L3
Urinary Excretion of α-Methylstyrene, Urinary Excretion of α-Methylstyrene Metabolite, and Tissue Concentrations of α-Methylstyrene in Male F344/N Rats after a Single Oral Gavage Dose of 1,000 mg/kg α-Methylstyrene
Solid lines represent the predicted best-fit curves from the PBPK model plotted through the observed data points (*).

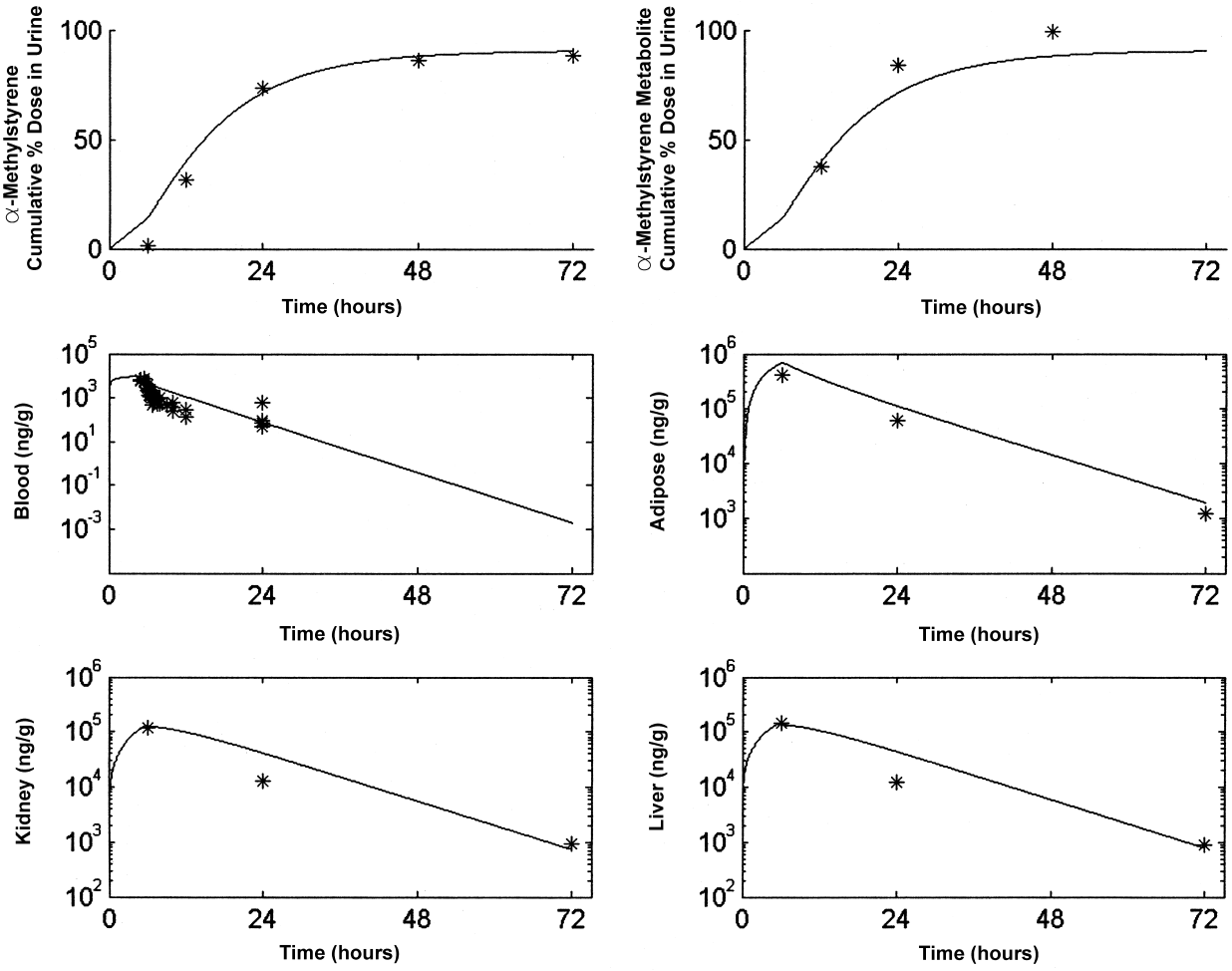


FIGURE L4
Urinary Excretion of α-Methylstyrene, Urinary Excretion of α-Methylstyrene Metabolite, and Tissue Concentrations of α-Methylstyrene in Male F344/N Rats after a Single 6-Hour Exposure to 300 ppm α-Methylstyrene by Inhalation
Solid lines represent the predicted best-fit curves from the PBPK model plotted through the observed data points (*).

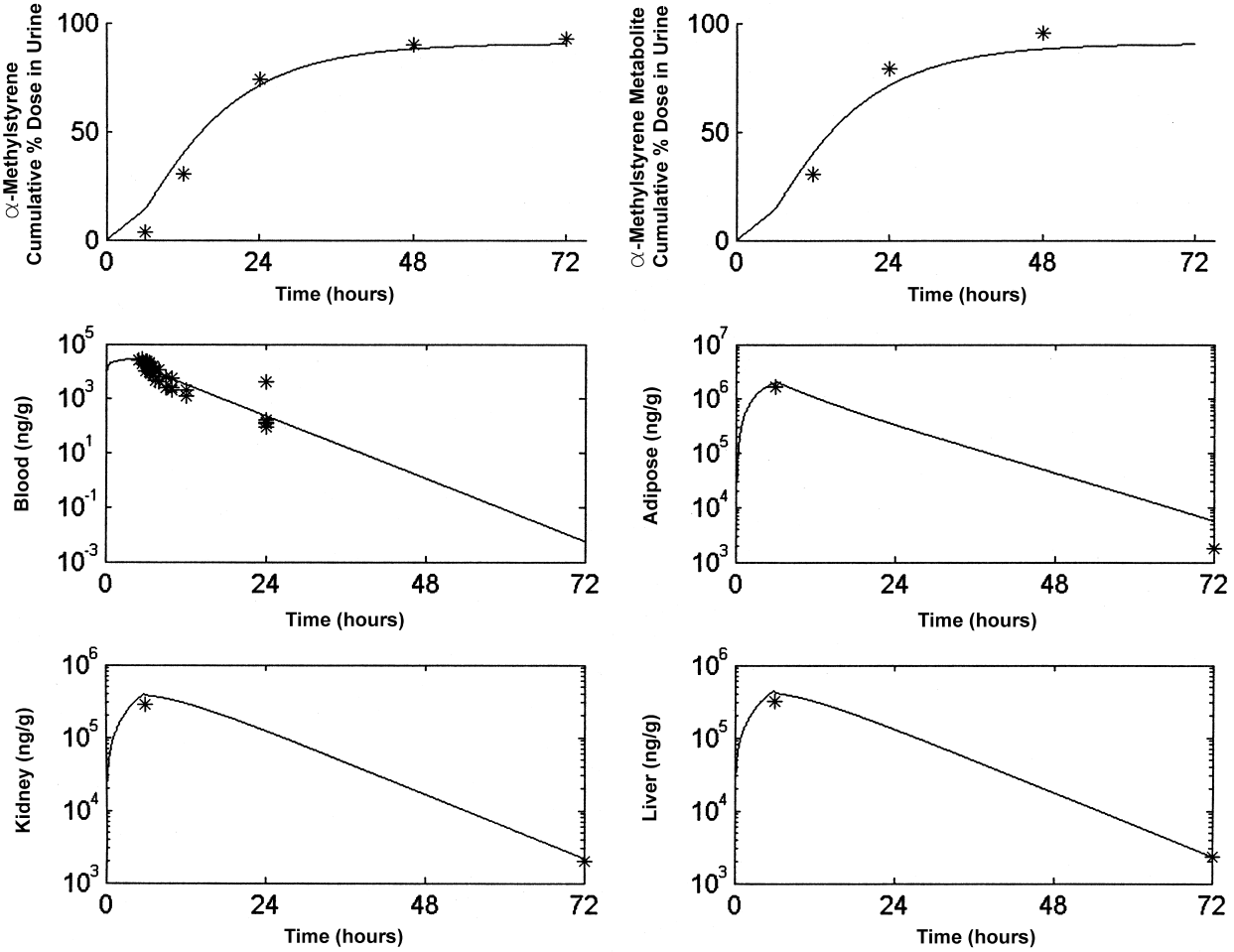


FIGURE L5
Urinary Excretion of α-Methylstyrene, Urinary Excretion of α-Methylstyrene Metabolite, and Tissue Concentrations of α-Methylstyrene in Male F344/N Rats after a Single 6-Hour Exposure to 900 ppm α-Methylstyrene by Inhalation
Solid lines represent the predicted best-fit curves from the PBPK model plotted through the observed data points (*).

APPENDIX M
METABOLISM AND CHEMICAL DISPOSITION OF α-METHYLSTYRENE

METABOLISM AND CHEMICAL DISPOSITION OF α-METHYLSTYRENE

INTRODUCTION

α-Methylstyrene is a volatile hydrocarbon (boiling point 165° C) that is used primarily in the production of specialty polymers and resins. The National Institute for Occupational Safety and Health (1990) reports that heavy construction, paper and allied products, and other business services are industries with the largest number of workers exposed to α-methylstyrene (NTP, 1985).

In preliminary studies conducted by Morgan *et al.* (1995) for the National Toxicology Program (NTP), inhalation exposure of rats and mice to concentrations up to 500 ppm of α-methylstyrene for 6 hours per day, 5 days per week for 2 weeks caused no significant effects on body or organ weights or on serum chemistries. Atrolactic acid (2-phenyl-2-hydroxy-propanoic acid), a major oxidative metabolite of α-methylstyrene, was not detected in the urine of female mice (urine from male mice was not analyzed) but was detected in significant amounts in the urine of male rats. The epoxide of α-methylstyrene was not detected in the blood of rats exposed to 500 ppm α-methylstyrene (mouse blood was not analyzed), but a 50% depletion of hepatic glutathione was observed. There was a narrow "therapeutic index" of sedation in mice exposed to α-methylstyrene by inhalation, and lethalities occurred at concentrations as low as 600 ppm. Mice "adapted" to the sedative effects of α-methylstyrene by the second week of exposure. No sedation or mortality occurred in rats at α-methylstyrene concentrations ranging up to 1,000 ppm. Relative liver weights in rats and mice were significantly increased after exposure to 600 ppm or greater, but no changes in serum chemistries were observed.

Citing a need to supplement data from its toxicity studies and limited information available in the literature detailing α-methylstyrene disposition and metabolism, the NTP nominated α-methylstyrene for study. The present studies investigated α-methylstyrene disposition, excretion, and metabolism after intravenous administration, oral administration, and nose-only inhalation exposure. The goal of the studies was to determine the disposition and metabolism of α-methylstyrene in rats.

MATERIALS AND METHODS

Adult male F344/N rats were purchased from Charles River Laboratories, Inc. (Raleigh, NC). Animals were quarantined at least 1 week before they were used in a study. Rats were fed Certified Purina Rodent Chow #5002 and were furnished tap water *ad libitum*. Rats were housed in standard polycarbonate cages and moved to individual glass metabolism chambers the day before study start for acclimation. These chambers provided for separate collection of urine, feces, and breath components for the oral and intravenous exposures.

For all studies, rats were sacrificed by an intracardiac injection of sodium pentobarbital (300 mg/kg). Prior to administration of sodium pentobarbital, animals were anesthetized with an intramuscular injection of 60 mg/kg ketamine and 8.6 mg/kg xylazine. All studies were approved by the Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

For nose-only exposures, Battelle® restrainers (Geneva) were used during the exposure phase of the inhalation study. Animals were acclimated to the restrainers for 2 hours each day for 2 days prior to exposure. Restrainers afforded separate collection of urine and feces excreted during exposure. Upon termination of the 6-hour exposure and removal from restrainers, a stream of warm air was briefly directed over each rat to remove [^{14}C]α-methylstyrene deposited on the fur and to prevent ingestion of [^{14}C]α-methylstyrene through grooming. Rats were then immediately transferred to glass metabolism chambers.

An indwelling jugular cannula was surgically implanted into animals used in the inhalation study to facilitate serial collection of blood samples. The technique was similar to that reported by McKenna and Bieri (1984). Animals were anesthetized with ketamine/xylazine/acepromazine (10:1:0.1, 60-80 mg/kg or to effect). A small incision at the level of the clavicle, approximately 0.5 cm from the midline, afforded access to the right, external jugular vein. The vein was blunt dissected, and the cannula (prefilled with sterile saline containing 20 IU/mL of sodium heparin) was inserted to a depth of approximately 28 mm. Placement in the atrium was confirmed by patency. The cannula was secured in the vein at the insertion point by sutures to the surrounding musculature. The cannula was then exteriorized by passing the distal end subcutaneously around the neck to exit the dorsal surface via a small incision between the scapulae. The ventral incision was closed with sutures. The distal end of the cannula was sutured to the base of the neck between the scapulae. Animals were allowed to recover approximately 24 hours prior to exposure.

Nonradiolabeled α-methylstyrene (lot #0922OLG, CAS No. 98-83-9) was purchased from the Aldrich Chemical Company (Milwaukee, WI) with a stated purity of 99% or greater. Identity of nonradiolabeled α-methylstyrene was confirmed by nuclear magnetic resonance (NMR) spectrometry. The 500 MHz ^{1}H NMR spectrum of α-methylstyrene was consistent with the literature spectrum (*Aldrich*, 1993). Signals were at 2.16 ppm (three methyl protons), 5.08 and 5.37 ppm (two methylene protons), and 7.26, 7.33, and 7.46 ppm (five aromatic protons). Radiolabeled α-methylstyrene (lot #950612), randomly radiolabeled with ^{14}C in the phenyl ring, was received from Wizard Laboratories, Inc. (West Sacramento, CA), at a specific activity of 1.0 mCi/mmol. [^{14}C]α-methylstyrene was initially determined to be approximately 99% radiochemically pure by high performance liquid chromatography (HPLC). Purity of [^{14}C]α-methylstyrene in dose formulations was reestablished at 98% or greater during the studies.

The following were purchased from the Sigma Chemical Company (St. Louis, MO): β-glucuronidase (prepared from *Escherichia coli*), sulfatase (prepared from *Aerobacter aerogenes*), acylase (prepared from porcine kidney), Trizma® buffer, and phosphate buffered saline (pH 7.4). Bis(trimethylsilyl)trifluoroacetamide (BSTFA), used for preparation of trimethylsilane derivatives of urinary metabolites, was purchased from Supelco, Inc. (Bellefonte, PA). Ketamine and xylazine were obtained from Aveco (Ft. Dodge, IA) and Butler (Columbus, OH), respectively. Sodium heparin and sodium pentobarbital were obtained from Elkins-Sinn, Inc. (Cherry Hill, NJ), and ANPRO Pharmaceuticals (Arcadia, CA), respectively. Emulphor EL-620 was obtained from the GAF Chemical Corporation (Wayne, NJ).

Standards of 2-phenylpropionic acid, atrolactic acid (2-hydroxy-2-phenylpropionic acid), and 2-phenyl-1,2-propanediol were purchased from the Aldrich Chemical Company (Milwaukee, WI). Ultima Gold scintillation cocktail and Soluene-350® tissue solubilizer were obtained from the Packard Instrument Company (Meriden, CT). HPLC grade solvents used for extraction of blood samples and HPLC mobile phases were supplied by Fisher Scientific (Fair Lawn, NJ). All other chemicals mentioned in this report were either reagent grade or HPLC grade.

All HPLC was performed utilizing modular instrumentation consisting of two Waters Model 510 or 600A dual piston solvent pumps, a gradient controller (Waters Corporation, Milford, MA), a Rheodyne Model 7125 injector, an ultraviolet detector set to 256 or 257 nm (Kratos/Applied Biosystems, Foster City, CA), and an in-line radioactivity detector (Ramona LS; Raytest or β-Ram Model 2, IN/US Instruments, Tampa, FL) fitted with either 500- or 600-µL lithium glass flow-through solid scintillator cells. Data were acquired with Scintflow for β-Ram (Version 3.14; Scintco, Augusta, NJ) or Ramona acquisition software (Version 11.2; IN/US Instruments). A Microsorb MV Phenyl column (Varian, Inc., Palo Alto, CA) (phenyl stationary phase) was used for all analyses. The mobile phase flow rate was 1 mL/minute, and solvents used to prepare the mobile phases were mixed by volume. Linear gradients were used to make changes in mobile phase composition. After flowing through the detectors, column effluent was collected in fractions and assayed for ^{14}C using liquid scintillation spectrometry (LSS).

For all dose formulations, the radiochemical purity of [^{14}C]α-methylstyrene was determined using an isocratic mobile phase consisting of acetonitrile:1% aqueous acetic acid, 60:40. Initial mobile phase composition for determination of the urinary ^{14}C profile, urinary metabolite isolation, and determination of the ^{14}C profile in blood was acetonitrile:1% aqueous acetic acid, 20:80. For determination of the urinary ^{14}C profile, composition was changed linearly over 30 minutes to acetonitrile:1% aqueous acetic acid, 60:40, with no hold at initial conditions. For isolation of urinary metabolites, initial conditions were maintained for 5 minutes, followed by a change to 30:70 over 20 minutes preceding another change to 80:20 over a 5 minute interval. Characterization of the ^{14}C profile in blood extracts obtained from inhalation studies was performed holding initial conditions for 2.5 minutes, followed by a linear gradient to 60:40 over 7.5 minutes preceding another linear change to 98:2 or 100:0 over 3 minutes.

Intravenous dose formulations contained 13.3 to 23.6 µCi [^{14}C]α-methylstyrene and an appropriate amount of Emulphor EL-620 and phosphate buffered saline (1:20, respectively) in a dose volume of 1 mL/kg. Oral dose formulations contained 43.8 µCi [^{14}C]α-methylstyrene, an appropriate amount of nonradiolabeled α-methylstyrene, and sufficient corn oil for a dose volume of 5 mL/kg. α-Methylstyrene used for generation of atmospheres during nose-only inhalation exposures contained appropriate masses of radiolabeled and nonradiolabeled α-methylstyrene to deliver approximately 20 µCi per rat at 300 or 900 ppm.

Intravenous doses (10.8 mg α-methylstyrene/kg body weight) were drawn into a syringe equipped with a Teflon®-tipped plunger (Hamilton #1750, Hamilton Co., Reno, NV) and a 27-gauge hypodermic needle. Excess dose formulation was wiped off the needle before weighing the filled dosing apparatus. Intravenous doses were injected into a lateral tail vein. After dosing, the needle was wiped clean with a Kimwipe®, and the empty dosing apparatus was reweighed. The Kimwipe® was placed into a vial containing 2 mL methanol and analyzed by LSS. Each dose was calculated as the difference between the weights of the filled and empty dosing apparatus, less the amount found in the wipes. To determine the concentration of [^{14}C]α-methylstyrene in the dose formulation, two weighed aliquots were taken before, two after, and one in the midst of dosing a series of animals. These aliquots were assayed for ^{14}C using LSS.

The oral dose (1,000 mg/kg) was administered to one rat by intragastric gavage. The dose, contained in a 2.5-mL syringe fitted with a Teflon®-tipped plunger (Hamilton #1002) and a gavage needle (16-gauge ball-tipped), was weighed after excess dose was removed from the outside with a Kimwipe®. The wipe was placed into a scintillation vial containing 2 mL of methanol and analyzed by LSS after addition of fluor. The determination of actual dose delivered was performed as for the intravenous study.

For generation and delivery of α-methylstyrene vapor to animals during 6-hour, nose-only inhalation exposures, conditioned room air was pumped into the inlet stream using a Gast Model 0531 rotary, vane type pump (Gast Manufacturing, Inc., Benton Harbor, MI). Mass flow controllers regulated total inlet and outlet flow to deliver a constant flow (400 mL/minute) of freshly generated vapor to each nose port while removing this flow from the ports at a slightly lower rate. This resulted in a slightly positive pressure in the apparatus [1 to 2 inches H_2O, Dwyer Magnehelic pressure gauge (Dwyer Instruments, Inc., Michigan City, IN)]. Liquid α-methylstyrene was metered from a syringe [Harvard Apparatus Model 22 infusion pump (Harvard Apparatus, Holliston, MA)] in the generation column. The appropriate infusion rate was determined experimentally during preliminary trial runs. The column was wrapped with a specially designed heating mantle to maintain a temperature of 155° ± 5° C, sufficient for vapor generation. α-Methylstyrene vapor from the generation column entered a glass mixing chamber (600 mm × 11 mm interior diameter) prior to passing through a 0.5µ PTFE membrane filter and into the inlet manifold. The inlet manifold equally divided total flow into 400 mL/minute portions delivered to each nose port through equal lengths of stainless steel tubing. Noninhaled and expired vapor exited the nose ports through equal lengths of copper tubing passing into an outlet manifold where the portions were recombined into a single air stream. This single air stream then passed through eight vapor scrubbing traps filled with acetonitrile to remove residual radiolabel prior to exiting via another Gast vacuum pump to the laboratory fume hoods. The first four scrubbing traps were cooled to 0° C, and the remaining four traps were cooled to 60° C with a dry ice/isopropanol slurry. A constant 30 mL/minute flow of α-methylstyrene vapor derived from the inlet flow

stream just prior to the inlet manifold was continuously directed through 1/8 inch outside diameter stainless steel tubing into a series of gas sampling valves and a 1 mL sample loop before venting to the laboratory fume hood. Actuation of the gas sampling valve transferred the sample loop contents into the carrier gas stream of the Varian 3700 gas chromatograph (Varian Instrument Group, Walnut Creek, CA), initiating analysis of the 1 mL vapor sample.

α-Methylstyrene vapor concentration in exposure atmospheres was quantitated by an automated gas chromatographic system. This system was designed to accept 1 mL injections of α-methylstyrene vapor directly from the nose-only exposure apparatus, and it utilized a Varian 3700 gas chromatograph fitted with a DB-1 megabore capillary column (30 m × 0.53 mm interior diameter, J&W Scientific, Folsom, CA) and flame ionization detector. Helium, hydrogen, and airflow rates were 18, 30, and 300 mL/minute, respectively. Standard curves were constructed encompassing the mass of α-methylstyrene expected in 1 mL of vapor at 300 and 900 ppm.

Absorbed doses (mg α-methylstyrene/kg body weight) in the inhalation studies were calculated using the total mass (mg) of α-methylstyrene absorbed per animal during exposure. These mass values were determined by dividing the total ^{14}C (μCi) recovered in tissues, residual carcass, volatile breath, and excreta by the specific activity (μCi/mg) of the solution used for α-methylstyrene vapor generation (four animals per study). Total masses of α-methylstyrene absorbed by animals sacrificed immediately following cessation of exposure (three animals per study) were calculated as noted above except no excreta were collected from this group during or after exposure. Exhaled volatile components eliminated in the short interval between cessation of exposure and sacrifice were also not accounted for in this group, thereby precipitating their exclusion in the calculation of the mean absorbed dose reported per study. The mean mass of α-methylstyrene absorbed by four rats per study used for excreta collection was used to estimate the absorbed dose (mg/kg) for the five rats per study used for blood collection.

Urine and feces were collected separately into round-bottom flasks, cooled with dry ice, and removed at 6, 12 (urine only), 24, 48, and 72 hours postdosing in the intravenous study and postinitiation of exposure in the inhalation studies. Urine and feces were collected at 6, 24, and 48 hours postdosing in the oral study. Residual bladder urine was removed at sacrifice and combined with the 72-hour collection. In the inhalation studies, 6-hour samples contained all urine and feces excreted into restrainers during exposure. During exposure, urine and feces excreted into restrainers were immediately transferred to scintillation vials. Samples were stored in the dark at approximately –20° C until analyzed.

Expired volatile compounds and carbon dioxide (intravenous study only) were collected by passing room air through the metabolism chambers and a series of traps. The first trap was filled with 40 to 75 mL absolute ethanol and cooled to 0° C in an ice/water bath. The second trap, also containing absolute ethanol, was cooled to –60° C using a dry ice/isopropanol slurry. The third and fourth traps (intravenous study only) were filled with approximately 500 mL of 1 N NaOH. Traps were changed at 6, 12, 24, 48, and 72 hours postdose and 12, 24, 48 (900 ppm only), and 72 hours (900 ppm only) postinitiation of dose during the intravenous and inhalation studies, respectively.

Serial blood samples (300 μL each) were collected through the jugular cannulae from five animals for 24 hours, beginning 5 hours after initiation of exposure, in the 300 and 900 ppm inhalation studies. Immediately following collection, two approximately 75 μL aliquots were transferred to scintillation vials filled with 2 mL of Soluene-350® for ^{14}C quantitation. The remaining approximately 150 μL was transferred to a microcentrifuge tube for extraction according to the method described above.

Adipose tissues (two samples), muscle (two samples), skin (ears), as well as entire kidney, liver, spleen, lung, testes, bladder, heart, and brain were removed and assayed for ^{14}C content. Additionally, stomach, small intestine, cecum, and large intestine were removed and assayed for ^{14}C content. No tissues were excised in the oral or pilot intravenous pharmacokinetic studies. During the inhalation studies, skin samples were removed from the hind leg instead of the ears.

Aliquots of urine, ethanol, and NaOH were added directly to vials containing scintillation cocktail (Ultima Gold, Packard Instrument Company, Inc., Meriden, CT). Samples of tissue, feces, and blood (0.1 to 0.3 g) were digested in Soluene-350® (2 mL). After digestion, samples requiring bleaching were decolorized with perchloric acid/hydrogen peroxide prior to addition of scintillation cocktail. Stomach, small intestine, cecum, large intestine, and the carcass were digested in 2 N ethanolic sodium hydroxide, and aliquots were added to scintillation cocktail for analysis by LSS.

Samples from each urinary collection interval up to 48 hours from one rat (10 mg/kg intravenous) were analyzed by HPLC. Analysis of the urine by HPLC identified six metabolites: A (unknown), B (2-phenyl-1,2-propanediol glucuronide), C (2-phenyl-1,2-propanediol), D (atrolactic acid), E [S-(2 hydroxy-2-phenylpropyl)-N-acetylcysteine], and F (2-phenyl propionic acid). For the inhalation studies, urine from a different rat for each collection interval between 6 and 48 hours was analyzed. The urine was filtered through a Millex HV 0.45 µm filter. Radiochemical concentrations of the samples were determined by LSS. Profiles of ^{14}C-labeled components were determined by HPLC as described above.

After the urinary metabolite profiles had been established in the intravenous study, attempts were made to determine which metabolites were present as glucuronide, sulfate, or other conjugates. Aliquots of urine from the intravenous study were incubated with β-glucuronidase, sulfatase, or acylase. To a vial containing 1,000 units of β-glucuronidase was added 420 µL of deionized distilled water; 200 µL of this solution was boiled for 10 minutes and then flash frozen to deactivate the enzyme. 200 µL of either the heat deactivated enzyme or the active enzyme was added to 50 µL of urine from the 0 through 6 and 6 through 12 hour collections and to 100 µL of urine from the 12 through 24 and 24 through 48 hour collections. The mixtures were then incubated for 1 hour at 37° C. Sulfatase incubations contained 50 µL of urine (0 through 6 or 12 through 24 hour collection), 250 µL of Trizma® buffer (pH 7.6), and 250 µL of sulfatase solution (10 to 20 units/mL). Controls were prepared with heat deactivated and flash frozen enzyme. The incubations were heated at 37° C for 1 hour. Alternately, urine samples (100 µL) were incubated at 37° C for 6 hours with 100 µL acylase solution [2 mg/mL containing 7,000 units/mg in 0.9 M potassium phosphate buffer (pH 7.4)]. These incubation mixtures were chromatographed as described previously, and the profiles compared with those from untreated urine. There was no noticeable change in the profile following treatment with sulfatase or acylase. However, following treatment with β-glucuronidase, metabolite B was transferred to a compound that coeluted with metabolite C.

Urine collected 6 to 24 hours postdosing from a rat administered α-methylstyrene by oral gavage (1,000 mg/kg) was used for isolation and purification of metabolites using the HPLC method described previously. Metabolites were manually collected from the radioactivity detector following multiple runs. Eluant containing a given metabolite was pooled; the organic solvent was removed by rotary evaporation; and the residue (except for metabolite D) was brought to dryness by lyophilization. With metabolite D, the residue was basified to pH 10 with 1 N NaOH prior to lyophilization, and the resulting residue was reconstituted in methanol. A portion of this methanolic solution was reanalyzed by HPLC to confirm that the isolation procedures caused no degradation of the metabolite, and the remainder of the solution was dried under a stream of nitrogen. Metabolites C, D, E, and F were further purified by use of a Waters C18 Sep-Pak Plus (Waters Corporation, Milford, MA) extraction column prior to analysis by gas chromatography-mass spectrometry (GC/MS). Metabolite E was rechromatographed to eliminate contamination with metabolite D using the method described above for the initial isolation; then metabolite E was incubated with acylase. Metabolite B was treated with β-glucuronidase and rechromatographed using the same HPLC conditions. A new peak appeared that coeluted with metabolite C, and this analyte was collected and concentrated to dryness. The trimethylsilane derivatives of metabolite C, the aglycone of metabolite B, a standard of 2-phenyl-1,2-propanediol, metabolite D, and a standard of atrolactic acid were prepared and analyzed by GC/MS. A standard of 2-phenylpropionic acid and metabolite F were analyzed by GC/MS. A sample of metabolite B was further purified using a Microsorb-MV (Varian, Inc.) phenyl analytical column with an isocratic mobile phase of 10% acetonitrile in aqueous 1% acetic acid, with a flow rate of 1 mL/minute. This sample of metabolite B was analyzed by ^{1}H- and ^{13}C-Distortionless Enhanced Polarization Transfer (DEPT) NMR. Metabolite E was analyzed by ^{1}H- and ^{13}C-NMR, and its trimethylsilane derivative was analyzed by GC/MS.

Aliquots (150 µL) of blood from the inhalation studies were extracted with 300 µL of acetonitrile and then centrifuged at 14,000 or 16,000 g; the supernatants were transferred to 0.5 dram vials. Nonradiolabeled α-methylstyrene, atrolactic acid, and 2-phenylpropionic acid standards were added to each vial. Immediately prior to analysis by HPLC, 300 µL of water was added to each extract.

Whole blood obtained from rats immediately after 6-hour nose-only inhalation exposure (900 ppm) was centrifuged to obtain red blood cells. Red blood cells were washed with 0.9% saline, lysed with distilled deionized water, and centrifuged. A 20 µL aliquot of supernatant was diluted with 300 µL of the mobile phase (initial conditions) used for analysis by HPLC. To determine whether bound radiolabel was associated with heme, an HPLC method that separates heme from globin was used (Masalas and Manca, 1994).

Blood α-methylstyrene concentration versus time data obtained during and postexposure in the 300 and 900 ppm inhalation studies were analyzed by noncompartmental and compartmental techniques using WinNonlin software (Version 1.0; Scientific Consulting, Inc., Apex, NC). Noncompartmental analysis of data from individual animals was performed with WinNonlin Model 202 for infusion, where the inhaled dose was used as the infused dose.

Data sets from individual animals were also analyzed by compartmental techniques. The data were initially fit to a two-compartment model with zero-order absorption and first-order elimination (WinNonlin Model 9). Two additional two-compartment models were written to simultaneously solve pooled data from all animals within an exposure concentration from each of the two inhalation experiments. Each of these models permitted zero-order absorption. The models differed in that the first model contained a description of first-order elimination and the second contained a description of nonlinear (Michaelis-Menten) elimination. All pharmacokinetic analyses were conducted on weighted data (1/YHAT, where YHAT is the predicted α-methylstyrene concentration). For models in which data from two exposure concentrations were solved simultaneously, the dose rate (mg/kg per hour, calculated as dose received in mg/kg divided by the duration of exposure in hours) was required as model input.

Results and Discussion

Intravenous Study for Urinary Metabolite Identification

Intravenous doses of α-methylstyrene (10 mg/kg) were mainly excreted in the urine with 76% ± 2% excreted in the first 24 hours postdosing and 86% ± 1% by 72 hours (Table M1). Fecal elimination accounted for 2% of the dose. Exhalation of volatile organics and carbon dioxide accounted for only 2% and 0.02% of the dose, respectively.

The distribution of radioactivity in tissues 72 hours postdosing is shown in Table M2. Concentrations of α-methylstyrene equivalents were low, and only 0.3% of the radioactivity was recovered in the tissues. The concentration of α-methylstyrene and/or its metabolites in blood was 16 ng Eq per gram.

The profiles of metabolites present following an intravenous dose of α-methylstyrene (10 mg/kg) were determined for each urinary collection interval up to 48 hours postdosing for one rat. The relative amounts of these metabolites are shown in Table M3. The peak eluting at 10:25 (metabolite D) coelutes with atrolactic acid, and over 20% of the administered dose was excreted as this metabolite. Metabolite E was most abundant in the early urine collection (0 to 6 hours), with considerably less detected in later collections, suggesting that it may be an early intermediate metabolite of α-methylstyrene.

Oral Study for Urinary Metabolite Identification

α-Methylstyrene was administered orally to one rat at a dose of 1,000 mg/kg in an effort to obtain as much urinary metabolite as possible. Urine from this experiment was chromatographed and gave a similar profile as the urine from the intravenous study. Five of the metabolite peaks were identified by GC/MS. Metabolite B was treated

with β-glucuronidase, and the aglycone was analyzed by GC/MS. The spectrum of the bis-trimethylsilane derivative of a 2-phenyl-1,2-propanediol standard had no M+ at 296 but had peaks at m/z 281 (loss of a methyl), 193 (loss of CH_2O-trimethylsilane), and 147. The spectra of metabolite C and the aglycone of metabolite B were virtually identical to that of the bis-trimethylsilane derivative of a 2-phenyl-1,2-propanediol standard. Therefore, it was concluded that metabolite B is a glucuronide of 2-phenyl-1,2-propanediol and that metabolite C is 2-phenyl-1,2-propanediol.

Metabolite B was analyzed by ^{13}C-DEPT NMR in an effort to determine the position of attachment of the glucuronide group in the conjugate. Of the two oxygenated carbon atoms, only the terminal methylene group had protons with which the pulsed carbon nucleus could relax. Therefore, the position of the methylene resonance was determined in these characterization experiments using NMR. The spectra displayed a doublet for each carbon bearing a proton, possibly indicating that this metabolite was present as a pair of diasteriomers. The ^{13}C-DEPT NMR spectrum of 2-phenyl-1,2-propanediol was determined in order to interpret the spectra for metabolite B, and the methylene resonance appeared at 72 ppm. If the glucuronide conjugate were attached to the carbon alpha to the phenyl ring, the chemical shift of the methylene carbon would have been approximately 3 ppm upfield (at 69 ppm) relative to that for the unconjugated 2-phenyl-1,2-propanediol; had it been at the beta carbon, the shift would have been approximately 10 ppm downfield (at 82 ppm) (Silverstein *et al.*, 1981). In the ^{13}C-DEPT NMR spectrum of metabolite B, the methylene resonances were at 79 ppm, consistent with the attachment of the glucuronide moiety to the carbon beta to the phenyl ring.

A bis-trimethylsilane derivative of an atrolactic acid standard and metabolite D were analyzed by GC/MS, and the spectra were identical. A standard of 2-phenylpropionic acid and metabolite F were analyzed by GC/MS, and both spectra contained a molecular ion at m/z 150 and signals at m/z 105 (loss of COOH) and 77. An analogous urinary metabolite was identified after administration of 4-isopropenyltoluene to rabbits (Matsumoto *et al.*, 1994). Metabolite E was treated with acylase, and the incubation mixture was chromatographed as described previously for isolation of the peak. The profile was compared with the HPLC profile of the untreated urine. Treatment with acylase converted metabolite E to a new component that eluted between metabolites A and B. The ^{1}H NMR spectrum of metabolite E was consistent with a N-acetylcysteine conjugate resulting from reaction of glutathione with the epoxide of α-methylstyrene, followed by further metabolism to the mercapturate. The ^{13}C-DEPT NMR also corroborated this finding. The ^{13}C chemical shift of the methylene group beta to the ring was consistent with attachment of the sulfur atom to this carbon atom. Analogous metabolites were found for the metabolism of the vinyltoluenes (Bergemalm-Rynell and Steen, 1982). Trimethylsilane derivatives of the mercapturate(s) were prepared for analysis by GC/MS. Two di-derivatized and two tri-derivatized products were present in roughly equal amounts. The fragmentation patterns (in particular the ion at 193 a.m.u.) in these spectra were also consistent with the formation of just one of two possible positional isomers for the mercapturate. The NMR and mass spectral data indicated that a diasteriomeric pair of mercapturates was formed as metabolites.

The proposed metabolic pathway for α-methylstyrene is shown in Figure M1. The presence of roughly equal amounts of the diasteriomeric mercapturates suggests that the initial epoxidation of α-methylstyrene is not sterioselective and proceeds with no marked preference for the antarafacial or suprafacial addition of active oxygen to yield enantiomeric epoxides. Both enzymatic hydrolysis and glutathione conjugation of epoxides are known to proceed by S_N2 reactions. Therefore, enzymatic hydrolysis can yield enantiomeric diols. Further oxidation of the terminal hydroxy group of these diols to form atrolactic acid does not affect the chiral center at the benzyl position, and the potential products are enantiomers. However, conjugation with a chiral molecule such as glutathione or glucuronic acid would produce diasteriomeric metabolites from the enantiomeric products, as is the case with the mercapturates and glucuronides characterized in these studies.

Inhalation Study

The time weighted average α-methylstyrene vapor concentrations during exposure for the 300 and 900 ppm groups were 304 and 900 ppm. Animals exposed to 300 or 900 ppm received mean doses of 130.8 and 340.1 mg/kg, or 26.63 and 20.05 μCi, respectively.

For the 300 ppm group, urinary excretion was the main route of elimination, comprising 88.2% of the absorbed dose, with volatile breath and feces accounting for 3.1% and 2.2%, respectively (Table M4). These trends were consistent for all collection intervals. Over 90% of the absorbed dose was eliminated within 48 hours postinitiation of exposure. The same pattern of elimination was observed in the 900 ppm group; 92.4% was excreted in urine, with volatile breath and feces accounting for 2.5% and 2.6%, respectively.

Tissue distribution of radioactivity at 6, 24 (300 ppm only), and 72 hours after initiation of inhalation exposure are shown in Tables M5 and M6. At 72 hours after initiation of exposure, 2.6% to 10.1% and 1.1% to 2.4% of the inhaled radiolabeled α-methylstyrene was recovered in the residual carcass and tissues in the 300 and 900 ppm groups, respectively. The highest concentrations of radiolabeled α-methylstyrene (µg Eq/g tissue) were observed in adipose, bladder, liver, kidney, and skin at 6, 24 (300 ppm only), and 72 hours after initiation of exposure. This is consistent with the lipophilic nature of α-methylstyrene and the fact that most of the dose was eliminated in urine. Elevated levels of radiolabel were present in the small intestine compared to the stomach and large intestine, suggesting biliary excretion and reabsorption of [^{14}C]α-methylstyrene-derived metabolite(s). Tissues for the 24-hour time point in the 300 ppm group were removed from animals used for serial blood sampling.

The metabolite profile observed in urine from 300 and 900 ppm male rats was qualitatively the same as that in the intravenous study. Table M7 shows the percent of the absorbed dose excreted as each metabolite per collection interval. Much more atrolactic acid was observed in urine collected during the first 24 hours in the inhalation studies compared to the intravenous study. Rats exposed to 900 ppm exhibited nearly a twofold increase in excretion of atrolactic acid, accompanied by a corresponding drop in excretion of 2-phenyl-1,2-propanediol glucuronide, in urine collected between 12 and 24 hours compared to rats exposed to 300 ppm.

Characterization of the ^{14}C Profile in Blood

In extraction method development experiments, recovery of carbon-14 from blood spiked with [^{14}C]α-methylstyrene was 95% ± 6%. Recoveries of carbon-14 from blood samples obtained from the 300 and 900 ppm groups were 74% ± 10% and 82% ± 9%, respectively. These recoveries suggest sequestration of metabolites by red blood cells. Chromatographic analysis of red blood cell lysate showed that no radioactivity was associated with heme.

α-Methylstyrene concentrations in blood dropped precipitously in the 300 ppm rats upon cessation of exposure, from more than 6 µg/mL just prior to termination of exposure (5.5 hours) to an average of 0.97 µg/mL at 7 hours (Table M8). From 7 to 24 hours, α-methylstyrene concentrations in blood decreased at a much slower rate. α-Methylstyrene concentration in blood dropped from more than 24 µg/mL at 5.5 hours into the exposure to approximately 10 µg/mL in the first hour after cessation of exposure in the 900 ppm rats.

Four metabolites were extracted from blood obtained in the inhalation study. The major component at all time points was 2-phenyl-1,2-propanediol. Atrolactic acid, 2-phenylpropionic acid, and an additional radiolabeled component noted as blood metabolite 1 were also observed. Identities of atrolactic acid, 2-phenyl-1,2-propanediol, 2-phenylpropionic acid, and α-methylstyrene peaks were established by coelution with nonradiolabeled standards.

Pharmacokinetic Analysis

The following definitions of the pharmacokinetic parameters were derived from noncompartmental analyses.

$$AUC_{last} \text{ (hours} \times \text{mg/L)} = \Sigma\ (t_i - t_{i-1})(C_i + C_{i-1})/2$$

where AUC_{last} is the area under the blood concentration-time curve from time zero to the last measurable concentration and i = 1 to n (last time point).

β is the terminal elimination rate constant; $\beta(hour^{-1})$ is estimated via linear regression of time versus log concentration.

$t_{½}$ (hours) = $-\ln(2)/\beta$

where $t_{½}$ is the elimination half-life.

AUC_{INF} (hours × mg/L) = $AUC_{last} + C_n/\beta$

where AUC_{INF} is the area under the blood concentration-time curve extrapolated to time infinity and C_n is the last measurable concentration.

V_z (L/kg) = Dose/($\beta \times C_{INF}$)

where V_z is the volume of distribution based on the terminal phase.

Cl (L/hour per kg) = Dose/AUC_{INF}

where Cl is clearance.

Mean parameter estimates obtained from noncompartmental analysis of complete time courses from individual animals within an exposure group (300 and 900 ppm) are provided in Table M9. Parameter estimates appeared to be dependent upon dose, suggesting nonlinear pharmacokinetics.

Two two-compartment models were written to simultaneously solve data from the 300 and 900 ppm groups (Table M10). One model was written with zero-order absorption and first-order elimination; the other model was written with zero-order absorption and saturable (Michaelis-Menten) elimination.

REFERENCES

The Aldrich Library of 13C and 1H FT NMR Spectra (1993). 1st ed. (C.J. Pouchert, Ed.), Vol. 2, p. 23. Aldrich Chemical Company, Inc., Milwaukee, WI.

Bergemalm-Rynell, K., and Steen, G. (1982). Urinary metabolites of vinyltoluene in the rat. *Toxicol. Appl. Pharmacol.* **62**, 19-31.

McKenna, M.C., and Bieri, J.G. (1984). Multilayer cannula for long term infusion of unrestrained rats. *Lab. Anim. Sci.* **34**, 308-310.

Masalas, B., and Manca, L. (1994). Detection of globin chains by reverse phase high performance liquid chromatography. *Methods Enzymo.* **231**, 21-44.

Matsumoto, T., Ishida, T., Takeda, Y., and Yagi, J. (1994). The enantioselective metabolism of 4-isopropenyltoluene in rabbits. *Biol. Pharm. Bull.* **17**, 1441-1445.

Morgan, D., Burka, T., and Mahler, J. (1995). Comparative inhalation studies of styrene, α-methylstyrene, and divinylbenzene. Minutes, Project Review Committee meeting, February 16, 1995. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983) unpublished provisional data as of July 1, 1990. [database online (http://www.cdc.gov/noes/)]. NIOSH, Cincinnati, OH.

National Toxicology Program (NTP) (1985). NTP Executive Summary. Support for Chemical Nomination and Selection Process of the National Toxicology Program, Executive Summary of Data, alpha-Methylstyrene, NTP DRAFT Report, September 30, 1985. National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

Silverstein, R.M., Bassler, G.C., and Morrill, T.C. (1981). *Spectrometric Identification of Organic Compounds*, 4th ed. John Wiley & Sons, Inc., New York.

TABLE M1
Cumulative Excretion of Radioactivity by Male Rats after Intravenous Administration of [^{14}C]α-Methylstyrene (10 mg/kg)[a]

End of Collection Period (hours)	Percent of Dose Recovered in: Urine	Feces	Breath	CO_2	Total
6	34.1 ± 3.9	0.0468 ± 0.0696	2.01 ± 0.76	0.00666 ± 0.00268	36.1 ± 4.4
12	53.0 ± 7.3	—[b]	2.08 ± 0.77	0.0101 ± 0.0037	55.1 ± 8.0
24	76.4 ± 2.1	1.20 ± 0.33	2.14 ± 0.78	0.0132 ± 0.0025	79.8 ± 2.7
48	84.9 ± 1.6	1.78 ± 0.72	2.15 ± 0.78	0.0200 ± 0.0093	88.9 ± 0.7
72	86.0 ± 1.4	1.88 ± 0.73	2.16 ± 0.78	0.0236 ± 0.0078	90.0 ± 0.4
Cage wash	86.2 ± 1.4	1.88 ± 0.73	2.16 ± 0.78	0.0236 ± 0.0078	90.3 ± 0.4

[a] n=4
[b] Not collected

TABLE M2
Tissue Distribution of Radioactivity in Male Rats after Intravenous Administration of [^{14}C]α-Methylstyrene (10 mg/kg)[a]

Tissue	ngEq AMS/g Tissue	Tissue to Blood Ratio	% of Dose in Total Tissue
Adipose	25.3 ± 2.7	1.75 ± 0.74	0.0170 ± 0.002
Bladder	95.3 ± 96.6	7.47 ± 9.69	0.000630 ± 0.0006
Blood	16.4 ± 6.7	Unity	0.00811 ± 0.0032
Brain	4.53 ± 0.94	0.319 ± 0.160	0.000298 ± 0.00005
Heart	73.2 ± 21.9	4.74 ± 1.51	0.00203 ± 0.0006
Kidney	160 ± 52	11.6 ± 6.1	0.0113 ± 0.0039
Liver	72.8 ± 14.2	4.85 ± 1.78	0.0304 ± 0.0039
Lung	87.7 ± 36.7	6.11 ± 3.24	0.00608 ± 0.0027
Muscle	4.50 ± 1.05	0.310 ± 0.150	0.0205 ± 0.0041
Skin	55.4 ± 26.4	4.09 ± 3.15	0.0892 ± 0.0406
Spleen	233 ± 81	16.6 ± 8.1	0.00616 ± 0.0024
Testis	4.49 ± 0.56	0.310 ± 0.134	0.000454 ± 0.00006
Stomach[b]			0.00234 ± 0.00104
Small intestine[b]			0.0479 ± 0.0145
Cecum[b]			0.0219 ± 0.0092
Large intestine[b]			0.00843 ± 0.0032
Total in Tissue			0.276 ± 0.074

[a] n=4
[b] Includes contents

TABLE M3
Urinary Metabolite Profile in a Male Rat after Intravenous Administration of [^{14}C]α-Methylstyrene (10 mg/kg)

Hours Postdosing	% of Dose Excreted	Metabolite (% of dose)					
		A[a]	B[b]	C[c]	D[d]	E[e]	F[f]
0 to 6	34.2	0.65	16.86	0.51	7.46	6.94	0.21
6 to 12	19.6	0.51	10.17	0.61	5.34	1.81	0.33
12 to 24	21.4	0.71	10.87	0.88	6.96	1.31	0.26
24 to 48	7.23	0.3	2.97	0.75	2.1	0.63	0.086
Total	82.4	2.17	40.87	2.75	21.86	10.69	0.89

[a] Metabolite is unknown.
[b] Metabolite is 2-phenyl-1,2-propanediol glucuronide.
[c] Metabolite is 2-phenyl-1,2-propanediol.
[d] Metabolite is atrolactic acid.
[e] Metabolite is S-(2 hydroxy-2-phenylpropyl)-N-acetylcysteine.
[f] 2-Phenyl propionic acid elutes at this retention time.

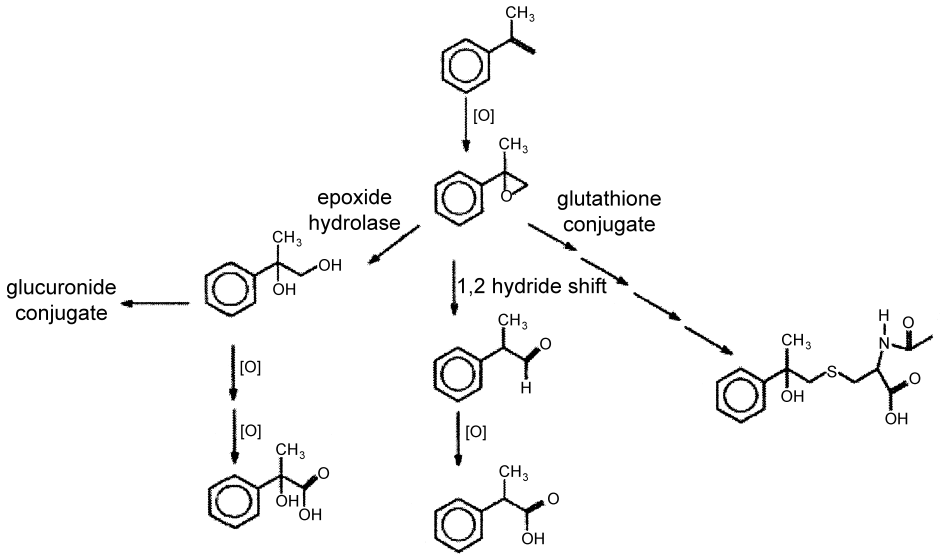


FIGURE M1
Proposed Metabolic Pathway for α-Methylstyrene

TABLE M4
Radioactivity Recovered from Male Rats Exposed to [^{14}C]α-Methylstyrene by Nose-Only Inhalation for 6 Hours[a]

Collection Interval (hours)[b]	Volatile Breath	Urine	Cage Rinse	Feces	Carcass and Tissues	Total Recovered Dose
300 ppm						
0 to 6	—[c]	1.3 ± 1.3		0.3 ± 0.3		1.6 ± 1.6
6 to 12	3.0 ± 0.8	30.1 ± 6.4 (31.3)		—		33.0 ± 5.6 (34.6)
12 to 24	0.2 ± 0.1	42.1 ± 5.0 (73.5)		1.0 ± 0.2 (1.4)		43.3 ± 4.9 (77.9)
24 to 48	—	12.4 ± 3.8 (85.9)		0.7 ± 0.0[d] (2.1)		13.2 ± 3.4 (91.4)
48 to 72	—	2.3 ± 0.7 (88.2)	0.6 ± 0.2	0.1 ± 0.0[d] (2.2)		3.1 ± 0.7 (100[d])
Overall Recovery	3.1 ± 0.9	88.2 ± 3.9	0.6 ± 0.2	2.2 ± 0.3	5.9 ± 3.8	100[d]
900 ppm						
0 to 6	—	3.6 ± 3.6		0.0 ± 0.0		3.7 ± 3.6
6 to 12	2.1 ± 0.4	27.0 ± 4.5 (27.0)		—		29.1 ± 4.3 (32.8)
12 to 24	1.1 ± 0.2 (3.2)	43.5 ± 5.1 (70.5)		1.1 ± 0.2 (1.4)		44.8 ± 5.0 (77.5)
24 to 48	0.2 ± 0.0 (3.4)	15.8 ± 2.9 (86.3)		1.3 ± 0.2 (2.1)		17.2 ± 3.1 (94.7)
48 to 72	0.0 ± 0.0 (2.5)	2.5 ± 0.6 (92.4)	0.9 ± 0.6	0.2 ± 0.1 (2.6)		3.7 ± 0.8 (100[d])
Overall Recovery	2.5 ± 0.4	92.4 ± 1.0	0.9 ± 0.6	2.6 ± 0.2	1.6 ± 0.6	100[d]

[a] Data are presented as percent of the recovered dose (mean ± standard deviation). n=4. Values in parentheses are cumulative percent of total radiolabeled dose excreted.
[b] Time since initiation of exposure
[c] No samples collected
[d] Administered dose is calculated as the absorbed dose (total radioactivity in the residual carcass, tissues, and all excreta).

TABLE M5
Tissue Distribution of Radioactivity in Male Rats Exposed by Nose-Only Inhalation to 300 ppm [^{14}C]α-Methylstyrene for 6 Hours[a]

	Time Since Initiation of Exposure		
	6 Hours	24 Hours[b]	72 Hours
n	3	5	4
Adipose			
µg-Eq α-methylstyrene/g tissue	412.3 ± 58.74	59.98 ± 82.35	1.203 ± 0.604
Tissue/blood ratio	7.89 ± 1.07	18.8 ± 1.48	7.88 ± 3.96
% Dose in total tissue	27.85421 ± 8.67878	2.90159 ± 0.41629	0.06092 ± 0.02965
Blood			
µg-Eq α-methylstyrene/g tissue	52.09 ± 1.555	3.079 ± 0.635	0.156 ± 0.056
Tissue/blood ratio	Unity	Unity	Unity
% Dose in total tissue	2.33139 ± 0.16343	0.10988 ± 0.02476	0.00578 ± 0.00168
Brain			
µg-Eq α-methylstyrene/g tissue	36.51 ± 1.276	1.841 ± 0.351	0.159 ± 0.064
Tissue/blood ratio	1.039 ± 0.351	0.577 ± 0.086	1.039 ± 0.351
% Dose in total tissue	0.24563 ± 0.04178	0.00938 ± 0.00167	0.00079 ± 0.00028
Cecum[c]			
µg-Eq α-methylstyrene/g tissue	27.71 ± 0.591	6.601 ± 0.573	0.278 ± 0.142
Tissue/blood ratio	0.530 ± 0.107	2.09 ± 0.264	1.74 ± 0.331
% Dose in total tissue	6.39434 ± 2.08418	1.14698 ± 0.10379	0.04796 ± 0.02062
Heart			
µg-Eq α-methylstyrene/g tissue	43.58 ± 0.412	2.832 ± 0.314	0.386 ± 0.472
Tissue/blood ratio	0.834 ± 0.012	0.894 ± 0.100	2.03 ± 1.70
% Dose in total tissue	0.12430 ± 0.02716	0.00600 ± 0.00063	0.00080 ± 0.00091
Large intestine[c]			
µg-Eq α-methylstyrene/g tissue	2.889 ± 1.205	2.789 ± 0.976	0.090 ± 0.038
Tissue/blood ratio	0.055 ± 0.022	0.918 ± 0.468	0.571 ± 0.073
% Dose in total tissue	0.63875 ± 0.23418	0.47356 ± 0.17072	0.01485 ± 0.00520
Small intestine[c]			
µg-Eq α-methylstyrene/g tissue	122.8 ± 4.970	14.52 ± 1.414	0.441 ± 0.142
Tissue/blood ratio	2.35 ± 0.141	4.62 ± 0.848	2.89 ± 0.584
% Dose in total tissue	29.48797 ± 3.79862	2.46813 ± 0.42324	0.07811 ± 0.01804
Kidney			
µg-Eq α-methylstyrene/g tissue	114.9 ± 6.141	12.79 ± 2.006	0.928 ± 0.228
Tissue/blood ratio	2.20 ± 0.082	4.00 ± 0.240	6.18 ± 1.39
% Dose in total tissue	0.82861 ± 0.11538	0.06785 ± 0.01439	0.00504 ± 0.00089
Liver			
µg-Eq α-methylstyrene/g tissue	142.8 ± 20.62	11.98 ± 3.868	0.876 ± 0.209
Tissue/blood ratio	2.73 ± 0.345	3.74 ± 0.928	5.79 ± 0.692
% Dose in total tissue	5.13890 ± 1.42606	0.31899 ± 0.09041	0.02413 ± 0.00419
Lung			
µg-Eq α-methylstyrene/g tissue	51.03 ± 7.700	3.288 ± 0.492	0.427 ± 0.472
Tissue/blood ratio	0.975 ± 0.132	1.03 ± 0.082	2.31 ± 1.63
% Dose in total tissue	0.25580 ± 0.10187	0.01267 ± 0.00486	0.00231 ± 0.00284

TABLE M5
Tissue Distribution of Radioactivity in Male Rats Exposed by Nose-Only Inhalation to 300 ppm [^{14}C]α-Methylstyrene for 6 Hours

	Time Since Initiation of Exposure		
	6 Hours	24 Hours	72 Hours
n	3	5	4
Muscle			
µg-Eq α-methylstyrene/g tissue	40.84 ± 3.677	2.228 ± 0.665	0.138 ± 0.039
Tissue/blood ratio	0.781 ± 0.058	0.686 ± 0.106	0.899 ± 0.078
% Dose in total tissue	18.71510 ± 4.38545	0.74002 ± 0.22493	0.04742 ± 0.01063
Skin			
µg-Eq α-methylstyrene/g tissue	85.40 ± 7.349	6.430 ± 7.043	1.060 ± 0.424
Tissue/blood ratio	1.64 ± 0.138	1.85 ± 1.72	6.77 ± 0.897
% Dose in total tissue	13.92857 ± 3.60203	0.75241 ± 0.81271	0.12834 ± 0.04199
Spleen			
µg-Eq α-methylstyrene/g tissue	41.65 ± 5.144	3.010 ± 0.839	0.440 ± 0.540
Tissue/blood ratio	0.797 ± 0.089	0.929 ± 0.152	2.30 ± 1.95
% Dose in total tissue	0.07538 ± 0.00447	0.00437 ± 0.00114	0.00085 ± 0.00098
Stomach[c]			
µg-Eq α-methylstyrene/g tissue	9.193 ± 4.624	0.648 ± 0.522	0.025 ± 0.018
Tissue/blood ratio	0.175 ± 0.085	0.203 ± 0.172	0.179 ± 0.149
% Dose in total tissue	1.99003 ± 0.74909	0.10994 ± 0.08874	0.00434 ± 0.00316
Testis			
µg-Eq α-methylstyrene/g tissue	39.20 ± 1.239	4.149 ± 1.492	0.339 ± 0.347
Tissue/blood ratio	0.751 ± 0.034	1.32 ± 0.523	1.871 ± 1.189
% Dose in total tissue	0.40964 ± 0.07401	0.03046 ± 0.01229	0.00252 ± 0.00241
Urinary bladder			
µg-Eq α-methylstyrene/g tissue	211.5 ± 70.74	52.44 ± 28.41	1.064 ± 0.660
Tissue/blood ratio	4.04 ± 1.32	17.2 ± 10.8	7.63 ± 5.64
% Dose in total tissue	0.07259 ± 0.03346	0.01599 ± 0.01019	0.00032 ± 0.00012

[a] Data are presented as mean ± standard deviation.
[b] Tissues obtained at 24 hours were from rats used for serial blood sampling.
[c] Includes contents

TABLE M6
Tissue Distribution of Radioactivity in Male Rats Exposed by Nose-Only Inhalation to 900 ppm [^{14}C]α-Methylstyrene for 6 Hours[a]

	Time Since Initiation of Exposure	
	6 Hours	72 Hours
n	3	4
Adipose		
µg-Eq α-methylstyrene/g tissue	1,710.0 ± 73.7	1.770 ± 1.590
Tissue/blood ratio	12.2 ± 0.999	3.84 ± 3.37
% Dose in total tissue	35.0 ± 1.76	0.0329 ± 0.0300
Blood		
µg-Eq α-methylstyrene/g tissue	141.0 ± 7.3	0.447 ± 0.0599
Tissue/blood ratio	Unity	Unity
% Dose in total tissue	2.14 ± 0.238	0.00623 ± 0.000235
Brain		
µg-Eq α-methylstyrene/g tissue	124.0 ± 1.540	0.336 ± 0.0727
Tissue/blood ratio	0.881 ± 0.0398	0.753 ± 0.139
% Dose in total tissue	0.251 ± 0.0369	0.000665 ± 0.000116
Cecum[b]		
µg-Eq α-methylstyrene/g tissue	75.60 ± 21.1	0.921 ± 0.203
Tissue/blood ratio	0.542 ± 0.172	2.11 ± 0.653
% Dose in total tissue	5.12 ± 1.14	0.0630 ± 0.0193
Heart		
µg-Eq α-methylstyrene/g tissue	138.0 ± 2.660	0.490 ± 0.0712
Tissue/blood ratio	0.983 ± 0.0627	1.10 ± 0630
% Dose in total tissue	0.120 ± 0.0159	0.000481 ± 0.000042
Large intestine[b]		
µg-Eq α-methylstyrene/g tissue	10.8 ± 4.690	0.179 ± 0.159
Tissue/blood ratio	0.0769 ± 0.0358	0.389 ± 0.302
% Dose in total tissue	0.716 ± 0.309	0.0114 ± 0.00906
Small intestine[b]		
µg-Eq α-methylstyrene/g tissue	219.0 ± 33.5	0.898 ± 0.193
Tissue/blood ratio	1.56 ± 0.251	2.02 ± 0.431
% Dose in total tissue	15.7 ± 2.41	0.0626 ± 0.0143
Kidney		
µg-Eq α-methylstyrene/g tissue	282.0 ± 10.8	2.020 ± 0.221
Tissue/blood ratio	2.00 ± 0.103	4.56 ± 0.716
% Dose in total tissue	0.606 ± 0.0652	0.00407 ± 0.000551
Liver		
µg-Eq α-methylstyrene/g tissue	321.0 ± 11.4	2.330 ± 0.277
Tissue/blood ratio	2.28 ± 0.144	5.25 ± 0.531
% Dose in total tissue	3.53 ± 0.203	0.0221 ± 0.00133
Lung		
µg-Eq α-methylstyrene/g tissue	159.0 ± 12.7	0.522 ± 0.0622
Tissue/blood ratio	1.13 ± 0.111	1.18 ± 0.141
% Dose in total tissue	0.174 ± 0.0121	0.000833 ± 0.000183

TABLE M6
Tissue Distribution of Radioactivity in Male Rats Exposed by Nose-Only Inhalation to 900 ppm [^{14}C]α-Methylstyrene for 6 Hours

	Time Since Initiation of Exposure	
	6 Hours	72 Hours
n	3	4
Muscle		
µg-Eq α-methylstyrene/g tissue	129.0 ± 7.140	0.326 ± 0.0798
Tissue/blood ratio	0.916 ± 0.0660	0.723 ± 0.0951
% Dose in total tissue	18.1 ± 2.02	0.0417 ± 0.00687
Skin		
µg-Eq α-methylstyrene/g tissue	33.7 ± 82.3	1.670 ± 0.614
Tissue/blood ratio	2.42 ± 0.690	3.83 ± 1.64
% Dose in total tissue	16.6 ± 3.32	0.0778 ± 0.0329
Spleen		
µg-Eq α-methylstyrene/g tissue	124.0 ± 8.40	0.503 ± 0.151
Tissue/blood ratio	0.876 ± 0.0413	1.15 ± 0.420
% Dose in total tissue	0.0676 ± 0.006	0.000373 ± 0.000178
Stomach[b]		
µg-Eq α-methylstyrene/g tissue	13.8 ± 1.310	0.0535 ± 0.0261
Tissue/blood ratio	0.0980 ± 0.00476	0.118 ± 0.0507
% Dose in total tissue	0.958 ± 0.133	0.00349 ± 0.00161
Testis		
µg-Eq α-methylstyrene/g tissue	112.0 ± 6.390	0.422 ± 0.119
Tissue/blood ratio	0.794 ± 0.0338	0.934 ± 0.170
% Dose in total tissue	0.377 ± 0.0474	0.00139 ± 0.000306
Urinary bladder		
µg-Eq α-methylstyrene/g tissue	897.0 ± 377.0	1.060 ± 0.622
Tissue/blood ratio	6.40 ± 2.88	2.29 ± 0.999
% Dose in total tissue	0.142 ± 0.064	0.000118 ± 0.000069

[a] Data are presented as mean ± standard deviation.
[b] Includes contents

TABLE M7
Urinary Metabolite Profile in Male Rats Exposed by Nose-Only Inhalation to [^{14}C]α-Methylstyrene for 6 Hours[a]

Collection Interval[b]	300 ppm	900 ppm
6 to 12 hours		
% of dose excreted	38.13	30.54
Metabolite A (unknown)	0.54	0.65
Metabolite B (2-phenyl-1,2-propanediol glucuronide)	16.59	9.55
Metabolite C (2-phenyl-1,2-propanediol)	0.64	0.52
Metabolite D (atrolactic acid)	15.09	14.94
Metabolite E [S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine]	4.46	4.51
Metabolite F (2-phenyl propionic acid)	0.37	0.16
12 to 24 hours		
% of dose excreted	46.87	49.12
Metabolite A (unknown)	0.74	1.08
Metabolite B (2-phenyl-1,2-propanediol glucuronide)	23.26	13.49
Metabolite C (2-phenyl-1,2-propanediol)	0.50	0.57
Metabolite D (atrolactic acid)	16.94	28.94
Metabolite E [S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine]	4.38	4.30
Metabolite F (2-phenyl propionic acid)	0.40	0.40
24 to 48 hours		
% of dose excreted	15.41	16.37
Metabolite A (unknown)	0.61	0.51
Metabolite B (2-phenyl-1,2-propanediol glucuronide)	6.65	6.86
Metabolite C (2-phenyl-1,2-propanediol)	1.19	0.18
Metabolite D (atrolactic acid)	5.54	6.88
Metabolite E [S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine]	0.97	1.53
Metabolite F (2-phenyl propionic acid)	0.28	0.31

[a] All values are expressed as percent of dose.
[b] Time since initiation of exposure

TABLE M8
α-Methylstyrene Blood Concentration in Male Rats Exposed by Nose-Only Inhalation to [^{14}C]α-Methylstyrene for 6 Hours

Time Point[a] (hh:mm)	α-Methylstyrene Blood Concentration (μg/mL) by Animal				
	1	2	3	4	5
300 ppm					
5:00	—[b]	—	—	6.18	6.32
5:30	7.30	5.76	5.01	—	—
6:05	4.08	2.26	2.70	—	—
6:10	2.07	2.31	2.30	—	—
6:15	—	—	—	1.81	1.95
6:20	2.46	1.26	1.35	—	—
6:25	—	—	—	1.16	—
6:30	2.16	1.15	1.23	—	—
6:35	—	—	—	—	—
6:45	—	—	—	0.98	0.447
7:00	1.27	0.65	0.98	—	—
7:30	—	—	—	0.69	0.508
8:00	0.96	0.51	0.53	—	—
9:00	—	—	—	0.47	0.50
10:00	0.64	0.38	0.25	—	—
12:00	—	—	—	0.14	0.28
24:00	0.09	0.61	0.07	0.05	0.05
900 ppm					
5:00	—	—	—	24.75	24.84
5:00	—	—	—	24.75	24.84
5:30	18.27	26.72	29.16	—	—
6:05	10.8	21.92	22.58	—	—
6:10	14.94	17.07	23.42	—	—
6:15	—	—	—	19.73	14.91
6:20	12.97	14.01	15.47	—	—
6:25	—	—	—	16.98	12.38
6:30	11.23	9.13	20.52	—	—
6:35	—	—	—	18.19	8.99
6:45	—	—	—	14.94	8.68
7:00	6.64	8.11	15.33	—	—
7:30	—	—	—	11.00	4.87
8:00	4.27	4.30	11.11	—	—
9:00	—	—	—	6.19	2.55
10:00	2.11	2.53	5.54	—	—
12:00	—	—	—	2.18	1.26
24:00	0.13	4.28	0.17	0.14	0.09

[a] Time since initiation of exposure in hours and minutes
[b] No sample drawn

TABLE M9
Noncompartmental Analysis of Blood α-Methylstyrene Concentration versus Time Data from Male Rats Exposed by Nose-Only Inhalation for 6 Hours[a]

Parameter	300 ppm	900 ppm
n	5	5
Dose (mg/kg)	137.6 ± 7.0	345.3 ± 12.9
AUC_{last} (hours × mg/L)	25.9 ± 5.3	130.8 ± 34.3
β(hour^{-1})	0.144 ± 0.028	0.256 ± 0.060
$t_{½}$ (hours)	4.99 ± 1.14	2.81 ± 0.54
AUC_{INF} (hours × mg/L)	26.8 ± 4.9	132.6 ± 33.5
V_Z (L/kg)	38.6 ± 15.1	11.2 ± 4.1
Cl (L/hour per kg)	5.3 ± 0.9	2.7 ± 0.7

[a] Data are presented as mean ± standard deviation.

TABLE M10
Compartmental Analyses of Pooled Blood α-Methylstyrene Concentration (mg/L) versus Time Data for Male Rats Exposed to 300 or 900 ppm by Nose-Only Inhalation for 6 Hours[a]

Parameter	First-Order Elimination	Michaelis-Menten Elimination
Cl (L/hour per kg)	2.25 ± 0.20	—[b]
K_{12} (hour^{-1})[c]	0.723 ± 0.321	1.96 ± 0.43
K_{21} (hour^{-1})	0.321 ± 0.123	0.250 ± 0.065
V (L/kg)	1.68 ± 0.51	1.31 ± 0.27
K_M (mg/L)	—	3.58 ± 1.42
V_{MAX} (mg/hour per kg)	—	31.9 ± 7.0

[a] Data are presented as mean ± standard error, as determined in the optimization routine.
[b] Not applicable
[c] K is a rate constant