NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 278

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 2,6-XYLIDINE (2,6-DIMETHYLANILINE) (CAS NO. 87-62-7) IN CHARLES RIVER CD RATS (FEED STUDIES)

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

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a comprehensive audit before being presented for public review. This Technical Report has been reviewed and approved by the NTP Board of Scientific Counselors' Peer Review Panel in public session; the interpretations described herein represent the official scientific position of the NTP.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

Special Note: This Technical Report underwent peer review in public session and was approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee on September 22, 1982 [see pages 7-8]. Thereafter, the NTP adopted the policy that the experimental data and laboratory records from all NTP toxicology and carcinogenesis studies not yet printed and distributed would be audited. [A summary of the data audit is presented in Appendix G.] Consequently, printing and distribution of this Technical Report have been delayed, and the format differs from that of Technical Reports that underwent peer review more recently. The categories of evidence of carcinogenicity adopted by the NTP in June 1983 were not used to evaluate these data. This final Technical Report supersedes all previous drafts of this report which have been distributed.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 2,6-XYLIDINE

(2,6-DIMETHYLANILINE)

(CAS NO. 87-62-7)

IN CHARLES RIVER CD RATS

(FEED STUDIES)

Mary Kornreich, Ph.D., and
Charles A. Montgomery, Jr., D.V.M., Study Scientists

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

January 1990

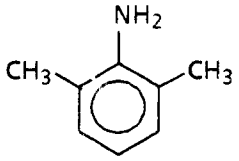
NTP TR 278

NIH Publication No. 90-2534

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

CONTENTS

APPENDIXES



2,6-XYLIDINE

(2,6-DIMETHYLANILINE)

CAS No. 87-62-7

ABSTRACT

2,6-Xylidine is a chemical intermediate used principally in the production of dyes. It is also a component of tobacco smoke, a degradation product of aniline-based pesticides, and a metabolite of certain drugs, particularly the xylide group of local anesthetics. The National Toxicology Program (NTP) sponsored single-administration, 2-week, and 13-week studies of 2,6-xylidine by gavage in F344/N rats. The U.S. Environmental Protection Agency (EPA) sponsored short-term gavage studies and 10-week range-finding feed studies in Charles River CD rats (a Sprague Dawley-derived strain). A carcinogenesis study of 2,6-xylidine was initiated by the EPA, which designed and monitored the study during the 2-year exposure period. The NTP then assumed responsibility for the study, conducting terminal kill, necropsy, histopathologic evaluation, data analysis, and report preparation.

Oral LD_{50} values of 1.2-1.3 g/kg were calculated for F344/N and Charles River CD rats administered single doses of 2,6-xylidine. Marginally toxic effects occurred in the hepatic, renal, and hematopoietic systems of dosed rats in the single-administration, 2-week, 10-week, and 13-week studies.

The 56 male and 56 female Charles River CD rats used in the 104-week carcinogenesis studies were the offspring of animals fed diets containing 0, 300, 1,000, or 3,000 ppm 2,6-xylidine before breeding, during pregnancy, and through the lactation period. The concentrations of 2,6-xylidine offered to animals in the 104-week studies were the same as those given to their parents.

During most of the 2-year studies, high dose male and female rats showed a reduction (greater than 10%) in body weight gain. Survival in high dose male rats was significantly reduced ($P < 0.001$) relative to that in controls. Survival also was reduced in the 1,000-ppm group. There was no significant relationship between concentration and mortality in female rats, but mortality was high for all groups of female rats during the second year of the study.

The epithelium of the nasal cavity was the primary site of compound-related neoplastic and nonneoplastic lesions. The incidences of both papillomas and carcinomas of the nasal cavity were significantly increased in high dose male and female rats. Carcinomas or adenocarcinomas (combined) occurred in 28/56 high dose males, 24/56 high dose females, and 1/56 mid dose females. Papillary adenomas occurred in 10/56 high dose males, 2/56 mid dose males, and 6/56 high dose females. None occurred in the other groups. The carcinomas were highly invasive and frequently destroyed the nasal turbinates and nasal septum. Metastasis to the brain was present in 5/56 male and 7/56 female high dose rats.

Malignant mesenchymal tumors were observed in the nasal cavity. Rhabdomyosarcomas occurred in two high dose male rats and two high dose female rats. These rare malignant tumors have not been previously reported at this site in Sprague Dawley rats. Malignant mixed tumors having features of adenocarcinomas and rhabdomyosarcomas were reported in one high dose male and one high dose

female rat. One undifferentiated sarcoma was seen in a high dose female rat. The nonneoplastic lesions observed in the nasal cavity included acute inflammation, epithelial hyperplasia, and squamous metaplasia.

The incidences of subcutaneous tissue fibromas were increased in high dose male and female rats (male: control, 0/56; low dose, 1/56; mid dose, 2/56; high dose, 4/56; female: 0/56; 2/56; 1/56; 4/56) and were dose related. Subcutaneous fibrosarcomas were observed in three high dose females, one high dose male, one mid dose female, one low dose male, and one control female.

A significant dose-related increase occurred in the incidence of female rats with neoplastic nodules of the liver (0/56; 1/56; 2/56; 4/55). This increase was significant in the high dose group by the incidental tumor test.

Conclusions: Under the conditions of these 2-year feed studies, 2,6-xylidine was *clearly carcinogenic** for male and female Charles River CD rats, causing significant increases in the incidences of adenomas and carcinomas of the nasal cavity. A rhabdomyosarcoma, a rare tumor of the nasal cavity, was observed in dosed rats of each sex. In addition, the increased incidences of subcutaneous fibromas and fibrosarcomas in male and female rats and the increased incidence of neoplastic nodules of the liver in female rats may have been related to the administration of 2,6-xylidine.

*A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 7-8.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,6-Xylidine is based on the short-term studies conducted by EG&G Mason Research Institute (Worcester, MA) from August 1980 to June 1981 and on studies conducted by Litton Bionetics, Inc. (Rockville, MD) from May 1977 until September 1980.

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

Mary Kornreich, Ph.D., and Charles A. Montgomery, Jr., D.V.M., Study Scientists

Gary A. Boorman, D.V.M., Ph.D.
Rajendra S. Chhabra, Ph.D.
Michael P. Dieter, Ph.D.
J. Fielding Douglas, Ph.D.
Charles K. Grieshaber, Ph.D.
Larry Hart, Ph.D.
Joseph K. Haseman, Ph.D.
James Huff, Ph.D.
C.W. Jameson, Ph.D.
Robert Maronpot, D.V.M.
E.E. McConnell, D.V.M.
John A. Moore, D.V.M.
Sherman Stinson, Ph.D.
Raymond W. Tennant, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report on 6/3/82)

Gary A. Boorman, D.V.M., Ph.D. (Chair) (NTP)
Bhola N. Gupta, D. Vet. Sci., Ph.D. (NTP)
Charles A. Montgomery, Jr., D.V.M. (NTP)
James Popp, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology
Michael Stedham, D.V.M. (Tracor Jitco, Inc.)

Principal Contributors at EG&G Mason Research Institute
(Conducted Study and Evaluated Tissues)

H. Lilja, Ph.D.
A. Block, Ph.D.
J. Esber, Ph.D.
R. Fleischman, D.V.M.
R. Grant, M.A.
M. Hagopian, Ph.D.
G. Wade, B.A.

Principal Contributors at Litton Bionetics, Inc.
(Conducted Study and EvaluatedTissues)

W. Cole, M.S.
H. Eskildson
J. Fritz, Ph.D.
E. Gordon, Ph.D.
E. Hart, Ph.D.
D. Jones
C. Lunchick, M.S.
D. Maloney, B.A.

Principal Contributor at Experimental Pathology Laboratory
(Provided Pathology Quality Assurance)

Miriam Anvers, D.V.M., Ph.D.

Principal Contributors at the U.S. Environmental Protection Agency
(Designed and Monitored Studies of Charles River CD Rats)

K. Blackburn
K. Campbell, M.P.H., D.V.M.
J. Stara, D.V.M.

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D. Theriault, Ph.D.
Abigail C. Jacobs, Ph.D.
John Warner, M.S.
Naomi Levy, B.A.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on 2,6-xylidine on September 22, 1982, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Margaret Hitchcock, Ph.D. (Chair)
Pharmacology Toxicology
John B. Pierce Foundation Laboratory
New Haven, CT

Curtis Harper, Ph.D.
Associate Professory of Pharmacology
University of North Carolina
Chapel Hill, NC

Alice Whittemore, Ph.D.
Biostatistics
Stanford University School of Medicine
Palo Alto, CA

Ad Hoc Subcommittee Panel of Experts

Norman Breslow, Ph.D.*
Biostatistics
University of Washington
Seattle, WA

Robert M. Elashoff, Ph.D.
Biostatistics
University of California at Los Angeles
Jonsson Comprehensive Cancer Center
Los Angeles, CA

Joseph Highland, Ph.D.*
Toxicology, Environmental Defense Fund
Washington, DC

J. Michael Holland, Ph.D., D.V.M.
Department of Biology
Oak Ridge National Laboratory
Oak Ridge, TN

Frank Mirer, Ph.D.
International Union, United Auto Workers
Detroit, MI

Robert A. Scala, Ph.D.
Exxon Corporation
East Millstone, NJ

Bernard Schwetz, Ph.D., D.V.M. (Principal Reviewer) Toxicology Research Laboratory, Dow Chemical U.S.A.
Midland, MI

James Swenberg, Ph.D., D.V.M. (Principal Reviewer) Chief of Pathology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Stan D. Vesselinovitch, D.V.Sc.
Departments of Radiology and Pathology
University of Chicago
Chicago, IL

Mary Vore, Ph.D. (Principal Reviewer)
Pharmacology, University of Kentucky
College of Medicine
Lexington, KY

*Unable to attend

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF 2,6-XYLIDINE

On September 22, 1982, the draft Technical Report on the toxicology and carcinogenesis studies of 2,6-xylidine received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M. Kornreich, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clearly carcinogenic for male and female Charles River CD rats).

Dr. Swenberg, a principal reviewer, agreed with the conclusions. As the 2-year studies were conducted in rats only, he questioned whether data from short-term studies in $B6C3F_1$ mice should be included. Dr. Kornreich indicated that these data are important and should be in the Technical Report. Dr. Swenberg said that some of the statistics used were confusing; he asked for more details on the various studies done on the stability and volatility of the chemical in feed and that a list be provided of references to other systemically administered agents that have induced nasal cavity tumors. In response to Dr. Swenberg, Dr. J. Haseman, NIEHS, said that NTP would revise the format for presenting the statistical analyses of tumor incidence data and make it consistent with the format used in most other NTP Technical Reports.

Dr. Kornreich said that three short-term studies were conducted by gavage in rats and mice and the studies conducted by the U.S. Environmental Protection Agency included two short-term studies and a 2-year study using different protocols, a different route of administration, and different strains of rats. The information from all these studies was included in the board draft because it would enhance the understanding of the toxicology of 2,6-xylidine. She acknowledged that there was considerable loss of chemical due to evaporation from the formulated diets and, to a lesser extent, through chemical reaction with feed components, resulting in a less than certain dose per rat. Mr. R. Smith, Ethyl Corporation, and Dr. C. Jameson, NTP, described studies done by Ethyl and NTP to measure loss of compound from the diets.

As a second principal reviewer, Dr. Schwetz said that although he agreed with the conclusions regarding the nasal tumors, the actual dose level was not determined in the 2-year studies and the route of exposure was a mixture of oral administration and vapor inhalation. Dr. Schwetz commented that there was too much information included which might be reported elsewhere, especially the data from studies in mice.

As a third principal reviewer, Dr. Vore agreed with the conclusions as stated. She requested that a statement be made concerning the high mortality in high dose male rats. Death did not appear to be due to nasal tumors, since the incidence of tumors in female rats was similar to that in males, and yet there was no increased mortality in female rats. She opined that there was a low probability that inhalation was a realistic route of exposure and did not need to be invoked as a mechanism for the nasal tumors.

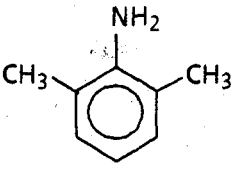
In response to a question by Dr. Mirer about data on fertility and birth defects, Dr. Kornreich said that the 2-year studies were done in conjunction with a multigeneration reproduction study. Dr. Scala emphasized the lack of quantitation of the doses and commented on the distinct possibility that topical and inhalation exposure was involved. Dr. Holland indicated that too much emphasis was being given to the actual dose to the target tissue. Further, he said that delaying release of the data pending the acquisition of more detailed information about the proportion of exposure of the nasal

route vs. the oral route would be unwarranted. Dr. Hitchcock said that data developed by NTP and others on the rate of loss of chemical through volatility from the diet should be highlighted in the Report (page 21) and some reasonable estimate of exposure derived. Dr. Vore requested that the data on mice not be deleted from the Technical Report.

Dr. Swenberg moved that the Technical Report on the studies of 2,6-xylidine be accepted with the revisions indicated. Dr. Vore seconded the motion. The Technical Report was approved unanimously by the Peer Review Panel.

I. INTRODUCTION

I. INTRODUCTION



2,6-XYLIDINE

(2,6-DIMETHYLANILINE)

CAS No. 87-62-7

There are six isomeric xylidines (dimethylanilines). Commercially available mixtures of these isomers are referred to as "xylidine." At least 2,300 kg was produced in the United States in 1976, and approximately 9,300 kg was imported the same year (USITC, 1977a,b). The annual production of a xylidine mixture consisting almost exclusively of 2,4- and 2,6-xylidine was 10,000-100,000 kg in France, Italy, Switzerland, and the United Kingdom and 100,000-1,000,000 kg in the Federal Republic of Germany (IARC, 1978).

Use of 2,6-xylidine as a chemical intermediate, principally in the production of dyes, may result in occupational exposure. 2,6-Xylidine is an intermediate in the production of C.I. Acid Orange 134 (used to color water-based cosmetics) and C.I. Direct Red 150 (used to color textiles) (Colour Index, 1977).

The general population may be exposed to xylidine-yielding compounds in tobacco smoke, drugs, and dyes. Several investigators have identified 2,6-xylidine as a component of tobacco smoke (Patrianakos and Hoffman, 1979; Petterson et al., 1980; Thelestam et al., 1980; Florin et al., 1980). Xylidine metabolites are released by drugs, particularly the xylidine group of local anesthetics. 2,6-Xylidine has been identified as a metabolite in the urine of rats, guinea pigs, dogs, and humans administered lidocaine, a local anesthetic that is clinically effective in controling ventricular arrhythmia in humans (Keenaghan and Boyes, 1972). 2,6-Xylidine is also a metabolite excreted in the urine of humans exposed to etidocaine, another member of the xylidine group of local anesthetics (Thomas et al., 1976). 2,6-Xylidine is the major degradation product of the hydrolytic reaction of lidamidine hydrochloride, an antidiarrheal agent (Zalipsky et al., 1978).

2,6-Xylidine may also enter the environment through the degradation of aniline-based pesticides such as CGA 17020 (Teridox®) (Bollag et al., 1978). Twenty-four hours after ^{14}C-labeled 2,6-xylidine was applied to soil, 66% of the applied radioactivity was bound to the soil. Acetylation apparently occurs easily in soil, and *N*-acetyl-2,6-dimethylanilide was isolated from the methanol extract of soil treated with 2,6-xylidine.

Intestinal microflora reductively cleave azo dyes to aromatic amines (Chung et al., 1978). Ponceau 3R is a mixture of dyes which, upon reductive splitting at the azo linkage, yields the water soluble salt of 1-amino-2-naphthol-3,6-disulfonic acid and several water insoluble anilines. Among the aniline derivatives, more than half are 2,4-, 2,5-, and 2,6-xylidines. 2,6-Xylidine accounts for 4%-12% of the metabolites of Ponceau 3R (Lindstrom, 1961).

2,6-Xylidine is produced by the reduction of Ponceau 3R by *Fusobacterium* sp. 2, a human intestinal anaerobe (Hartman et al., 1979). Ponceau 3R has been shown to be carcinogenic in the liver of rats when administered orally (Mannell, 1964). It was used as a food colorant (FD & C Red No. 1) until 1960 and is currently used as a biologic stain (IARC, 1978; Hartman et al., 1979). Although Ponceau 3R is not mutagenic per se by the Ames test (rat liver S9 and *Salmonella typhimurium* strain TA100), reduction of the azo dye leads to a mutagenic derivative (Hartman et al., 1979). Gas chromatographic analysis of reduced and extracted Ponceau 3R showed peak areas for 2,4-xylidine (38.4%) and 2,6-xylidine (3.4%).

Neither compound was mutagenic in the amounts formed under the conditions studied. The mutagenicity of Ponceau 3R was attributed to 2,4,5-trimethylaniline.

When administered orally to male Osborne-Mendel rats at doses of 200 mg/kg per day for up to 8 days, 2,6-xylidine was metabolized by oxidation to aminoxylols and traces of aminotoluic acid (Lindstrom et al., 1963). The aminoxylols formed from 2,6-xylidine were 4-hydroxy-2,6-dimethylaniline and 3-methyl-2-amino benzoic acid. At concentrations of less than 50 ppm in the diet, 2,6-xylidine seems to be completely oxidized. At high concentrations, a small amount of xylidine is not oxidized but is conjugated at the amino group and may be excreted as a conjugate (Lindstrom, 1961; Lindstrom et al., 1963; Magnusson et al., 1979).

Oral LD_{50} values have been determined for several ring-substituted aniline derivatives in male Sprague Dawley CD rats (Jacobson, 1972). The LD_{50} value for 2,6-xylidine was 0.84 g/kg. Although cyanosis was observed in some of the severely intoxicated animals, methemoglobin-induced hypoxia did not appear to be severe enough to be the cause of death. Single-dose oral LD_{50} values of 1.23 g/kg for male Sprague Dawley rats and 0.71 g/kg for male CF-1 mice have been reported (Vernot et al., 1977). The single oral LD_{50} of 2,6-xylidine hydrochloride in male Osborne-Mendel rats was determined to be 2.05 g/kg (Lindstrom et al., 1969).

In rats, the xylidine isomers differ from each other in toxicity. Hepatotoxicity occurred in rats fed 2,4-xylidine at concentrations of 375-10,000 ppm for 3-6 months (Lindstrom et al., 1963). In rats fed 2,6-xylidine at the same concentrations, hepatomegaly was the only abnormality seen. Inhibition of growth and target cell anemia at the affected site was less extensive in animals receiving 2,6-xylidine than in those given 2,4-xylidine.

Studies of 2,4-, 2,5-, and 2,6-xylidine in dogs and rats revealed that the toxicity of xylidine isomers is species specific. When oral doses of 2, 10, or 50 mg/kg per day were administered to dogs and doses of 20, 100, and 500-700 mg/kg were administered to rats for 4 weeks, dogs were found to be about 10 times more susceptible than rats to hepatotoxicity (Magnusson et al., 1971). In dogs, all three isomers induced fatty degeneration of the liver, the 2,6-isomer being the most toxic. In rats, all three isomers caused hepatomegaly, although the liver cells appeared normal. Liver enlargement was greatest for rats administered the 2,4-isomer and least for those receiving the 2,6-isomer; this finding was confirmed by Lindstrom et al. (1963). Although Magnusson et al. (1971) found no renal lesions in rats and dogs at any dose level, Lindstrom et al. (1963) observed slight or moderate pitting or depressed scarring of the kidneys after 6 months in most rats administered 10,000 ppm. The incidence of this lesion was reduced sharply with the concentration. Both investigators observed hyperkeratosis of the forestomach in rats administered any of the three isomers. Magnusson et al. (1971) considered this effect to be the result of stomach irritation. Repeated administration of 2,6-xylidine at 100 mg/kg was found to induce gastric ulcers in dogs.

The effects of 2,4-, 2,5-, and 2,6-xylidines on the structure of rat liver and on the drug-metabolizing activity of microsomal enzymes was studied by Magnusson et al. (1979). When administered orally at doses of 400-500 mg/kg for 4 weeks, each isomer caused hepatomegaly; this effect was considered to be due to the proliferation of the smooth endoplasmic reticulum. Decreases in glycogen content and glucose-6-phosphatase activity were demonstrated histochemically. Levels of microsomal cytochrome P450 were increased in rats given 2,4- or 2,5-xylidine but not in those given the 2,6-isomer. Concentrations of aniline hydroxylase were increased in all dosed animals except those receiving 2,6-xylidine. Glucuronyltransferase activity was increased in rats given any of the three isomers. The results of this study indicate that all isomers of xylidine can induce the drug-metabolizing microsomal enzymes.

Aromatic amines and their derivatives characteristically produce methemoglobinemia in several species. Cats are more susceptible than humans to the formation of methemoglobin by aromatic amines and amides and dogs are less susceptible (McLean et al., 1969). Xylidine reportedly causes anoxia (due to the formation of

methemoglobin) in humans (Gosselin et al., 1976; Proctor and Hughes, 1978). 2,6-Xylidine, administered intravenously at 0.28 mmol/kg, caused formation of 10% methemoglobin in cats; methemoglobin did not form in dogs (McLean et al., 1969). To correlate chemical structure with methemoglobin-forming activity, a series of ring-substituted derivatives was administered to cats. After 0.25 mmol/kg of 2,6-xylidine had been administered, methemoglobin reached a maximum of 10%; maximum levels were 20% for 2,3-dimethylaniline, 10% for 2,4-dimethylaniline, 36% for 2,5-dimethylaniline, 70% for 2-methylaniline, 60% for 3-methylaniline, 40% for 4-methylaniline, and 72% for aniline. These results demonstrate that methyl substituents tend to lower the methemoglobin-forming activity of aniline.

Methemoglobin formation was measured in male Osborne-Mendel rats given maximum tolerated doses (20 mg) of 2,4-, 2,5-, or 2,6-xylidine by injection into the femur (Lindstrom et al., 1969). The xylidines were not effective methemoglobin formers. 2,6-Xylidine produced less than 3% methemoglobin, whereas 2,4- and 2,5-xylidine produced between 3% and 4%.

2,6-Xylidine was not found to be mutagenic in the Ames test with or without S9 from Aroclor-induced rats in *S. typhimurium* strains TA100, TA98, TA1535, and TA1537 (Zimmer et al., 1980; Florin et al., 1980). Negative results were also obtained in *S. typhimurium* with or without S9 from Aroclor-induced rats and hamsters (Appendix C).

Carcinogenicity and long-term toxicity tests were conducted on male Charles River CD rats and male and female CD-1 HaM/ICR mice administered 2,4-xylidine hydrochloride or 2,5-xylidine hydrochloride (Weisburger et al., 1978). Increased incidences of lung tumors occurred in female mice administered 250 mg/kg for 18 months; no increased tumor incidences were found in dosed male rats or male mice. 2,5-Xylidine caused significant increases in the incidences of subcutaneous fibromas and fibrosarcomas in male rats relative to pooled controls. An increase in hepatic tumors in male mice was not significant, but the incidence of vascular tumors was increased when compared with that in pooled controls.

An IARC working group evaluated data on xylidine but decided not to publish a monograph because of the lack of carcinogenicity information. This lack of information and the potential for human exposure were the primary reasons for placing this chemical on test.

2,6-Xylidine was placed on study by the National Toxicology Program (NTP) at EG&G Mason Research Institute in August 1980. Single-dose, 14-day, and 13-week studies were performed on rats and mice. A 2-year study was planned but not conducted. At the time NTP chose 2,6-xylidine for study, the U.S. Environmental Protection Agency (EPA) had conducted single-dose and range-finding studies in rats (May to December 1977) and had initiated a 2-year study in rats at Litton Bionetics, Inc. (September 1978). In September 1980, sponsorship of the study was transferred from the EPA to NTP. NTP authorized Experimental Pathology Laboratories, Inc., to conduct the terminal kill, perform gross necropsies and histopathologic examinations, evaluate data, and prepare reports.

II. MATERIALS AND METHODS

STUDIES PERFORMED BY GAVAGE AT EG&G MASON RESEARCH INSTITUTE

CHEMICAL ANALYSES

PREPARATION AND ANALYSES OF GAVAGE DOSES

SINGLE-ADMINISTRATION STUDIES

TWO-WEEK STUDIES

THIRTEEN-WEEK STUDIES

STUDIES PERFORMED AT LITTON BIONETICS, INC.

CHEMICAL ANALYSES

SINGLE-ADMINISTRATION STUDIES

RANGE-FINDING STUDIES

TWO-YEAR STUDIES

Study Design
Source and Specifications of Animals
Diet Formulation and Feed Stability Studies
Animal Maintenance
Clinical Examinations and Pathology
Statistical Methods

STUDIES PERFORMED BY GAVAGE AT EG&G MASON RESEARCH INSTITUTE

CHEMICAL ANALYSES

2,6-Xylidine (lot no. E121279), used by EG&G Mason for the single-dose, 14-day, and 13-week studies, was obtained from Ethyl Corporation (Baton Rouge, LA) and analyzed by Midwest Research Institute (MRI).

Results of the elemental analysis performed at MRI agreed with theoretical values (Appendix D). The water content was 0.105% by Karl Fischer titration. Nonaqueous titration of amine groups indicated that the chemical was 98.8% pure. Thin-layer chromatography was performed with two different systems; each one indicated one major spot, one minor impurity, and two trace impurities. Gas chromatography with two different systems indicated a single impurity with an area 0.87%-0.89% that of the major peak. The infrared, ultraviolet, and nuclear magnetic resonance spectra were consistent with those expected for the structure and with literature spectra. A stability study indicated that 2,6-xylidine is stable for 2 weeks when stored as the bulk chemical under nitrogen and protected from light at temperatures up to 60° C. The bulk 2,6-xylidine was stored at EG&G Mason in a cold box maintained at 0° ± 5° C.

2,6-Xylidine from the same lot was reanalyzed by EG&G Mason. The results of gas chromatography indicated that the chemical was 99.06% pure (Appendix D). The infrared spectrum from EG&G Mason was consistent with that from MRI. Analyses performed 4 and 8 months after the chemical was received indicated that the bulk chemical remained stable during the studies.

Comparisons of analyses performed at EG&G Mason and Litton Bionetics are presented in Appendix D.

PREPARATION AND ANALYSES OF GAVAGE DOSES

The results of a stability study performed by the National Toxicology Program (NTP) indicated that 2,6-xylidine was not stable when mixed with feed (Appendix E). For this reason, the gavage route of administration was used for the studies conducted at EG&G Mason.

Corn oil (the gavage vehicle) was analyzed monthly for peroxides. 2,6-Xylidine, a clear, dark-brown, slightly viscous liquid, was dissolved in corn oil on a molar basis by shaking until it was dissolved. Completed preparations were placed in serum vials fitted with Teflon® stoppers and sealed. Appropriate amounts of 2,6-xylidine were weighed and then mixed with enough corn oil to obtain the desired concentrations (Table 1); rats received 5 ml/kg body weight. 2,6-Xylidine/corn oil mixtures at a concentration of 100 mg/ml were analyzed at MRI and showed no loss of stability after being stored in the dark for 14 days at room temperature. Samples stored in the open air and light for 3 hours also showed no loss of stability. Randomly selected samples of the mixtures prepared by EG&G Mason were analyzed, and the results indicated that all analyzed mixtures were properly formulated. The results from a referee sample sent to MRI from EG&G Mason confirmed that the dose mixtures were prepared properly. Gavage doses were administered with disposable plastic syringes and stainless steel, ball-tipped needles. Rats were fasted overnight before being dosed. Gavage formulations were prepared weekly, stored at 5° C, and allowed to equilibrate to room temperature before being administered.

SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats were obtained from Harlan Laboratories (Indianapolis, IN) and held in quarantine for 8-12 days. Rats were housed five per cage by sex. All animals received feed and water ad libitum. Details of animal maintenance are presented in Table 1.

Groups of five animals of each sex received 2,6-xylidine in corn oil by gavage at single doses of 0.31, 0.62, 1.25, 2.5, or 5 g/kg. Animals were observed once per hour for the first 3 hours after dosing and twice per day thereafter for the 14-day study period. Individual initial and final body weights were recorded. A necropsy was performed on at least one animal of each sex in each dose group.

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS OF SHORT-TERM GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON

Single-Adminstration Studies	Two-Week Studies	Thirteen-Week Studies
EXPERIMENTAL DESIGN		
Size of Study Groups 5 males and 5 females	5 males and 5 females	10 males and 10 females
Doses 0.31, 0.62, 1.25, 2.5, or 5 g/kg 2,6-xylidine in corn oil	0, 0.08, 0.16, 0.31, 0.62, or 1.25 g/kg 2,6-xylidine in corn oil	0, 0.02, 0.04, 0.08, 0.16, or 0.31 g/kg 2,6-xylidine in corn oil
Duration of Dosing Single dose	5 d/wk for 2 wk	5 d/wk for 13 wk
Type and Frequency of Observation 1 × h for the first 3 h and then 2 × d; weighed on d 0 and d 14	2 × d; weighed on d 1, d 7, and d 14; urine and blood collected 18-24 h before death	2 × d; urine and blood collected on d 90
Necropsy and Histologic Examinations Necropsy of at least 1 male and 1 female at each dose	Necropsy performed on all animals	Necropsy performed on all animals. All vehicle control and highest dose animals examined histologically
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species F344/N rats	F344/N rats	F344/N rats
Animal Source Harlan Industries (Indianapolis, IN)	Male--Charles River Breeding Laboratories (Portage, MI); female--Harlan Industries (Indianapolis, IN)	Male--Harlan Industries (Indianapolis, IN) and Charles River (Kingston, NY); female--Harlan Industries (Indianapolis, IN)
Time Held Before Study 8-12 d	20 d	3 wk
Age When Placed on Study 7 wk	7-8 wk	5-8 wk
Age When Killed 9 wk	9-10 wk	18-21 wk
Method of Animal Distribution After animals with extreme weights were culled, distributed so that cage weights	Same as single-administration studies	Animals with extreme weights were culled; the remaining animals were distributed so that cage weights were approximately equal
Feed NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as single-administration studies	Same as single-administration studies
Bedding Aspen Bed® (American Excelsior Co., Baltimore, MD); changed 2 × wk	Same as single-administration studies	Same as single-administration studies
Water Tap water supplied by automatic watering system (Edstrom Industries, Waterford, WI)	Same as single-administration studies	Same as single-administration studies

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS OF SHORT-TERM GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON (Continued)

Single-Administration Studies	Two-Week Studies	Thirteen-Week Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Cages		
Polycarbonate (Lab Products, Inc., Rochelle Park, NJ); changed 2 × wk	Same as single-administration studies	Same as single-administration studies
Cage Filters		
Nonwoven fiber filters (Lab Products, Rochelle Park, NJ)	Same as single-administration studies	Nonwoven fiber filters (Lab Products, Rochelle Park, NJ, or Snow Filtration, Cincinnati, OH)
Animals per Cage		
5	5	5
Other Chemicals on Study in the Same Room		
None	None	None
Animal Room Environment		
Temp--21.6°-22.7° C; hum--57%-77%; fluorescent light 12 h/d; 10-15 room air changes/h	Temp--22.2°-26.1° C; hum--43%-73%; fluorescent light 12 h/d; 12-15 room air changes/h	Temp--18.9°-23.3° C; hum--32.7%-77.0%; fluorescent light 12 h/d; 12-15 room air changes/h
CHEMICAL/VEHICLE		
Lot Number		
E121279	Same as single-administration studies	Same as single-administration studies
Source		
Ethyl Corporation (Baton Rouge, LA)	Same as single-administration studies	Same as single-administration studies
Preparation		
2,6-xylidine solutions in corn oil prepared so that animals received desired dose when 10 ml/kg body weight given by gavage	2,6-xylidine solutions in corn oil prepared to allow administration of desired dose when 5 ml/kg body weight given by gavage	Same as 2-wk studies
Maximum Storage Time		
2 wk	2 wk	2 wk
Storage Conditions		
Sealed in labeled serum vials with a nitrogen head space and stored at 0°-5°C	Same as single-administration studies	Same as single-administration studies

TWO-WEEK STUDIES

Male F344/N rats (from Charles River Breeding Laboratories) and female F344/N rats (from Harlan Industries) were quarantined for 20 days before the study began. The animals were housed five per cage by sex and received water and feed ad libitum. Details of animal maintenance are presented in Table 1.

Groups of five males and five females were administered doses of 0, 0.08, 0.16, 0.31, 0.62, or 1.25 g/kg of 2,6-xylidine in corn oil by gavage 5 days per week for 2 weeks. All animals were observed twice per day, and clinical observations were recorded. Body weights were recorded on the 1st, 7th, and 14th days and on the day the animals were killed (day 16 or 17).

Urine was collected for 18-24 hours before the animals were killed. Blood for hematologic studies and pH and carbon dioxide determinations was obtained from the tails of unanesthetized animals. On day 16 or 17, all survivors were anesthetized with a barbiturate anesthetic and killed by exsanguination. Complete necropsies were performed.

THIRTEEN-WEEK STUDIES

Male and female F344/N rats were obtained from Harlan Industries (Indianapolis, IN). Additional males were obtained from Charles River Breeding Laboratories (Kingston, NY). Animals were quarantined and observed for 3 weeks before the study began. A necropsy was performed on five males and five females, and the animals were examined for infectious and parasitic disease. The rest of the animals of each sex were caged by 1 g weight classes and then distributed into groups so that the average cage weights were approximately equal for animals of the same sex. Five additional animals of each sex were maintained in the same manner as the study animals and served as sentinels for viral screening. The rats were 5-8 weeks old when the study began.

Rats were housed five per cage in polycarbonate cages covered with nonwoven fiber filter sheets and suspended from perforated shelves. Cage racks and filters were changed once every 2 weeks. Clean cages and bedding were provided twice per week. Feed hoppers were filled twice per week and replaced once per week. Water was available ad libitum. Details of animal maintenance are provided in Table 1.

Ten animals of each sex received 2,6-xylidine in corn oil by gavage at doses of 0, 0.02, 0.04, 0.08, 0.16, or 0.31 g/kg. The doses were administered 5 days per week for 13 weeks. Animals were observed twice per day. At the end of the 13-week period, survivors were anesthetized with barbiturates and killed by exsanguination. A necropsy was performed on all animals immediately thereafter.

Tissues were preserved in 10% neutral buffered formalin and fixed for at least 96 hours. Tissues collected for histopathologic examinations were dehydrated, embedded in paraffin, sectioned at 4-6 microns, and stained with hematoxylin and eosin.

Blood and urine samples were collected on day 90 from 10 males and 10 females from each vehicle control and dosed group. Urine from each animal was collected in 15-ml polystryene tubes held over a cold pack at 1°-3° C for 18-24 hours. Blood for hematologic examinations and for pH and serum carbon dioxide determinations was obtained from the tails of unanesthetized animals. Samples were collected in potassium EDTA-coated or plain 1-ml capillary pickup pediatric tubes. Blood for clinical chemical analyses was drawn from the jugular vein after the animals had been anesthetized with sodium thiamylal. Except for the tests for total and direct bilirubin levels, all clinical chemical assays were performed on the day that samples were collected.

Hematologic Studies. The Clay Adams Ultra Logic 800 Hematology Analyzer was used to electronically count leukocytes and erythrocytes and to measure hemoglobin levels by the cyanmethemoglobin photometry method. The analyzer was calibrated on each day of analysis, and reference samples were used to check performance.

Urinalysis. The appearance of the urine was described and specific gravity and pH were determined. A microscopic examination was performed to determine the presence of erythrocytes, leukocytes, casts, or other formed elements. The Ames Clini-Tek® was used with N-Multistix® urine reagent strips to analyze for protein, glucose, ketone bodies, bilirubin, blood nitrite, and urobilinogen. Ames Performance Capsules® were used each day of analysis. Instrument calibration was automatic.

Serum Chemistry and Enzyme Analysis. The Gemeni Miniature Centrifugal Analyzer® was used to perform analyses for lactic dehydrogenase isoenzymes (LDH), sorbitol dehydrogenase (SDH), serum glutamic oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum carbon dioxide, cholinesterase, alkaline phosphatase, albumin, globulin, creatinine, blood urea nitrogen, calcium, inorganic phosphate, total bilirubin, and direct

bilirubin. Specific standards for each test were used for calibration on the day of analysis, and two reference material samples (normal and abnormal levels) were analyzed as performance checks. Ornithine carbamoyltransferase (OCT) levels were measured colorimetrically.

The Photovolt PVA-4M Electrolyte Analyzer was used to measure sodium, potassium, and chloride concentrations by the use of specific ion electrodes. Two standards, Photovolt Calibration Standard Serum and Photovolt Buffered Linearity Reference, were used to calibrate the instrument on each day of analysis. Two reference material samples, Hyland-O Pak-Chemistry Control Serum I (normal level) and Serum II (abnormal level) were analyzed on each day of analysis to check performance. An IL Blood Gas Analyzer Model 303 was used to measure serum pH.

STUDIES PERFORMED AT LITTON BIONETICS, INC.

CHEMICAL ANALYSES

Three lots of 2,6-xylidine were received from Pfaltz and Bauer (Stamford, CT). The first lot (number not available) was received in multiple batches from November 1976 to March 1978. Lot no. 37220 was received in three separate batches between October 1979 and June 1980 and was used for the short-term and 2-year studies. Lot no. B37220 was received in a single batch in July 1980 and was used for the 2-year studies. The chemical was stored at less than 4° C throughout the studies.

Representative samples from several lots and batches were analyzed by Litton Bionetics, Inc. Analyses included infrared spectroscopy and gas chromatography with a 10% FFAP column. The results of analyses indicated that the infrared spectra of the batches analyzed were consistent with each other and with an Aldrich reference spectrum (Reference no. D14, 600-5). Gas chromatographic analyses indicate that the purity of the batches analyzed ranged from 98.7% to greater than 99% based on peak area comparisons.

To compare the study materials used at Litton Bionetics and at EG&G Mason, investigators sent a sample used at Litton (lot no. 37220) to MRI to be analyzed comparatively with a sample used at EG&G Mason (lot no. E121279). The results of elemental analysis for carbon, hydrogen, and nitrogen agreed with the theoretical values for both lots. Karl Fischer analysis indicated that the Litton sample contained 0.29% water and the EG&G Mason sample 0.11%. Nonaqueous titration of the amine group with perchloric acid indicated a purity of 98.8% for both lots. Results of thin layer chromatography of the Litton lot by two different systems indicated a major spot and one minor impurity. The same thin-layer chromatographic systems indicated a major spot, a minor impurity, and two trace impurities from the Mason lot. Results of gas chromatographic analysis of the Litton lot, with a 20% SP2100/0.1% Carbowax 1500 column, indicated that there were no impurities having areas that were greater than or equal to 0.1% of the major peak. Chromatographic analysis of the Mason lot on the same column indicated one impurity with an area 0.89% that of the major peak. A second gas chromatography system, with a 5% SP1200/1.75% Bentone 34 column, indicated that there were no impurities having areas that were greater than or equal to 0.1% of the peak for the Litton lot; analysis of the Mason lot on the same system indicated one impurity with an area 0.87% that of the major peak. The infrared, ultraviolet/visible, and nuclear magnetic resonance spectra for both lots were consistent with that expected for the structure of 2,6-xylidine and with each other. The purity of both lots was considered to be comparable.

SINGLE-ADMINISTRATION STUDIES

Charles River CD strain rats were obtained from Charles River Breeding Laboratories and acclimated to laboratory conditions for 8 days. Animals were approximately 8 weeks old when the study began. Rats were housed individually

in wire-bottom cages. Water and feed were available ad libitum except during the night before dosing, when feed was removed from the cages. Details of animal maintenance are presented in Table 2.

Groups of five rats of each sex received 2,6-xylidine in corn oil by gavage in a single dose of 0.215, 0.316, 0.681, 1.00, 1.47, 2.15, or 3.16 g/kg. Rats were observed frequently on the day of dosing and once per day thereafter. Animals were weighed on the day of dosing and 7 and 14 days thereafter. A necropsy was performed on all animals.

RANGE-FINDING STUDIES

Weanling male and female Charles River CD rats were obtained from Charles River Breeding Laboratories and acclimated to laboratory conditions at least 1 week before the study began. Groups of five males and five females were fed diets containing 0, 100, 300, 1,000, or 3,000 ppm 2,6-xylidine. Additional control groups and 10,000-ppm groups were added later.

Formulated diets were prepared by suspending 2,6-xylidine in corn oil and incorporating it into the basal diet (Purina Laboratory Lab Chow® meal). Control diets were prepared by mixing 100 ml of corn oil with 7 kg of Chow. Rats were individually housed in plastic shoebox cages with filter tops on Absorb-Dri® bedding. Details of animal maintenance are presented in Table 2.

After being fed control feed or formulated diets for 10 weeks, males and females from each dose group were mated for 2 weeks. The females were allowed to litter and nurse their offspring for 10 days. The offspring were observed once per day. The numbers of living and dead pups were recorded on days 0 and 10 of nursing; body weights of the litter were recorded on day 10. Both parents and pups were killed on day 10 of lactation and gross necropsies were performed.

TWO-YEAR STUDIES

Study Design

Groups of 56 male and 56 female rats were fed diets containing 2,6-xylidine at concentrations of 0, 300, 1,000, or 3,000 ppm for 102 weeks. Water and feed were available ad libitum.

Source and Specifications of Animals

For each dose group, 28 male and 56 female weanling Charles River CRL:COBS CD (SD) BR outbred albino rats were obtained from Charles River Breeding Laboratories. These animals constituted the F_0 generation in this multigeneration study. Beginning at 5 weeks of age, the animals were fed diets containing 0, 300, 1,000, or 3,000 ppm 2,6-xylidine. They were mated at 16 weeks of age and pregnant females were allowed to deliver naturally over a 2-week period. The F_0 females continued to receive dosed or control diets during pregnancy and lactation. Progeny of this mating, designated the F_{1a} generation, were weaned at 21 days of age, and groups of 56 males and 56 females were fed the same study diets as their parents for 102 weeks.

Diet Formulation and Feed Stability Studies

2,6-Xylidine was suspended in corn oil, mixed with a small amount of Purina Laboratory Chow® ground meal, combined with additional feed, and then mixed in a blender until homogeneous. Control and formulated diets contained 50 ml of corn oil per kg of feed. At each concentration, samples were taken at distinct locations in the blender and analyzed for homogeneity.

Stability studies were performed at Litton Bionetics on formulated diets stored in sealed containers at ambient temperatures (Appendix E). Dosed feed was extracted with benzene. Concentrations of 2,6-xylidine were determined by spectrophotometric analysis after reaction with diazotized sulfanilic acid. Analyses performed on days 1, 7, 14, 21, and 28 indicated respective recoveries of 90.1%, 81.2%, 81.0%, 85.1%, and 83% of the 2,6-xylidine from the 3,000-ppm diet. Formulated diets were prepared once per week, stored at $-20°$ C until used, and dispensed in glass feed cups.

The NTP had performed feed stability studies before selecting a route of administration for its series of toxicity tests at EG&G Mason. The stability of 2,6-xylidine in feed was determined by preparing a 1,000-ppm feed mixture using NIH 07 Rat and Mouse Ration and analyzing it at various intervals after open storage in a rat cage

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS OF GAVAGE AND FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

Single-Administration Studies	Range-Finding Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 males and 5 females	5 males and 5 females	56 males and 56 females
Doses		
0.215, 0.316, 0.681, 1.00, 1.47, 2.15, or 3.16 g/kg 2,6-xylidine in corn oil by gavage	0, 100, 300, 1,000, 3,000, or 10,000 ppm 2,6-xylidine in feed	0, 300, 1,000, or 3,000 ppm 2,6-xylidine in feed
Duration of Dosing		
Single dose	Male--10 wk; female--14 wk	102 wk
Type and Frequency of Observation		
Frequently on d of dosing and 1 × d thereafter; weighed on d 0, 7, and 14	Observed 1 × d; offspring weighed on d 10 of nursing	Observed 1 × d; weighed 1 × wk for 26 wk and 1 × mo thereafter; feed consumption (individually housed animals only) measured 1 × wk for 26 wk and 1 × mo thereafter
Supplemental Studies		
None	None	Complete blood count, blood urea nitrogen, glucose, SGOT, and alkaline phosphatase at 12 mo and 18 mo and at termination from randomly selected animals
Termination		
D 14	D 10 of lactation	Wk 102 after weaning
Necropsy and Histologic Examinations		
Necropsy on all animals	Necropsy on all animals	Necropsy and histopathologic exams on all animals
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
Charles River CD rats	Charles River CD rats	Charles River CD rats
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Bred in-house from Charles River CD stock administered 0, 300, 1,000, or 3,000 ppm 2,6-xylidine in feed
Time Held Before Study		
8 d	At least 7 d	Newborns held until weaning and then distributed to cages and study groups; administered same diet as parents
Age When Placed on Study		
8 wk	Young adults	4 wk
Age When Killed		
2 wk after dosing	Parent rats and pups were killed at d 10 of lactation	
Method of Animal Distribution		
Random	Random	Table of random numbers
Feed		
Purina Lab Chow® meal	Same as single-administration studies	Same as single-administration studies

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS OF GAVAGE AND FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS (Continued)

Single-Administration Studies	Range-Finding Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Bedding		
Absorb-Dri® hardwood chip bedding	Same as single-administration studies	Same as single-administration studies
Water		
Ad libitum	Same as single-administration studies	Same as single-administration studies
Cages		
Wire bottom	Plastic shoebox	Polycarbonate shoebox-type hanging cage; changed 1 × wk
Cage Filters		
Filters (type not specified)	Snow-Flo® nonwoven polyester filter sheets	
Animals per Cage		
1	1	18 rats/group housed individually; 38 rats/group housed 3 per cage
Other Chemicals on Study in the Same Room		
None	None	None; control animals housed in a separate room
Animal Room Environment		
Temp--74° ± 3° F; hum--30%-70%; 12 h light cycle	Same as single-administration studies	Same as single-administration studies
CHEMICAL/VEHICLE		
Lot Number		
Unnumbered	37220	37220, B37220
Source		
Pfaltz and Bauer (Stamford, CT)	Same as single-administration studies	Same as single-administration studies
Preparation		
Not available	2,6-xylidine suspended in corn oil and incorporated into Purina Lab Chow® meal	2,6-xylidine suspended in corn oil, mixed with small amount of ground meal, and then blended with rest of feed in blender until homogeneous

at ambient temperatures and after storage in sealed bottles kept in the dark at −20° C, 5° C, or room temperature (Appendix F). Feed samples were extracted with acetonitrile, and the 2,6-xylidine content of the extracts was determined by gas chromatography. Open storage samples were analyzed on days 1, 3, 5, and 7; samples stored in sealed bottles were analyzed on days 7 and 14.

The feed stability results indicated that 2,6-xylidine mixed with the basal diet was unstable under all conditions of storage. Formulated feed mixtures stored open for 7 days in a rat cage lost 44% of the 2,6-xylidine initially present. 2,6-Xylidine concentrations in samples stored for 14 days in the dark in sealed containers at −20° C, 5° C, or room temperature were reduced 1.5%, 3.2%, and 14.6%. The samples of feed mixtures stored in sealed bottles lost 2,6-xylidine by reacting with feed components. Losses from samples stored open in a rat cage were caused by reaction with feed and evaporation. An estimated 70%-80% of the loss from the rat cage was due to evaporation. Because of the results of these studies, the NTP decided to use gavage rather

than diet for oral administration of 2,6-xylidine in short-term studies. The 2-year studies at Litton Bionetics began under the EPA contract before the NTP feed stability studies were completed.

Upon assuming responsibility for the 2-year studies, the NTP conducted further studies on the loss of 2,6-xylidine from feed. In these studies, 2,6-xylidine was blended with a series of feed ingredients and stored in sealed containers for 12 days at room temperature or at −20° C. Little or no reactivity was observed when 2,6-xylidine was mixed with starch, fat, or protein. The compound reacted moderately with unbleached whole wheat flour and significantly with nonfat dry milk. The two reactive ingredients contained reducing compounds. Dried skim milk typically contains 50%-52% of the reducing carbohydrate, lactose. Flour contains enediol compounds that react like typical aldehydes. Significant reactivity was observed in 2,6-xylidine blends with both β-D-lactose and D-glucose, whereas no reactivity was observed with sucrose. Mixing 2,6-xylidine and D-glucose resulted in a color change from white to brown. This "browning" phenomenon is considered to be due to irreversible reaction with components containing aldehyde groups.

Animal Maintenance

Eighteen rats per group were housed individually, and 38 rats per group were housed 3 per cage in polycarbonate shoebox-type hanging cages covered with nonwoven polyester filter sheets (Table 2). Animal rooms were controlled for temperature, humidity, and duration of light. No other chemicals were on study in the animal room. Control animals were housed in a separate room.

Clinical Examinations and Pathology

Rats were observed once per day, and clinical signs were observed and recorded once per week. Body weight data for all animals and feed consumption data for animals individually housed were recorded once per week for 26 weeks and once per month thereafter, except for the last 5 months when two measurements for feed and three for body weight were recorded.

At 12 months, 18 months, and the end of the studies, 10 males and 10 females were selected randomly and bled via the orbital sinus route. Analyses were performed for total erythrocyte count, hemoglobin, hematocrit, total leukocyte count, differential leukocyte count, blood urea nitrogen, glucose, SGOT, and alkaline phosphatase.

A Coulter Counter® (Model FN) was used to determine total white blood cell counts and total red blood cell counts. A Coulter Hemoglobinometer was used to measure hemoglobin levels. Packed cell volume was determined after samples were centrifuged at 10,000 rpm in a microhematocrit centrifuge.

Peripheral blood smears were prepared and examined. The Baker Centrifichem Model 400® analyzer was used for blood urea nitrogen, SGOT, glucose, and alkaline phosphatase determinations. Specific standards for each test were used for calibration. Two reference material samples (normal and abnormal levels) supplied by the manufacturer were assayed in duplicate along with each run of 20 test samples.

Necropsies were performed at Litton Bionetics on all animals that died early or were killed while in a moribund state. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed by Experimental Pathology Laboratories, Inc., at the Litton Bionetics facility on all animals including those found dead, except for tissues that were excessively autolyzed or missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Portions of nasal tumors were retrieved from the posterior nasal cavities of three rats held in formalin fixation; these tumors were submitted for electron microscopy in an attempt to confirm diagnoses made by light microscopy. Recognizable nasal tumor tissue was minced into 1-mm cubes, washed in phosphate buffer, and fixed in 2.5% phosphate buffered glutaraldehyde. After

being washed in buffer three times, the specimens were fixed in 1% osmium tetroxide, dehydrated in ascending concentrations of ethanol, and embedded in Epon 812. One-micron sections of multiple blocks from each specimen were stained with toluidine blue and mounted on glass slides for examination by light microscopy. The optimal block for electron microscopy was selected from these slides, and each block was finely trimmed and thin-sectioned at 600-800 Å. These sections were collected on bare copper grids, doubly stained with uranyl acetate and lead citrate, and examined in a Hitachi 12A electron microscope operated at 12 kV.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

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 were censored from the survival analyses at the time they were found to be missing or dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the anaysis of tumor incidence, and reported P values are one-sided.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of primary tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984) are included for those tumors appearing to show compound-related effects.

For results of clinical laboratory examinations of blood and urine, the multiple comparison procedure of Williams (1971, 1972) for statistical analysis of continuous variables was used. This procedure, a modification of Dunnett's (1955) test, asssumed that a response to a chemical will consistently increase or decrease in magnitude as the dose increases. This assumption seems reasonable for most of the data on organ weights, clinical chemistry, and hematologic parameters analyzed in this report. The Williams' procedure employs a smoothing algorithm to adjust for minor dose-response inversions. A multiple comparison procedure is deemed appropriate to help protect against false positives that may result because of the large number of parameters under investigation.

III. RESULTS

STUDIES PERFORMED BY GAVAGE AT EG&G MASON RESEARCH INSTITUTE

SINGLE-ADMINISTRATION STUDIES

TWO-WEEK STUDIES

THIRTEEN-WEEK STUDIES

STUDIES PERFORMED AT LITTON BIONETICS, INC.

SINGLE-ADMINISTRATION STUDIES

RANGE-FINDING STUDIES

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

III. RESULTS: MASON

STUDIES PERFORMED BY GAVAGE AT EG&G MASON RESEARCH INSTITUTE

SINGLE-ADMINISTRATION STUDIES

At doses of 0.31 and 0.62 g/kg, all rats survived until the end of the studies. At 1.25 g/kg, all male rats and 3/5 female rats died within 2 days of dosing. At 2.50 g/kg, all rats died within 2 days of dosing, and at 5.0 g/kg, all rats died within 1 day of dosing. The calculated LD_{50} value was 1.16 g/kg (0.83-1.63 g/kg) for female rats. No LD_{50} value could be calculated for male rats because survival in all dosed groups was either 0% or 100%. The LD_{50} value for male rats is probably between 0.62 and 1.25 g/kg.

All rats were inactive after receiving 2,6-xylidine. Dyspnea or shallow breathing occurred in all rats administered 1.25 g/kg or more. At gross necropsy, groups administered 0.62 g/kg or more had reddened renal medullae and groups administered 1.25 g/kg or more had reddened gastric mucosa and thick, oily, opaque yellow fluid in the stomach and intestines.

TWO-WEEK STUDIES

Compound-related deaths occurred in groups administered 0.62 g/kg or more (Table 3). All animals receiving 1.25 g/kg died before the termination of the studies. Depression in mean body weight relative to that of the vehicle controls was greater than 10% in male rats administered 0.31 g/kg or more and in female rats administered 0.62 g/kg. A generalized leukocytosis and an increase in the number of nucleated red blood cells were observed in male rats administered 0.31 or 0.62 g/kg. Slight anisocytosis, poikilocytosis, and polychromasia of the red blood cells occurred more frequently in dosed than in vehicle control animals. Moderate poikilocytosis occurred at 0.31 g/kg and moderate polychromasia at 0.31 and 0.62 g/kg. Slightly macrocytic erythrocytes were observed at the two highest doses. No significant differences in clinical chemical and urinalysis parameters were observed between dosed and vehicle control male rats.

TABLE 3. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE TWO-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON

Dose (g/kg)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	5/5	179.2	248.0	+68.8	
0.08	5/5	178.0	222.6	+44.6	90
0.16	5/5	179.7	244.2	+64.5	98
0.31	5/5	171.1	221.1	+50.0	89
0.62	3/5	179.7	198.5	+18.8	80
1.25	0/5	170.2	(c)	(c)	(c)
FEMALE					
0	(d) 4/5	109.8	139.6	+29.8	
0.08	5/5	109.9	141.5	+31.6	101
0.16	5/5	109.8	139.1	+29.3	99
0.31	(d) 4/5	109.3	139.4	+30.1	100
0.62	(d) 3/5	109.5	129.9	+20.4	93
1.25	0/5	109.1	(c)	(c)	(c)

(a) Number surviving/number in group
(b) Mean body weight change of the survivors
(c) No data are presented due to 100% mortality in this group.
(d) One animal in the group died after arterial blood was drawn.

Normal hematologic values were observed in female rats except for slight anisocytosis, poikilocytosis, and polychromasia at 0.31 and 0.62 g/kg. Fluctuations in clinical chemical measures for female rats appear to be due to sporadically occurring anemia rather than to a dose-related trend in the dosed groups. No significant differences were noted in results of urinalysis from dosed and vehicle control female rats.

THIRTEEN-WEEK STUDIES

No deaths occurred (Table 4). Mean body weight gains were over 10% less in male and female rats receiving the highest (0.31 g/kg) dose and in female rats administered 0.04 and 0.16 g/kg than in controls. No noteworthy clinical signs were observed in any of the animals in these studies.

At the highest dose (0.31 g/kg), increases in mean liver weights and decreases in body weights resulted in significant increases in the liver/body weight ratios ($P = 0.003$ for males and females) (Table 5). The liver weight to body weight ratio also was increased for male rats administered 0.16 g/kg. The liver weight to brain weight and kidney weight to brain weight ratios were significantly increased in females given 0.31 g/kg (Table 6).

Histologic examinations revealed minimal-to-moderate inflammatory changes in the nasal mucosa of each sex, but the vehicle controls had morphologically similar lesions of equal severity. Various inflammatory and degenerative lesions observed in other tissues were considered to be unrelated to administration of 2,6-xylidine.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON

Dose (g/kg)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	149.83 ± 4.71	337.34 ± 8.22	187.51 ± 6.52	
0.02	10/10	148.42 ± 4.16	343.21 ± 6.24	194.79 ± 5.19	102
0.04	10/10	150.15 ± 4.32	349.03 ± 9.38	198.88 ± 7.05	103
0.08	10/10	150.20 ± 4.04	350.75 ± 8.35	200.55 ± 6.11	104
0.16	10/10	148.86 ± 3.68	327.27 ± 4.67	178.41 ± 5.24	97
0.31	10/10	147.26 ± 3.18	289.73 ± 9.81	142.47 ± 11.40	86
FEMALE					
0	10/10	111.69 ± 2.23	197.82 ± 3.56	86.13 ± 3.35	
0.02	10/10	113.31 ± 2.23	202.68 ± 3.61	89.37 ± 3.06	102
0.04	10/10	111.87 ± 2.07	188.59 ± 1.97	76.72 ± 2.19	95
0.08	10/10	114.39 ± 2.66	193.56 ± 2.62	79.17 ± 2.25	98
0.16	10/10	113.77 ± 2.60	189.15 ± 4.30	75.38 ± 3.03	96
0.31	10/10	111.52 ± 2.51	167.15 ± 2.48	55.63 ± 2.71	84

(a) Number surviving/number in group
(b) Mean body weight change of the group ± standard error of the mean

TABLE 5. LIVER WEIGHT RELATIVE TO TERMINAL BODY WEIGHT OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON

Dose (g/kg)	Number Examined	Weight (grams) Liver (a)	Terminal Body (a)	Ratio (b) × 100	Liver (percent of controls) (c)
MALE					
0	10	11.475 ± 4.290	330.600 ± 8.124	3.469 ± 0.093	
0.02	10	12.603 ± 0.337	337.140 ± 6.336	3.736 ± 0.057	108
0.04	10	12.248 ± 0.511	344.900 ± 8.979	3.541 ± 0.073	102
0.08	10	12.099 ± 0.381	341.580 ± 8.141	3.539 ± 0.042	102
0.16	10	12.705 ± 0.494	319.960 ± 5.133	(d) 3.967 ± 0.127	114
0.31	10	13.777 ± 0.299	286.500 ± 4.159	(d) 4.810 ± 0.083	137
FEMALE					
0	10	6.149 ± 0.138	193.340 ± 3.540	3.182 ± 0.058	
0.02	10	6.398 ± 0.123	197.750 ± 3.462	3.239 ± 0.054	102
0.04	10	5.946 ± 0.244	179.040 ± 6.630	3.381 ± 0.226	106
0.08	10	6.425 ± 0.142	188.650 ± 2.053	3.405 ± 0.062	107
0.16	10	6.402 ± 0.175	183.310 ± 4.270	3.491 ± 0.035	110
0.31	10	8.259 ± 0.130	163.160 ± 2.128	(d) 5.067 ± 0.085	159

(a) Mean weight
(b) The mean of the ratio of the liver weight for each animal to its terminal body weight
(c) The mean ratio of a dosed group divided by the mean ratio of the control group
(d) Statistically significant ($P \leq 0.01$) by Williams' test (Williams, 1971, 1972)

TABLE 6. SUMMARY OF STATISTICALLY SIGNIFICANT RESULTS FROM THE ANALYSIS OF ORGAN AND BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON (a)

Parameters	Dose (g/kg) 0.16	0.31
MALE		
Body weight gain		(b) –
Liver weight/body weight	(b) +	(b) +
Brain weight/body weight	+	(b) +
Heart weight/body weight		(b) +
Kidney weight/body weight		(b) +
Lung weight/body weight	(b) +	(b) +
Heart weight/brain weight	–	–
FEMALE		
Body weight gain	–	(b) –
Liver weight/body weight		(b) +
Brain weight/body weight		(b) +
Heart weight/body weight		(b) +
Kidney weight/body weight		(b) +
Lung weight/body weight	(b) +	+
Liver weight/brain weight		(b) +
Kidney weight/brain weight		(b) +

(a) + indicates a significantly increased response ($P \leq 0.05$); – indicates a significantly decreased response ($P \leq 0.05$); organ weights with nonsignificant results are omitted from the list. No significant differences were observed at 0.02, 0.04, or 0.08 g/kg. P values vs. the vehicle controls by Williams' test (Williams, 1971, 1972)
(b) Also significant at $P \leq 0.01$

The results of hematologic and clinical chemistry studies indicate that male rats are more sensitive to 2,6-xylidine than are female rats (Table 7). Significant decreases in total leukocyte counts occurred in male rats at doses of 0.04 g/kg and above; they were accompanied by decreases in the percent of lymphocytes and increases in the percent of segmented neutrophils at doses of 0.08 g/kg and above. In male rats, significant decreases occurred in hemoglobin levels at 0.16 and 0.31 g/kg and in erythrocyte and hematocrit levels at 0.31 g/kg. A dose-related increase in polychromasia occurred in male rats. Significant decreases in serum glutamic oxaloacetate transaminase (SGOT) and lactic dehydrogenase levels occurred at all doses.

Decreases were also observed for potassium, calcium, and inorganic phosphorus levels in sera of male rats. Cholinesterase levels were increased in male rat sera. In female rats, significant decreases were observed in hemoglobin levels at 0.16 and 0.31 g/kg and in hematocrit levels at 0.31 g/kg. Significant decreases in cholinesterase, globulin, and creatinine levels occurred in the sera of female rats. Increases were observed in sorbitol dehydrogenase, serum glutamic pyruvate transaminase, blood urea nitrogen, chloride, and inorganic phosphorus levels in female rat sera.

The decreases in hemoglobin, erythrocyte, and hematocrit levels appear to be related to administration of 2,6-xylidine, but they are not severe enough to be considered indicators of anemia. The decreases in total leukocytes and lymphocytes may also be compound related. Decreases in SGOT, lactic dehydrogenase, direct bilirubin, and serum creatinine levels have no known clinical significance. The decreases in potassium, calcium, and inorganic phosphorus levels and in urine specific gravity, and the increase in cholinesterase levels are not biologically significant at the observed levels.

TABLE 7. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON

		Male		Female	
	Dose (g/kg)	Number (a)	Response (b)	Number (a)	Response (b)
Erythrocyte count (10^6/μl)	0.00	10	9.6 ± 0.2	9	8.2 ± 0.3
	0.02	10	9.0 ± 0.3	10	7.9 ± 0.5
	0.04	10	10.6 ± 0.5	10	8.2 ± 0.3
	0.08	10	9.2 ± 0.1	10	7.6 ± 0.4
	0.16	9	9.1 ± 0.5	10	7.7 ± 0.1
	0.31	10	(c) 8.0 ± 0.2	10	7.3 ± 0.2
Hemoglobin (g/dl)	0.00	10	17.6 ± 0.1	9	16.6 ± 0.1
	0.02	10	17.8 ± 0.2	10	16.6 ± 0.1
	0.04	10	17.5 ± 0.2	10	16.8 ± 0.3
	0.08	10	17.1 ± 0.2	10	16.5 ± 0.3
	0.16	9	(c) 16.6 ± 0.3	10	(c) 15.6 ± 0.2
	0.31	10	(c) 16.3 ± 0.3	10	(c) 15.4 ± 0.2
Hematocrit (percent)	0.00	10	46.9 ± 1.3	9	44.6 ± 1.1
	0.02	10	43.7 ± 1.2	10	45.0 ± 1.2
	0.04	10	45.5 ± 0.8	10	41.5 ± 0.7
	0.08	10	45.7 ± 0.8	10	41.5 ± 1.3
	0.16	9	44.0 ± 0.8	10	43.1 ± 1.0
	0.31	10	(c) 42.3 ± 0.9	10	(c) 39.7 ± 0.7
Leukocyte count (10^3/μl)	0.00	10	7.2 ± 0.3	9	5.1 ± 0.3
	0.02	10	6.8 ± 0.2	10	4.8 ± 0.3
	0.04	10	(c) 6.0 ± 0.2	10	4.4 ± 0.3
	0.08	10	(c) 5.3 ± 0.2	10	4.6 ± 0.3
	0.16	9	(c) 5.2 ± 0.2	10	4.9 ± 0.3
	0.31	10	(c) 6.5 ± 0.3	10	6.0 ± 0.4

TABLE 7. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON (Continued)

	Dose (g/kg)	Male		Female	
		Number (a)	Response (b)	Number (a)	Response (b)
Lymphocytes (percent)	0.00	10	82.1 ± 1.8	9	81.2 ± 1.4
	0.02	10	81.1 ± 1.8	10	85.5 ± 1.2
	0.04	10	81.1 ± 1.3	10	83.5 ± 1.5
	0.08	10	(c) 76.0 ± 1.2	10	81.9 ± 1.9
	0.16	9	(c) 70.7 ± 1.7	10	75.8 ± 2.6
	0.31	10	(c) 75.9 ± 2.4	10	80.8 ± 1.8
Monocytes (percent)	0.00	10	2.3 ± 0.6	9	1.3 ± 0.4
	0.02	10	2.1 ± 0.5	10	1.0 ± 0.4
	0.04	10	1.3 ± 0.3	10	0.9 ± 0.3
	0.08	10	1.3 ± 0.3	10	1.0 ± 0.4
	0.16	9	1.4 ± 0.5	10	1.0 ± 0.2
	0.31	10	(c) 0.8 ± 0.2	10	0.5 ± 0.2
Segmented neutrophils (percent)	0.00	10	15.5 ± 1.4	9	17.4 ± 1.6
	0.02	10	16.7 ± 1.8	10	13.4 ± 1.5
	0.04	10	17.6 ± 1.3	10	15.9 ± 1.8
	0.08	10	(c) 22.6 ± 1.3	10	17.1 ± 2.1
	0.16	9	(c) 28.0 ± 2.1	10	22.7 ± 2.7
	0.31	10	(c) 23.3 ± 2.5	10	18.7 ± 1.7
Band neutrophils (percent)	0.00	10	0.0 ± 0.0	9	0.0 ± 0.0
	0.02	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.04	10	0.1 ± 0.0	10	0.0 ± 0.0
	0.08	10	0.1 ± 0.1	10	0.0 ± 0.0
	0.16	9	0.0 ± 0.0	10	0.1 ± 0.1
	0.31	10	0.0 ± 0.0	10	0.0 ± 0.0
Eosinophils (percent)	0.00	10	0.1 ± 0.1	9	0.0 ± 0.0
	0.02	10	0.1 ± 0.1	10	0.0 ± 0.0
	0.04	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.08	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.16	9	0.0 ± 0.0	10	0.0 ± 0.0
	0.31	10	0.0 ± 0.0	10	0.0 ± 0.0
Basophils (percent)	0.00	9	0.0 ± 0.0	9	0.0 ± 0.0
	0.02	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.04	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.08	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.16	9	0.0 ± 0.0	10	0.0 ± 0.0
	0.31	10	0.0 ± 0.0	10	0.0 ± 0.0
Nucleated erythrocytes (percent of leukocytes)	0.00	9	1.1 ± 0.0	9	0.3 ± 0.3
	0.02	10	0.3 ± 0.2	10	0.3 ± 2.0
	0.04	10	0.2 ± 0.2	10	0.1 ± 1.0
	0.08	10	0.2 ± 0.2	10	0.3 ± 2.0
	0.16	9	0.6 ± 0.3	10	1.1 ± 0.3
	0.31	10	1.8 ± 0.8	10	2.9 ± 0.8
SGOT (d) (IU/liter)	0.00	10	113.2 ± 7.8	10	79.9 ± 3.7
	0.02	10	(c) 96.7 ± 3.9	10	71.7 ± 2.0
	0.04	10	(c) 90.9 ± 6.5	10	77.4 ± 2.6
	0.08	10	(c) 91.2 ± 3.3	10	80.2 ± 4.7
	0.16	10	(c) 89.8 ± 3.0	10	74.4 ± 6.0
	0.31	10	(c) 91.1 ± 5.2	10	73.4 ± 3.1

TABLE 7. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON (Continued)

	Dose (g/kg)	Male		Female	
		Number (a)	Response (b)	Number (a)	Response (b)
LDH (e)	0.00	10	1,022.0 ± 39.3	10	932.0 ± 124.4
(IU/liter)	0.02	10	(c) 677.0 ± 56.0	10	695.0 ± 41.1
	0.04	10	(c) 425.0 ± 37.1	10	544.0 ± 47.5
	0.08	10	(c) 916.0 ± 67.3	10	906.0 ± 160.7
	0.16	10	(c) 832.0 ± 51.1	10	770.0 ± 125.0
	0.31	10	(c) 878.0 ± 109.2	10	720.0 ± 94.2
SDH (f)	0.00	10	373.9 ± 59.4	10	375.8 ± 33.0
(SU/ml)	0.02	10	505.4 ± 109.8	10	286.2 ± 38.9
	0.04	10	610.5 ± 96.3	10	306.6 ± 29.3
	0.08	10	338.6 ± 52.6	10	427.5 ± 46.8
	0.16	10	344.6 ± 28.9	10	400.0 ± 48.2
	0.31	10	565.1 ± 90.1	10	(c) 508.2 ± 24.7
SGPT (g)	0.00	10	41.0 ± 3.5	10	25.9 ± 1.4
(IU/liter)	0.02	10	45.5 ± 3.7	10	23.5 ± 0.8
	0.04	10	53.5 ± 9.3	10	27.0 ± 0.9
	0.08	10	35.8 ± 1.9	10	24.2 ± 0.8
	0.16	10	34.4 ± 1.4	10	25.2 ± 0.9
	0.31	10	40.8 ± 4.3	10	(c) 31.5 ± 1.0
Cholinesterase	0.00	10	0.7 ± 0.0	10	3.8 ± 0.1
(IU/ml)	0.02	10	0.7 ± 0.0	10	3.9 ± 0.1
	0.04	10	0.8 ± 0.0	10	3.6 ± 0.2
	0.08	10	(c) 0.8 ± 0.0	10	3.6 ± 0.2
	0.16	10	(c) 0.8 ± 0.0	10	(c) 3.2 ± 0.2
	0.31	10	(c) 0.8 ± 0.0	10	(c) 1.9 ± 0.1
OCT (h)	0.00	10	4.2 ± 0.8	10	4.4 ± 0.3
(IU/ml)	0.02	10	5.5 ± 0.9	10	2.9 ± 0.4
	0.04	10	7.6 ± 1.1	10	4.4 ± 0.7
	0.08	10	3.6 ± 0.9	10	4.8 ± 0.8
	0.16	10	3.3 ± 0.7	10	3.6 ± 0.3
	0.31	10	3.0 ± 0.3	10	3.6 ± 0.5
Total protein	0.00	9	6.9 ± 0.0	10	6.8 ± 0.1
(g/dl)	0.02	10	7.1 ± 0.1	10	6.9 ± 0.1
	0.04	10	6.9 ± 0.0	10	6.5 ± 0.1
	0.08	10	6.7 ± 0.0	10	6.5 ± 0.1
	0.16	10	6.7 ± 0.1	10	6.5 ± 0.1
	0.31	10	6.9 ± 0.1	10	6.6 ± 0.1
Albumin (A)	0.00	9	4.2 ± 0.1	10	3.8 ± 0.1
(g/dl)	0.02	10	4.3 ± 0.0	10	3.9 ± 0.1
	0.04	10	4.3 ± 0.0	10	3.9 ± 0.0
	0.08	10	4.0 ± 0.1	10	3.9 ± 0.1
	0.16	10	4.1 ± 0.0	10	3.8 ± 0.1
	0.31	10	4.2 ± 0.0	10	3.8 ± 0.1
Globulin (G)	0.00	10	2.8 ± 0.1	10	3.0 ± 0.1
(g/dl)	0.02	10	2.9 ± 0.1	10	3.1 ± 0.1
	0.04	10	2.6 ± 0.1	10	(c) 2.6 ± 0.1
	0.08	10	2.8 ± 0.1	10	(c) 2.7 ± 0.0
	0.16	10	2.7 ± 0.1	10	(c) 2.7 ± 0.1
	0.31	10	2.8 ± 0.1	10	(c) 2.8 ± 0.0

TABLE 7. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON (Continued)

	Dose (g/kg)	Male		Female	
		Number (a)	Response (b)	Number (a)	Response (b)
A/G ratio	0.00	10	1.5 ± 0.1	10	1.3 ± 0.0
	0.02	10	1.5 ± 0.1	10	1.3 ± 0.0
	0.04	10	1.7 ± 0.0	10	1.5 ± 0.1
	0.08	10	1.4 ± 0.0	10	1.5 ± 0.0
	0.16	10	1.5 ± 0.1	10	1.4 ± 0.0
	0.31	10	1.5 ± 0.0	10	1.4 ± 0.0
Blood urea nitrogen (mg/dl)	0.00	10	17.0 ± 1.1	10	11.5 ± 0.6
	0.02	10	17.4 ± 0.9	10	12.5 ± 0.9
	0.04	10	15.3 ± 0.3	10	11.7 ± 0.5
	0.08	10	19.3 ± 1.0	10	12.0 ± 0.5
	0.16	10	19.7 ± 0.9	10	11.3 ± 0.5
	0.31	10	18.6 ± 0.4	10	(c) 13.8 ± 0.3
Creatinine (mg/dl)	0.00	10	0.6 ± 0.1	10	0.5 ± 0.0
	0.02	10	0.6 ± 0.0	10	0.6 ± 0.0
	0.04	10	0.4 ± 0.0	10	(c) 0.4 ± 0.0
	0.08	10	0.4 ± 0.0	10	(c) 0.4 ± 0.0
	0.16	10	0.4 ± 0.0	10	(c) 0.4 ± 0.0
	0.31	10	0.3 ± 0.0	10	(c) 0.3 ± 0.0
Total bilirubin (mg/dl)	0.00	10	0.3 ± 0.0	10	0.2 ± 0.0
	0.02	10	0.3 ± 0.0	10	0.2 ± 0.0
	0.04	10	0.2 ± 0.0	10	0.1 ± 0.0
	0.08	10	0.2 ± 0.0	10	0.2 ± 0.0
	0.16	10	0.2 ± 0.0	10	0.3 ± 0.1
	0.31	10	0.3 ± 0.0	10	0.2 ± 0.0
Direct bilirubin (mg/dl)	0.00	10	0.1 ± 0.0	10	0.0 ± 0.0
	0.02	10	0.1 ± 0.0	10	0.0 ± 0.0
	0.04	10	0.1 ± 0.0	10	0.0 ± 0.0
	0.08	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.16	10	0.0 ± 0.0	10	0.0 ± 0.0
	0.31	10	0.0 ± 0.0	10	0.0 ± 0.0
Sodium (Na^+) (mEq Na^+/liter)	0.00	10	146.4 ± 0.5	9	151.0 ± 0.3
	0.02	10	146.5 ± 0.7	10	153.7 ± 3.0
	0.04	10	146.5 ± 0.3	10	152.7 ± 2.3
	0.08	10	145.8 ± 0.3	10	153.8 ± 2.7
	0.16	10	145.8 ± 0.1	10	152.2 ± 2.0
	0.31	10	146.0 ± 0.3	10	155.8 ± 2.6
Potassium (K^+) (mEq K^+/liter)	0.00	10	3.7 ± 0.1	9	3.4 ± 0.1
	0.02	10	3.9 ± 0.2	10	3.2 ± 0.2
	0.04	10	4.0 ± 0.1	10	3.3 ± 0.1
	0.08	10	(c) 3.4 ± 0.1	10	3.1 ± 0.2
	0.16	10	(c) 3.4 ± 0.1	10	3.4 ± 0.3
	0.31	10	(c) 3.2 ± 0.1	10	3.0 ± 0.2
Chloride (Cl^-) (mEq Cl^-/liter)	0.00	10	111.0 ± 0.6	9	112.1 ± 1.3
	0.02	10	111.2 ± 0.6	10	115.1 ± 2.0
	0.04	10	111.8 ± 0.7	10	115.0 ± 1.7
	0.08	10	109.9 ± 0.6	10	115.8 ± 1.7
	0.16	10	111.2 ± 0.7	10	114.9 ± 1.7
	0.31	10	111.8 ± 0.8	10	(c) 117.8 ± 2.1

TABLE 7. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF 2,6-XYLIDINE AT EG&G MASON (Continued)

	Dose (g/kg)	Male		Female	
		Number (a)	Response (b)	Number (a)	Response (b)
Calcium (Ca^{++}) (mg/dl)	0.00	10	10.2 ± 0.1	10	9.4 ± 0.2
	0.02	10	10.3 ± 0.1	10	9.2 ± 0.4
	0.04	10	10.5 ± 0.1	10	9.2 ± 0.3
	0.08	10	(c) 9.7 ± 0.1	10	9.6 ± 0.2
	0.16	10	(c) 9.6 ± 0.1	10	9.5 ± 0.3
	0.31	10	(c) 10.0 ± 0.1	10	9.7 ± 0.3
Inorganic phosphorus (mg/dl)	0.00	10	6.1 ± 0.1	10	4.7 ± 0.2
	0.02	10	(c) 5.5 ± 0.2	10	4.7 ± 0.2
	0.04	10	(c) 5.2 ± 0.1	10	5.1 ± 0.2
	0.08	10	(c) 5.4 ± 0.2	10	4.3 ± 0.2
	0.16	10	(c) 5.4 ± 0.1	10	4.7 ± 0.1
	0.31	10	(c) 5.9 ± 0.2	10	(c) 5.3 ± 0.1
Carbon dioxide (mEq/liter)	0.00	10	15.1 ± 1.5	10	17.0 ± 2.3
	0.02	10	18.9 ± 1.7	10	16.0 ± 1.8
	0.04	10	17.9 ± 1.2	10	15.8 ± 2.4
	0.08	10	15.9 ± 0.3	10	14.4 ± 0.8
	0.16	9	17.6 ± 0.8	10	15.8 ± 1.0
	0.31	10	18.0 ± 0.6	10	17.8 ± 1.4
Serum pH	0.00	10	7.433 ± 0.012	10	7.433 ± 0.007
	0.02	10	7.443 ± 0.013	10	7.468 ± 0.037
	0.04	10	7.461 ± 0.014	10	7.448 ± 0.008
	0.08	10	7.447 ± 0.010	10	7.451 ± 0.010
	0.16	10	7.429 ± 0.018	10	7.438 ± 0.009
	0.31	10	7.399 ± 0.013	10	7.413 ± 0.005
Urine specific gravity	0.00	10	1.065 ± 0.003	10	1.041 ± 0.005
	0.02	10	1.047 ± 0.004	10	1.053 ± 0.004
	0.04	10	1.050 ± 0.004	10	1.047 ± 0.005
	0.08	10	1.052 ± 0.004	10	1.049 ± 0.004
	0.16	10	1.048 ± 0.002	10	1.048 ± 0.003
	0.31	10	1.048 ± 0.004	10	1.046 ± 0.002
Urine pH	0.00	10	6.050 ± 0.050	10	5.700 ± 0.153
	0.02	10	6.000 ± 0.000	9	5.667 ± 0.267
	0.04	10	6.000 ± 0.149	10	5.900 ± 0.100
	0.08	10	6.000 ± 0.000	10	5.800 ± 0.133
	0.16	10	5.900 ± 0.100	10	5.600 ± 0.163
	0.31	10	5.700 ± 0.153	10	0.560 ± 0.163

(a) Number of animals with values available
(b) Mean ± standard error
(c) Statistically significant ($P \leq 0.05$) by Williams' test (Williams, 1971, 1972)
(d) SGOT = serum glutamic oxaloacetate transaminase; IU = international unit of enzyme activity (the amount of an enzyme that will convert 1 µmol of substrate per minute in an assay system under optimum conditions).
(e) LDH = lactic dehydrogenase; for LDH, there is evidence that one of the underlying assumptions of Williams' test (monotonicity) is not satisfied.
(f) SDH = sorbitol dehydrogenase; SU = Sigma units (millimicromoles converted in 24 hours).
(g) SGPT = serum glutamic pyruvate transaminase
(h) OCT = ornithine carbamoyltransferase

STUDIES PERFORMED AT LITTON BIONETICS, INC.

SINGLE-ADMINISTRATION STUDIES

All male rats administered 2,6-xylidine at doses of 1.0 g/kg or less survived to the end of the 14-day observation period. Four of five male rats administered 1.47 g/kg died, and all males receiving doses of 2.15 or 3.16 g/kg died. All female rats given doses of 0.681 g/kg or less survived to the end of the 14-day observation period. At 1.47 and 2.15 g/kg, 4/5 female rats died. All female rats administered 3.16 g/kg died. The following LD_{50} values were calculated by a modification of Horn's method (1956): male--1.31 g/kg (95% confidence limits, 1.12-1.52 g/kg); female--1.27 g/kg (95% confidence limits, 0.944-1.71 g/kg).

The activity of animals given 0.215 g/kg was reduced, and prostration was observed at higher doses. Ptosis and a watery discharge from the eyes were seen at 3.16 g/kg.

Mottling of the kidney was the most notable lesion seen in survivors. Bright red lungs and pale livers and kidneys were observed in rats that died. Other changes were judged to be the result of postmortem degeneration.

RANGE-FINDING STUDIES

No animals died (Table 8). All animals appeared normal except for one male in the 10,000-ppm group; that animal had a slowly healing ulcerated cutaneous scab during the last 3 weeks of the study.

Mean body weight gain depressions greater than 10% occurred in female rats administered 2,6-xylidine at a concentration of 3,000 ppm and in male and female rats at 10,000 ppm. No other signs of toxicity were observed during the studies. No noteworthy lesions were seen during gross necropsy.

TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE RANGE-FINDING FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	152	474	+322	
100	5/5	133	457	+324	96
300	5/5	146	456	+310	96
1,000	5/5	147	493	+346	104
3,000	5/5	150	445	+295	94
0	5/5	90	458	+368	
10,000	5/5	93	366	+273	80
FEMALE					
0	5/5	121	274	+153	
100	5/5	121	283	+162	103
300	5/5	130	292	+162	107
1,000	5/5	130	284	+154	104
3,000	5/5	130	257	+127	94
0	5/5	80	246	+166	
10,000	5/5	74	201	+127	82

(a) Number surviving/number in group
(b) Mean body weight change of the group

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weight gains relative to those of controls were markedly reduced (greater than 10%) for high dose male and female rats throughout most of the studies (Table 9). Decreased mean body weight gain also was seen in mid dose female rats. Mid dose male rats and low dose male and female rats showed some evidence of reduced weight gains, but these decreases were generally in the 5%-9% range.

Feed consumption by dosed and control groups was comparable (Table 10). Feed consumption by low, mid, and high dose males was 102%, 100%, and 97% that of the controls; feed consumption by low, mid, and high dose female rats was 105%, 99%, and 98% that by controls.

No compound-related clinical signs were observed.

TABLE 9. BODY WEIGHT GAIN OF RATS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

Week	Weight Gain (grams) (a)				Percent Weight Change Relative to Controls (b)		
	Control	300 ppm	1,000 ppm	3,000 ppm	300 ppm	1,000 ppm	3,000 ppm
MALE							
1	63.9	49.7	49.0	47.2	−22	−23	−26
4	188.3	175.3	181.6	163.5	−7	−4	−13
8	285.5	259.5	267.0	247.6	−9	−6	−13
16	385.9	362.7	351.4	329.3	−6	−9	−15
24	441.7	417.3	414.8	389.2	−6	−6	−12
38	513.2	486.7	481.3	455.2	−5	−6	−11
54	566.7	530.8	524.8	489.5	−6	−7	−14
70	613.6	577.4	558.5	536.8	−6	−9	−13
78	613.9	585.1	575.8	542.2	−5	−6	−12
90	567.7	573.9	559.4	542.3	+1	−1	−4
99	525.7	554.7	539.1	522.0	+6	+3	−1
FEMALE							
1	40.4	32.1	34.0	30.5	−21	−16	−25
4	103.7	97.7	97.0	87.2	−6	−6	−16
8	146.6	134.0	131.8	121.8	−9	−10	−17
16	187.5	176.3	170.3	151.2	−6	−9	−19
24	214.0	198.3	194.4	176.1	−7	−9	−18
38	246.0	234.7	224.1	204.7	−5	−9	−17
54	294.1	276.1	252.1	230.5	−6	−14	−22
70	327.5	319.4	285.0	260.9	−2	−13	−20
78	327.4	346.3	293.5	279.7	+6	−10	−15
90	356.1	355.6	308.1	298.8	0	−13	−16
99	352.4	364.8	309.1	319.0	+4	−12	−9

(a) Relative to initial body weights, which were 119.6, 132.4, 121.4, and 122.0 g for control, 300-, 1,000-, and 3,000-ppm groups of male rats, respectively, and 103.8, 112.7, 106.2, and 106.0 g for groups of female rats

TABLE 10. FEED CONSUMPTION BY RATS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

Week	Control Grams Feed/ Day (a)	300 ppm Grams Feed/ Day (a)	300 ppm Low/ Control (b)	1,000 ppm Grams Feed/ Day (a)	1,000 ppm Mid/ Control (b)	3,000 ppm Grams Feed/ Day (a)	3,000 ppm High/ Control (b)
MALE							
1-4	22.2	22.2	1.00	21.3	0.96	20.7	0.93
5-8	25.1	25.2	1.00	24.6	0.98	23.4	0.93
9-12	23.8	24.7	1.04	24.5	1.03	23.4	0.98
13-16	23.8	23.8	1.00	23.3	0.98	22.0	0.92
17-20	23.2	22.7	0.98	22.8	0.99	21.7	0.94
21-24	23.3	22.7	0.98	22.7	0.98	22.3	0.96
25-30	23.6	24.4	1.03	23.8	1.01	23.3	0.98
34-42	22.8	24.6	1.08	24.2	1.06	23.4	1.03
46-54	23.4	24.0	1.03	23.9	1.02	22.8	0.98
58-66	23.2	23.9	1.03	22.9	0.99	23.3	1.00
70-78	23.7	23.6	1.00	23.6	0.99	23.7	1.00
82-99	21.9	22.7	1.04	22.9	1.04	22.1	1.01
Mean	23.3	23.7	1.0	23.4	1.0	22.7	1.0
Standard error	0.24	0.27	0.01	0.27	0.01	0.26	0.01
FEMALE							
1-4	18.0	17.6	0.98	17.4	0.96	17.0	0.94
5-8	18.5	18.4	1.01	19.3	1.04	18.2	0.98
9-12	17.8	17.7	0.99	17.3	0.97	17.0	0.95
13-16	16.7	17.5	1.05	16.2	0.97	16.2	0.97
17-20	15.7	15.7	1.00	15.4	0.98	15.4	0.98
21-24	15.8	16.3	1.04	15.2	0.96	15.3	0.97
25-30	16.0	17.4	1.09	16.3	1.02	15.9	0.99
34-42	17.0	18.8	1.11	17.4	1.02	17.0	1.00
46-54	17.8	18.4	1.03	16.9	0.95	16.6	0.93
58-66	17.0	19.4	1.14	17.4	1.02	17.7	1.04
70-78	15.1	17.9	1.18	16.2	1.07	15.3	1.01
82-99	17.0	17.8	1.05	16.2	0.96	17.8	1.05
Mean	16.9	17.7	1.1	16.8	1.0	16.6	1.0
Standard error	0.30	0.29	0.02	0.32	0.01	0.29	0.01

(a) Grams of feed consumed per animal per day; not corrected for scatter.
(b) Feed consumption for dosed group divided by feed consumption for control group

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing 2,6-xylidine in feed at the doses used in these studies and for the controls are shown in Table 11 and in the Kaplan-Meier curves in Figure 1. Survival in high dose male rats was significantly reduced relative to that of controls. Survival in mid dose males also was reduced. In groups of female rats, differences in survival were not significant. The increased mortality in high dose male rats does not appear to have been caused primarily by nasal cavity tumors, since a comparable increase in nasal cavity neoplasms in female rats was not associated with increased mortality.

Pathology and Statistical Analyses of Results

This section describes statistically significant or biologically noteworthy increases in the incidences of rats with neoplastic or nonneoplastic lesions in the nasal cavity, subcutaneous tissue, liver, pituitary gland, and adrenal gland.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes A and B for male and female rats, respectively.

TABLE 11. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

	Control	300 ppm	1,000 ppm	3,000 ppm
MALE (a)				
Animals initially in study	56	56	56	56
Nonaccidental deaths before termination (b)	13	16	23	42
Killed at termination	43	39	33	14
Died during termination period	0	1	0	0
Survival P values (c)	<0.001	0.470	0.053	<0.001
FEMALE				
Animals initially in study	56	56	56	56
Nonaccidental deaths before termination (b)	23	31	24	32
Killed at termination	33	24	30	23
Died during termination period	0	1	2	1
Survival P values (c)	0.227	0.209	0.911	0.143

(a) Start of termination period: week 102
(b) Includes animals killed in a moribund condition
(c) The results of the life table trend test are in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

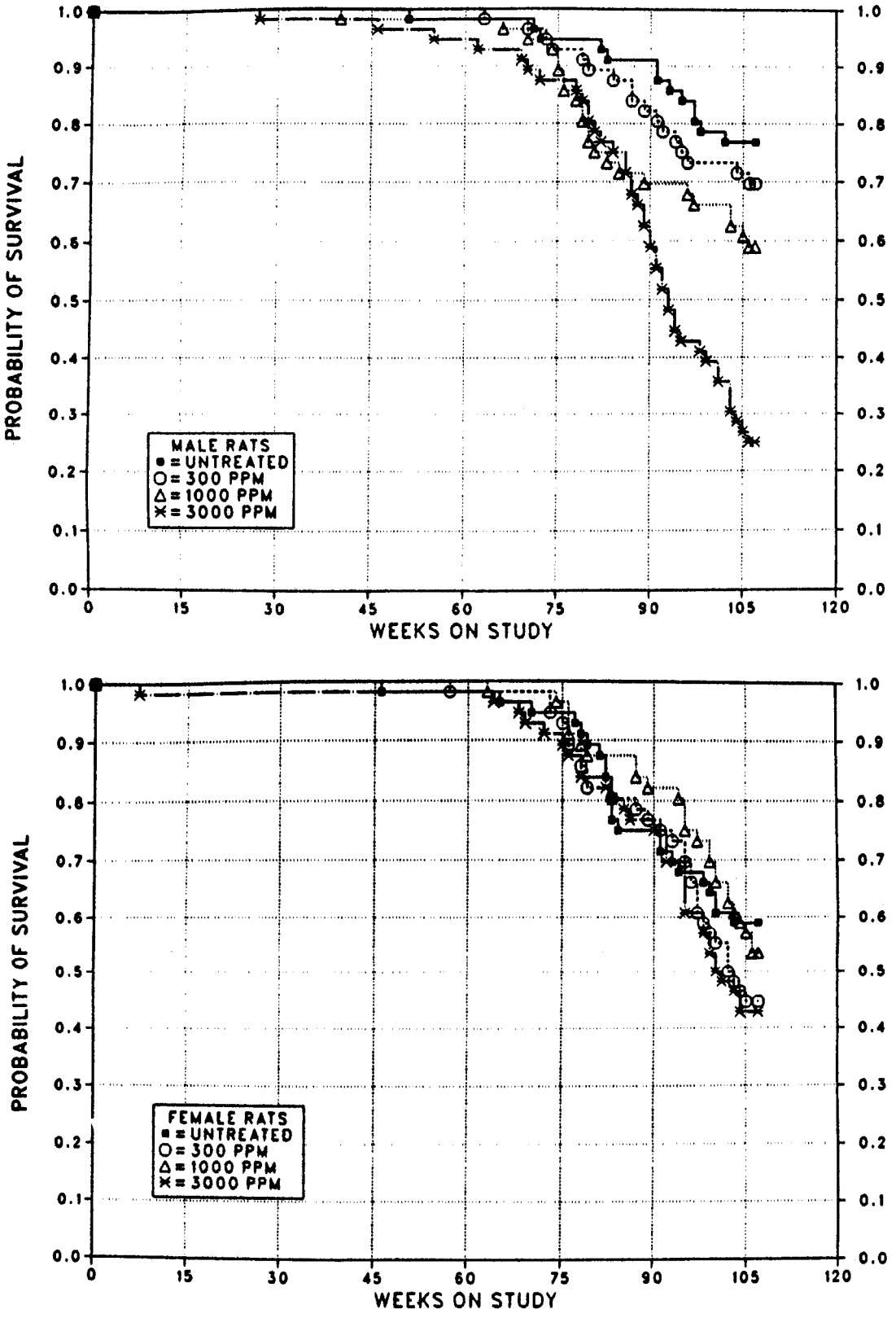


FIGURE 1. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING 2,6-XYLIDINE FOR TWO YEARS

Nasal Cavity: High dose male and female rats had significantly increased incidences of carcinomas (Table 12). Two adenocarcinomas were observed in high dose males. High dose rats of each sex had increased incidences of papillary adenomas. A few unusual neoplasms of the nasal cavity were observed in high dose male and female rats: One undifferentiated sarcoma was present in a high dose female; rhabdomyosarcomas occurred in two high dose male and two high dose females; and neoplasms with features associated with both adenocarcinomas and rhabdomyosarcoma (malignant mixed tumors) were observed in one high dose male and one high dose female rat.

The benign neoplasms (adenoma and papillary adenoma) were located in the anterior part of the nasal cavity in the region of the respiratory turbinates. The malignant neoplasms (carcinoma, adenocarcinoma, sarcoma, rhabdomyosarcoma, and malignant mixed tumors) were usually located in the posterior nasal cavity in the region of the ethmoturbinates and the posterior part of the nasal septum.

The adenocarcinomas and carcinomas varied in structure from well-differentiated growths composed of acini lined by cuboidal cells (adenocarcinomas) to poorly differentiated neoplasms in which a glandular pattern was not prominent (carcinomas). Most of these malignant neoplasms appeared to arise from the submucosal gland in the dorsal posterior portion of the nasal turbinates. As the neoplasms became more undifferentiated, it was difficult to determine whether they were arising from the surface epithelium or from the submucosal glands. The carcinomas were composed of infiltrating sheets of pleomorphic hyperchromic epithelial cells, which varied from small pleomorphic cells with indistinct basophilic cytoplasm and round basophilic nuclei to large anaplastic cells with abundant eosinophilic cytoplasm and large pleomorphic nuclei containing distinct nucleoli. Mitotic figures were numerous in malignant neoplasms; focal squamous metaplasia was sometimes present. The carcinomas were highly invasive, frequently destroying the nasal turbinates and nasal septum. Invasion into the adjacent maxillary bone and overlaying frontal bone occurred in several rats. Metastasis to the brain was detected in 5/56 male and 7/56 female rats fed 2,6-xylidine at 3,000 ppm.

The undifferentiated sarcoma was present in the olfactory region of the nasal cavity. The neoplasm was composed of interlacing bundles of spindle-shaped cells having little cytoplasm and elongated nuclei. Mitotic figures were numerous throughout the neoplasm. The center of the neoplasm was necrotic. Invasion into the nasal septum and frontal bone occurred.

The rhabdomyosarcomas were composed of anaplastic pleomorphic cells with abundant eosinophilic cytoplasm. The cells had round-to-oval vesicular nuclei with distinct nucleoli; multinucleated giant cells were common. The neoplastic cells varied from round to spindle-shaped, and elongated, "straplike" cells with centrally located multiple nuclei were present. In some areas, the neoplasms were less differentiated; in these areas, cells had hyperchromatic nuclei, numerous mitotic figures, and less cytoplasm. Cross striations were readily apparent in 1-μ sections stained with toluidine blue, whereas striations were barely visible in sections stained with hematoxylin and eosin.

Electron microscopy revealed Z bands; M bands were sometimes apparent. Some slightly elongated cells having filaments or myofibrils and no Z bands may be analogous to the "straplike" cells observed by light microscopy. Multinucleated giant cells contained a few myofibrils or filaments and a suggestion of Z bands. These findings confirm that these mesenchymal nasal tumors are rhabdomyosarcomas.

Neoplasms diagnosed as malignant mixed tumors of the nasal cavity had two distinct cellular components. Some portions of the neoplasm were composed of well-differentiated cuboidal epithelium arranged in a glandular pattern. Sarcomatous proliferations of elongated "straplike" cells (containing multiple elongated nuclei and abundant eosinophilic cytoplasm) were mixed with neoplastic glandular epithelium. Marked pleomorphism and numerous mitotic figures were present in all areas of this neoplasm. When this neoplasm was stained by Mallory's phosphotungstic acid hematoxylin,

TABLE 12. ANALYSIS OF NASAL CAVITY TUMORS IN RATS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS (a)

	Control	300 ppm	1,000 ppm	3,000 ppm
MALE				
Papillary Adenoma				
Overall Rates	0/56 (0%)	0/56 (0%)	2/56 (4%)	10/56 (18%)
Adjusted Rates	0.0%	0.0%	4.9%	42.7%
Terminal Rates	0/43 (0%)	0/40 (0%)	1/33 (3%)	4/14 (29%)
Life Table Tests	P<0.001	(b)	P=0.209	P<0.001
Incidental Tumor Tests	P<0.001	(b)	P=0.322	P=0.001
Cochran-Armitage Trend Test	P<0.001			
Fisher Exact Test		(b)	P=0.248	P<0.001
Carcinoma				
Overall Rates	0/56 (0%)	0/56 (0%)	0/56 (0%)	26/56 (46%)
Adjusted Rates	0.0%	0.0%	0.0%	68.6%
Terminal Rates	0/43 (0%)	0/40 (0%)	0/33 (0%)	5/14 (36%)
Life Table Tests	P<0.001	(b)	(b)	P<0.001
Incidental Tumor Tests	P<0.001	(b)	(b)	P<0.001
Cochran-Armitage Trend Test	P<0.001			
Fisher Exact Test		(b)	(b)	P<0.001
Carcinoma or Adenocarcinoma				
Overall Rates	0/56 (0%)	0/56 (0%)	0/56 (0%)	28/56 (50%)
Adjusted Rates	0.0%	0.0%	0.0%	73.1%
Terminal Rates	0/43 (0%)	0/40 (0%)	0/33 (0%)	6/14 (43%)
Life Table Tests	P<0.001	(b)	(b)	P<0.001
Incidental Tumor Tests	P<0.001	(b)	(b)	P<0.001
Cochran-Armitage Trend Test	P<0.001			
Fisher Exact Test		(b)	(b)	P<0.001
Adenoma, Adenocarcinoma, or Carcinoma				
Overall Rates	0/56 (0%)	0/56 (0%)	2/56 (4%)	33/56 (59%)
Adjusted Rates	0.0%	0.0%	4.9%	81.8%
Terminal Rates	0/43 (0%)	0/40 (0%)	1/33 (3%)	8/14 (57%)
Life Table Tests	P<0.001	(b)	P=0.209	P<0.001
Incidental Tumor Tests	P<0.001	(b)	P=0.322	P<0.001
Cochran-Armitage Trend Test	P<0.001			
Fisher Exact Test		(b)	P=0.248	P<0.001
FEMALE				
Adenoma				
Overall Rates	0/56 (0%)	0/56 (0%)	1/56 (2%)	6/56 (11%)
Adjusted Rates	0.0%	0.0%	3.1%	17.0%
Terminal Rates	0/33 (0%)	0/25 (0%)	1/32 (3%)	2/24 (8%)
Life Table Tests	P<0.001	(b)	P=0.494	P=0.013
Incidental Tumor Tests	P<0.001	(b)	P=0.494	P=0.019
Cochran-Armitage Trend Test	P<0.001			
Fisher Exact Test		(b)	P=0.500	P=0.014
Carcinoma				
Overall Rates	0/56 (0%)	0/56 (0%)	1/56 (2%)	24/56 (43%)
Adjusted Rates	0.0%	0.0%	1.8%	53.9%
Terminal Rates	0/33 (0%)	0/25 (0%)	0/32 (0%)	6/24 (25%)
Life Table Tests	P<0.001	(b)	P=0.504	P<0.001
Incidental Tumor Tests	P<0.001	(b)	P=0.393	P<0.001
Cochran-Armitage Trend Test	P<0.001			
Fisher Exact Test		(b)	P=0.500	P<0.001
Adenoma or Carcinoma				
Overall Rates	0/56 (0%)	0/56 (0%)	2/56 (4%)	29/56 (52%)
Adjusted Rates	0.0%	0.0%	4.9%	62.0%
Terminal Rates	0/33 (0%)	0/25 (0%)	1/32 (3%)	8/24 (33%)
Life Table Tests	P<0.001	(b)	P=0.238	P<0.001
Incidental Tumor Tests	P<0.001	(b)	P=0.179	P<0.001
Fisher Exact Test		(b)	P=0.248	P<0.001

(a) Statistical analyses used are discussed in Section II (Statistical Method) and Table A3 (footnotes).
(b) No P values are presented because no tumors were observed in the dosed and control groups.

rhabdomyosarcomas and cross striations in the cytoplasm of the "straplike" cells were observed.

Papillary adenomas of the nasal cavity varied in size and originated from the epithelium of the nasal cavity and maxillary turbinates or from the nasal septum. These neoplasms formed papillary projections into the lumen of the nasal cavity and were usually lined by single layers of respiratory epithelium. The neoplasms were well-differentiated and characterized by an absence of invasion into the underlying tissue. Multiple papillary adenomas were occasionally present; five high dose male and one high dose female rat with papillary adenomas also had malignant epithelial neoplasms in other regions of the nasal cavity.

Acute inflammation (rhinitis), epithelial hyperplasia, and squamous metaplasia occurred at increased incidences in high dose male and female rats (Table 13). Inflammation of the epithelium lining the nasal cavity occurred in both control and dosed rats. The increase in acute rhinitis was dose related. Animals with rhinitis had an accumulation of suppurative exudate and cellular debris in the lumen of the nasal cavity. Many of the rats with acute inflammatory changes in the nasal mucosa had erosion and ulceration of the epithelial lining of the nasal cavity and hyperplasia of the submucosal glands. In a few animals with subacute rhinitis, the cellular infiltrate was a mixture of neutrophilic and mononuclear cells; in some animals with acute rhinitis, the infiltrate was primarily mononuclear cells.

Subcutaneous Tissue: The incidences of high dose male and female rats with fibromas were significantly greater than those in controls (Table 14). Subcutaneous fibrosarcomas were observed in three high dose females, one high dose male, one mid dose female, one low dose male, and one control female. The incidences of high dose males and females with fibromas were significant by the life table test.

TABLE 13. SUMMARY OF NONNEOPLASTIC NASAL CAVITY LESIONS OBSERVED IN RATS IN THE TWO-YEAR STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

	Control	300 ppm	1,000 ppm	3,000 ppm
MALE				
Acute inflammation	12/56 (21%)	(a) 21/56 (38%)	(b) 32/56 (57%)	(b) 42/56 (75%)
Epithelial hyperplasia	0/56 (0%)	0/56 (0%)	1/56 (2%)	4/56 (7%)
Squamous metaplasia	0/56 (0%)	0/56 (0%)	0/56 (0%)	(a) 5/56 (9%)
FEMALE				
Acute inflammation	7/56 (13%)	14/56 (25%)	(a) 15/56 (27%)	(b) 38/56 (68%)
Epithelial hyperplasia	0/56 (0%)	0/56 (0%)	1/56 (2%)	(b) 11/56 (20%)
Squamous metaplasia	0/56 (0%)	0/56 (0%)	0/56 (0%)	4/56 (7%)

(a) $P<0.05$ relative to controls by Fisher exact test
(b) $P<0.01$ relative to controls by Fisher exact test

TABLE 14. SUBCUTANEOUS TISSUE TUMORS IN RATS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

	Control	300 ppm	1,000 ppm	3,000 ppm
MALE				
Fibroma				
Overall Rates	0/56 (0%)	1/56 (2%)	2/56 (4%)	4/56 (7%)
Adjusted Rates	0.0%	2.2%	4.9%	22.1%
Terminal Rates	0/43 (0%)	0/40 (0%)	1/33 (3%)	2/14 (14%)
Life Table Tests	P=0.002	P=0.483	P=0.211	P=0.005
Incidental Tumor Tests	P=0.058	P=0.617	P=0.322	P=0.042
Cochran-Armitage Trend Test	P=0.029			
Fisher Exact Test		P=0.500	P=0.248	P=0.059
Fibroma or Fibrosarcoma				
Overall Rates	0/56 (0%)	2/56 (4%)	2/56 (4%)	5/56 (9%)
Adjusted Rates	0.0%	4.4%	4.9%	26.4%
Terminal Rates	0/43 (0%)	0/40 (0%)	1/33 (3%)	2/14 (14%)
Life Table Tests	P<0.001	P=0.219	P=0.211	P=0.001
Incidental Tumor Tests	P=0.101	P=0.371	P=0.322	P=0.035
Cochran-Armitage Trend Test	P=0.022			
Fisher Exact Test		P=0.248	P=0.248	P=0.028
FEMALE				
Fibroma				
Overall Rates	0/56 (0%)	2/56 (4%)	1/56 (2%)	4/56 (7%)
Adjusted Rates	0.0%	6.6%	2.1%	15.5%
Terminal Rates	0/33 (0%)	1/25 (4%)	0/32 (0%)	3/24 (13%)
Life Table Tests	P=0.029	P=0.207	P=0.522	P=0.034
Incidental Tumor Tests	P=0.057	P=0.278	P=0.594	P=0.052
Cochran-Armitage Trend Test	P=0.048			
Fisher Exact Test		P=0.248	P=0.500	P=0.059
Fibrosarcoma				
Overall Rates	1/56 (2%)	0/56 (0%)	1/56 (2%)	3/56 (5%)
Adjusted Rates	3.0%	0.0%	2.2%	11.7%
Terminal Rates	1/33 (3%)	0/25 (0%)	0/32 (0%)	2/24 (8%)
Life Table Tests	P=0.055	P=0.555N	P=0.745N	P=0.208
Incidental Tumor Tests	P=0.085	P=0.555N	P=0.713N	P=0.277
Cochran-Armitage Trend Test	P=0.080			
Fisher Exact Test		P=0.500N	P=0.752	P=0.309
Fibroma or Fibrosarcoma				
Overall Rates	1/56 (2%)	2/56 (4%)	2/56 (4%)	6/56 (11%)
Adjusted Rates	3.0%	6.6%	4.3%	22.5%
Terminal Rates	1/33 (3%)	1/25 (4%)	0/32 (0%)	4/24 (17%)
Life Table Tests	P=0.010	P=0.433	P=0.529	P=0.025
Incidental Tumor Tests	P=0.026	P=0.514	P=0.606	P=0.049
Cochran-Armitage Trend Test	P=0.020			
Fisher Exact Test		P=0.500	P=0.500	P=0.057

Liver: Neoplastic nodules occurred in female rats with a significant positive trend (Table 15). Hepatocellular carcinomas were observed in one control, one mid dose, and one high dose female rat. Incidences of dosed male rats with liver tumors were not increased; a high dose male had a neoplastic nodule, and a control and a mid dose male had an hepatocellular carcinoma.

Pituitary Gland: Adenomas occurred in female rats with a significant positive trend, but pairwise comparisons did not yield significant results (control, 29/56; low dose, 32/55; mid dose, 30/56; high dose, 20/56; $P<0.05$). The incidence of male rats with pituitary gland adenomas was significantly increased only in the low dose group (9/55; 18/54; 13/54; 9/54; $P<0.06$).

Adrenal Gland: The increased incidence of high dose females with cortical adenomas was significant by the life table test (control, 7/56; low dose, 9/56; mid dose, 6/55; high dose, 12/55; $P<0.05$). Since this lesion is not usually life threatening, life table analysis may not be the most appropriate statistical method. No dose-related adrenal tumors were seen in male rats.

Other Sites: Several microscopic nonneoplastic changes were detected in dosed and control animals; e.g., nephropathy, alveolar macrophages, hemosiderosis and hematopoiesis in the spleen, foci of cellular alteration in the liver, cortical vacuolation and focal hyperplasia in the adrenal cortex and medulla, and cystic changes in the uterus and ovaries. These lesions are commonly observed in aging CD rats.

Hematologic and Clinical Chemistry Analyses

Decreases were detected in erythrocyte counts and hemoglobin levels at 18 months in the 3,000-ppm group of male rats and in erythocyte counts, hemoglobin levels, and hematocrit at 12 months in the 1,000- and 3,000-ppm groups of female rats (Table 16). These changes were not severe enough to be considered indicative of anemia. The biologic significance of changes in leukocyte counts during the last 6 months of the study is not known.

No biologically significant changes in clinical chemistry parameters were attributable to administration of 2,6-xylidine.

TABLE 15. LIVER TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

	Control	300 ppm	1,000 ppm	3,000 ppm
Neoplastic Nodule				
Overall Rates	0/56 (0%)	1/56 (2%)	2/56 (4%)	4/55 (7%)
Adjusted Rates	0.0%	4.0%	6.3%	16.7%
Terminal Rates	0/33 (0%)	1/25 (4%)	2/32 (6%)	4/24 (17%)
Life Table Tests	P=0.012	P=0.445N	P=0.231	P=0.029
Incidental Tumor Tests	P=0.012	P=0.445N	P=0.231	P=0.029
Cochran-Armitage Trend Test	P=0.027			
Fisher Exact Test		P=0.500	P=0.248	P=0.057
Neoplastic Nodule or Hepatocellular Carcinoma				
Overall Rates	1/56 (2%)	1/56 (2%)	3/56 (5%)	5/55 (9%)
Adjusted Rates	3.0%	4.0%	9.4%	20.8%
Terminal Rates	1/33 (3%)	1/25 (4%)	3/32 (9%)	5/24 (21%)
Life Table Tests	P=0.013	P=0.699	P=0.293	P=0.044
Incidental Tumor Tests	P=0.013	P=0.699	P=0.293	P=0.044
Cochran-Armitage Trend Test	P=0.033			
Fisher Exact Test		P=0.752	P=0.309	P=0.099

TABLE 16. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS

	Dose (ppm)	12 months Number (a)	12 months Response (b)	18 months Number (a)	18 months Response (b)	24 months Number (a)	24 months Response (b)
MALE							
Erythrocyte count (10^6/µl)	0	10	8.7 ± 0.2	10	7.9 ± 0.2	10	8.7 ± 0.3
	300	10	8.3 ± 0.2	10	7.7 ± 0.1	10	8.0 ± 0.3
	1,000	10	8.0 ± 0.3	10	7.8 ± 0.2	9	7.9 ± 0.2
	3,000	10	8.3 ± 0.2	9	7.0 ± 0.3	10	8.0 ± 0.2
Hemoglobin (g/dl)	0	10	16.5 ± 0.3	10	15.5 ± 0.3	10	16.2 ± 0.6
	300	10	16.5 ± 0.3	10	15.6 ± 0.2	10	15.3 ± 0.5
	1,000	10	15.5 ± 0.9	10	15.1 ± 0.4	9	14.8 ± 0.7
	3,000	10	16.3 ± 0.2	9	14.3 ± 0.6	10	15.0 ± 0.3
Hematocrit (percent)	0	10	47.5 ± 0.7	10	45.8 ± 0.9	10	47.0 ± 1.6
	300	10	46.3 ± 0.9	10	45.5 ± 0.6	10	45.8 ± 1.5
	1,000	10	43.8 ± 2.4	10	44.3 ± 1.1	9	42.6 ± 2.0
	3,000	10	46.3 ± 0.7	9	42.4 ± 1.9	10	43.6 ± 1.0
Leukocyte count (10^3/µl)	0	10	10.5 ± 1.1	10	10.8 ± 1.1	10	8.5 ± 1.5
	300	10	11.2 ± 0.7	10	12.4 ± 2.1	10	6.7 ± 0.4
	1,000	9	15.0 ± 5.7	10	11.1 ± 1.2	8	11.7 ± 2.0
	3,000	10	10.7 ± 0.5	9	12.7 ± 1.0	10	11.0 ± 1.6
Lymphocytes (percent)	0	10	69.8 ± 4.4	10	70.8 ± 3.4	10	70.1 ± 4.7
	300	10	73.5 ± 4.0	10	74.1 ± 3.8	10	64.8 ± 3.9
	1,000	10	69.9 ± 3.2	10	69.3 ± 2.2	9	69.3 ± 3.9
	3,000	10	72.0 ± 3.1	9	58.9 ± 5.3	10	67.1 ± 4.8
Segmented neutrophils (percent)	0	10	25.9 ± 4.3	10	27.3 ± 3.1	10	28.0 ± 4.7
	300	10	22.1 ± 3.4	10	24.5 ± 3.9	10	32.0 ± 3.8
	1,000	10	24.4 ± 3.2	10	29.1 ± 2.0	9	29.8 ± 3.8
	3,000	10	25.0 ± 2.5	9	39.1 ± 5.4	10	31.0 ± 4.6
Glucose (mg/dl)	0	10	96.5 ± 1.8	10	103.9 ± 7.2	10	74.7 ± 9.2
	300	10	104.3 ± 3.9	10	107.6 ± 5.2	10	95.4 ± 6.4
	1,000	10	115.8 ± 6.3	10	111.7 ± 2.9	9	(c) 115.6 ± 4.2
	3,000	9	113.0 ± 6.3	9	105.7 ± 3.1	9	(c) 115.3 ± 10.7
Alkaline phosphatase (IU/liter)	0	10	107.1 ± 8.5	10	121.4 ± 14.4	10	113.2 ± 18.9
	300	10	112.8 ± 12.0	10	95.5 ± 9.2	10	92.9 ± 11.1
	1,000	10	105.9 ± 13.9	10	102.4 ± 9.8	9	160.7 ± 19.4
	3,000	10	112.2 ± 8.9	9	110.8 ± 11.3	9	145.4 ± 12.1
SGOT (c) (IU/liter)	0	10	112.5 ± 9.6	10	101.3 ± 12.7	10	93.7 ± 8.4
	300	10	93.8 ± 9.1	10	80.9 ± 5.4	10	85.7 ± 6.7
	1,000	10	98.1 ± 12.9	10	70.0 ± 3.4	9	108.1 ± 8.5
	3,000	10	93.7 ± 6.2	9	74.7 ± 2.9	9	94.0 ± 9.5
Blood urea nitrogen (mg/dl)	0	10	12.3 ± 0.6	8	12.0 ± 0.6	9	9.9 ± 0.8
	300	10	12.5 ± 0.3	10	11.7 ± 0.4	10	9.2 ± 1.0
	1,000	10	13.5 ± 1.1	10	12.0 ± 0.6	9	11.0 ± 0.8
	3,000	10	11.8 ± 0.4	9	11.2 ± 0.4	9	13.3 ± 2.4

TABLE 16. HEMATOLOGIC AND CLINICAL CHEMISTRY RESULTS IN THE TWO-YEAR FEED STUDIES OF 2,6-XYLIDINE AT LITTON BIONETICS (Continued)

	Dose (ppm)	12 months Number (a)	12 months Response (b)	18 months Number (a)	18 months Response (b)	24 months Number (a)	24 months Response (b)
FEMALE							
Erythrocyte count (10^6/µl)	0	10	8.0 ± 0.1	10	7.2 ± 0.1	10	7.1 ± 0.2
	300	10	(d) 7.7 ± 0.1	10	6.6 ± 0.3	9	7.3 ± 0.2
	1,000	10	(d) 7.3 ± 0.1	8	6.9 ± 0.2	10	6.5 ± 0.4
	3,000	10	(d) 7.2 ± 0.1	10	6.9 ± 0.1	10	7.0 ± 0.3
Hemoglobin (g/dl)	0	10	16.6 ± 0.2	10	15.1 ± 0.3	10	14.5 ± 0.4
	300	10	16.3 ± 0.2	10	14.3 ± 0.6	9	14.2 ± 0.6
	1,000	10	(d) 15.5 ± 0.2	8	14.4 ± 0.3	10	14.0 ± 0.8
	3,000	10	(d) 15.3 ± 0.2	10	14.7 ± 0.2	10	13.8 ± 0.5
Hematocrit (percent)	0	10	46.5 ± 0.4	10	44.2 ± 0.8	10	42.1 ± 1.1
	300	10	45.6 ± 0.8	10	41.6 ± 1.8	10	41.4 ± 1.6
	1,000	10	(d) 44.5 ± 0.4	8	42.9 ± 1.0	10	39.9 ± 2.5
	3,000	10	(d) 43.8 ± 0.5	10	43.7 ± 0.5	10	39.6 ± 1.5
Leukocyte count (10^3/µl)	0	10	7.5 ± 0.6	10	8.3 ± 0.8	9	4.9 ± 0.5
	300	10	7.2 ± 0.9	10	9.1 ± 0.5	9	4.8 ± 1.0
	1,000	10	7.1 ± 0.5	8	8.4 ± 1.3	9	5.8 ± 0.7
	3,000	10	7.8 ± 0.5	10	9.0 ± 1.2	10	(d) 9.2 ± 1.0
Lymphocytes (percent)	0	10	71.2 ± 3.0	10	64.3 ± 3.9	10	68.5 ± 5.5
	300	10	74.9 ± 2.9	10	67.6 ± 5.1	9	70.6 ± 8.0
	1,000	10	73.4 ± 2.4	9	69.3 ± 7.2	10	66.3 ± 4.8
	3,000	10	74.9 ± 2.0	10	66.2 ± 3.5	10	70.9 ± 3.4
Segmented neutrophils (percent)	0	10	24.6 ± 3.0	10	34.0 ± 4.0	10	29.5 ± 5.5
	300	10	19.8 ± 2.5	10	30.6 ± 4.9	9	29.7 ± 7.8
	1,000	10	23.4 ± 2.1	9	26.1 ± 5.2	10	32.6 ± 4.8
	3,000	10	23.3 ± 2.0	10	32.9 ± 3.3	10	27.4 ± 3.5
Glucose (mg/dl)	0	10	89.3 ± 3.8	10	90.7 ± 4.3	9	88.8 ± 6.7
	300	10	(d) 101.9 ± 3.0	10	97.9 ± 5.5	10	100.1 ± 7.5
	1,000	10	(d) 102.6 ± 2.5	10	99.1 ± 4.5	10	99.7 ± 8.0
	3,000	10	(d) 112.4 ± 3.4	10	97.1 ± 2.6	9	105.6 ± 4.3
Alkaline phosphatase (IU/liter)	0	10	73.2 ± 10.1	10	66.7 ± 9.4	9	83.3 ± 12.5
	300	10	73.2 ± 10.1	10	69.0 ± 4.1	9	88.2 ± 10.1
	1,000	10	62.6 ± 5.7	10	71.4 ± 4.5	10	97.1 ± 7.8
	3,000	10	66.9 ± 7.8	10	64.0 ± 5.9	9	99.7 ± 14.7
SGOT (IU/liter)	0	10	117.1 ± 9.8	10	105.3 ± 8.4	9	91.3 ± 9.9
	300	10	98.9 ± 6.5	10	94.5 ± 8.2	9	74.7 ± 5.3
	1,000	10	114.2 ± 12.9	10	98.1 ± 4.9	9	122.0 ± 22.8
	3,000	10	88.4 ± 4.2	10	92.6 ± 9.8	9	109.4 ± 16.3
Blood urea nitrogen (mg/dl)	0	10	14.3 ± 0.6	10	12.3 ± 0.7	9	14.3 ± 1.1
	300	10	13.1 ± 0.7	10	12.6 ± 0.8	10	12.0 ± 0.9
	1,000	10	13.4 ± 0.4	10	13.0 ± 0.6	10	12.1 ± 1.0
	3,000	10	(d) 12.3 ± 0.7	10	13.2 ± 0.7	9	12.9 ± 1.4

(a) Number of animals with values available
(b) Mean ± standard deviation
(c) SGOT = serum glutamic oxaloacetate transaminase; IU = international unit of enzyme activity (the amount of an enzyme that will convert 1 µmol of substrate per minute in an assay system under optimum conditions).
(d) Statistically significant ($P \leq 0.05$)

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

A series of preliminary and 2-year studies were conducted to determine the toxicity and carcinogenicity of 2,6-xylidine. The epithelium of the nasal cavity was the primary site affected, both neoplastic and nonneoplastic lesions being observed in rats fed diets containing up to 3,000 ppm 2,6-xylidine for 2 years. Toxic effects were also seen in the liver, kidney, and hematopoietic system.

The results of the single-dose studies of male rats agreed with those of previous studies. The following LD_{50} values were calculated: 1.31 g/kg for male Charles River CD rats; 0.62-1.25 g/kg for male F344/N rats. LD_{50} values of 0.84-2.06 g/kg had been calculated from previous studies (Lindstrom et al., 1969; Jacobson, 1972; Vernot et al., 1977). The LD_{50} value for female Charles River CD rats (1.27 g/kg) was close to that for female F344/N rats (1.16 g/kg).

The toxic effects of 2,6-xylidine on the hematopoietic system were more severe in male rats than in female rats. A dose-related leukocytosis and an increase in the number of nucleated erythrocytes occurred in male F344/N rats fed diets containing 2,6-xylidine for 2 weeks. Slight-to-moderate anisocytosis, poikilocytosis, and polychromasia occurred in dosed groups of male rats and in female rats fed diets containing the higher doses (0.32 and 0.62 g/kg). Administration of 2,6-xylidine for 13 weeks to male F344/N rats was associated with slight decreases in total leukocyte counts and the percentage of lymphocytes. Slight decreases in hemoglobin and hematocrit levels and in erythrocyte counts occurred in dosed male rats; hemoglobin and hematocrit levels were decreased in dosed female rats. In the 2-year studies conducted in CD rats, decreases in erythrocyte counts, accompanied by decreases in hemoglobin and hematocrit levels, occurred in males fed diets containing 3,000 ppm 2,6-xylidine (month 18) and in females fed diets containing 1,000 or 3,000 ppm (month 12). These changes are not considered to be biologically significant. Changes in the leukocyte count were not severe enough to have clear-cut biologic significance. Previous investigators observed anemia and methemoglobinemia in rats exposed to 2,6-xylidine (Lindstrom et al., 1963, 1969). 2,6-Xylidine also produces methemoglobinemia in cats (McLean et al., 1967).

Slight hepatic and renal toxicity was observed. Mottled kidneys were frequently observed in Charles River CD rats receiving 2,6-xylidine doses as low as 0.2 g/kg. Increased liver weight to body weight ratios occurred in male F344/N rats administered 0.16 or 0.31 g/kg by gavage for 13 weeks and in females administered 0.31 g/kg. The kidney weight to brain weight ratio was significantly increased in females at 0.31 g/kg. 2,6-Xylidine caused fatty degeneration of the liver in dogs (Magnusson et al., 1971), hepatomegaly in rats (Lindstrom et al., 1963; Magnusson et al., 1971), and pitting or depressed scarring of the kidneys in rats receiving 10,000 ppm for 6 months (Lindstrom et al., 1963). Renal lesions were not observed in dogs or rats administered 2,6-xylidine at doses of 500-700 mg/kg per day for 4 weeks (Magnusson et al., 1971).

The results of these studies indicate that 2,6-xylidine toxicity is qualitatively similar in CD and F344/N rats. Disposition studies of radiolabeled 2,6-xylidine suggested that absorption and elimination were faster in F344/N than in CD rats, but the two strains did not differ markedly in tissue retention of radioactivity 24 hours after single doses were administered (Ethyl Corp, 1982a).

The occurrence of nasal cavity neoplasms provided strong evidence for the carcinogencity of 2,6-xylidine in CD rats. The incidences of high dose male and female rats with nasal cavity carcinomas were significantly increased ($P < 0.001$). The carcinomas were highly invasive and frequently destroyed the nasal turbinates and the nasal septum. Metastasis to the brain occurred in 5/56 males and 7/56 females in the high dose groups. High dose rats of each sex also had significant increases of papillary adenomas of the nasal cavity ($P < 0.02$). Most of the carcinomas and adenomas of the nasal cavity occurred in animals in the highest dose groups. Nonneoplastic nasal cavity lesions in high dose male and female rats included acute inflammation (rhinitis), epithelial hyperplasia, and squamous metaplasia. A dose-related increase in the incidence of acute rhinitis was observed.

The occurrence of several unusual neoplasms of the nasal cavity was considered to be compound related. One undifferentiated sarcoma was observed in a high dose female rat. Rhabdomyosarcomas occurred in two high dose males and two high dose females. Malignant mixed tumors having features associated with both adenocarcinoma and rhabdomyosarcoma were observed in one high dose male and one high dose female.

Nasal cavity tumors have been associated with occupational exposure of humans and chemical administration to laboratory animals. Occupations associated with increased incidences of nasal tumors include those in the furniture industry (Mosbech and Acheson, 1971; Formbeur, 1972), shoe industry (Hadfield, 1972; Acheson, 1976) and nickel refineries (Pederson et al., 1973; Konetzke, 1974). In experimental animals, nasal cavity tumors have been associated with chemical administration in the diet or drinking water or by parenteral injection or inhalation. Several nitrosamine compounds have caused nasal tumors in rats when administered by parenteral injection (Garcia and Lijinsky, 1972; Reznik et al., 1975; Mohr et al., 1977; Bulay and Mirvish, 1979; Hecht et al., 1980) or when administered in drinking water (Goodall et al., 1968; Lijinsky and Taylor, 1975, 1979; Pelfrene and Garcia, 1976; Singer and Taylor, 1976). Nasal cavity tumors have also been induced in rats by administering dioxane in drinking water (Hoch-Ligeti et al., 1970) or by gavage (NCI, 1978) and by administering phenacetin (Isaka et al., 1979) and quinoxaline 1,4-dioxane (Tucker, 1975) in the diet.

Most chemically induced nasal tumors in humans and experimental animals arise from the respiratory or olfactory epithelium. Tumors of neural origin have also been reported. The presence of chemically induced malignant mesenchymal tumors, in addition to epithelial tumors, is an unusual feature of this study. The rhabdomyosarcomas are rare tumors of particular interest. Throughout the Carcinogenesis Program, none of the 205 nasal cavity tumors in 36,402 dosed or control male F344/N rats or of 178 nasal cavity tumors in 36,355 dosed or control female F344/N rats was a rhabdomyosarcoma. Rhabdomyosarcoma of the nasal cavity has not been reported in the literature. The finding of four of these tumors in the current studies is therefore remarkable.

Volatilization of 2,6-xylidine from the formulated diets may have occurred in these studies. Its vapor pressure is 1 mm at 44° C and 10 mm at 87° C (Weast, 1976). When formulated diets were stored open in a rat cage at room temperature, 44% of the compound was lost over a 7-day period (Appendix E). When 2,6-xylidine was protected from light and stored in a sealed container for 14 days at −20° C and 5° C and at room temperature, losses were 1.5%, 3.2%, and 14.6%, respectively. Approximately 70%-80% of the loss from the rat cage was due to evaporation, and the remaining loss was due to the reaction of the chemical with feed components. 2,6-Xylidine at concentrations of 0.24 ppm was detected in the air of animal cages containing 10,000 ppm of the chemical in feed (Ethyl Corp., 1982b).

Analyses of feed containing 2,6-xylidine indicate that the chemical reacts markedly with nonfat dry milk (which contains 50%-52% of the reducing sugar, lactose), moderately with wheat flour (which contains enediol compounds having reactivities similar to those of aldehydes), and markedly with the reducing sugars D-glucose and β-D-lactose. These results suggest that chemical decomposition occurs principally via the reaction of the amine group of 2,6-xylidine with carbonyl groups of feed components. When 2,6-xylidine was mixed with D-glucose, the color of the reaction mixture changed from white to brown. This type of "browning reaction" in foods is commonly caused by an initial aldehyde-amine reaction followed by a nonreversible rearrangement that yields polymeric products. The polymeric products of this secondary reaction have not been well characterized. Although the products of such a reaction involving 2,6-xylidine are not known, the biologic availability of such polymerized xylidine derivatives is likely to be low.

Studies have been conducted of the absorption, distribution, elimination, and bioretention of [^{14}C]2,6-xylidine in Sprague Dawley rats administered gavage doses of 63 mg/kg (Ethyl Corp., 1982a). One group of rats received 10 daily doses of the study chemical; another received 9 daily doses of the vehicle followed by a single dose of the study chemical. The single oral dose

IV. DISCUSSION AND CONCLUSIONS

of [^{14}C]2,6-xylidine was readily absorbed and distributed to organs and tissues. Most of the radiolabel was eliminated in the urine; some was eliminated in the feces and in expired air. Small amounts of the radiolabel were recovered in tissues 24 hours after the dose was administered. Accumulation of the radiolabel occurred when 10 daily doses were administered. Animals receiving 10 daily doses had higher levels of radioactivity in the blood and other tissues, and radioactivity disappeared more slowly. The percentage of radioactivity in the blood of rats administered the 10th dose was six to seven times higher than that in the blood of rats receiving the single dose; the radioactivity recovered in tissues was also higher in the group receiving repeated doses of 2,6-xylidine. Retention of the radiolabel in tissues after repeated dosing was not due to impairment of elimination. After receiving the 10th dose of radiolabeled 2,6-xylidine, male rats excreted the radiolabel more rapidly in urine and feces than did males receiving only one dose of the labeled compound. The retention of radioactivity suggests that 2,6-xylidine or its metabolites bind to certain blood and tissue components.

Distribution studies showed that the greatest concentrations of radioactivity 24 hours after the 10th dose was administered were found in red blood cells and the liver (Ethyl Corp., 1982a). High concentrations, relative to those in other tissues, were also found in the kidneys and whole blood. Leukocytes, skin, and the nasal cavity were not examined in these studies. Twenty-four hours after rats received 63 mg/kg by gavage, the concentration of radioactivity in nasal tissues was 2.5 times greater than that in the liver; concentrations in the olfactory bulb were only slightly less than those in the liver. These results were confirmed when high concentrations of radioactivity were found in the nasal tissue of rats administered 2,6-xylidine intraperitoneally. These studies indicate that 2,6-xylidine absorbed solely through the oral route concentrates in nasal cavity tissues where it can produce toxic effects. Therefore, it is not necessary to hypothesize inhalation exposure to account for the effects of 2,6-xylidine on nasal tissues.

Compounds related to 2,6-xylidine have also been shown to be carcinogenic. 2,4-Xylidine hydrochloride and 2,5-xylidine hydrochloride were found to be weakly carcinogenic when administered in the diet for 2 years to male Charles River rats and to male and female HaM/ICR mice (Weisburger et al., 1978; Table 17). Administration of 2,4-xylidine produced a significant increase in lung tumors in female mice only at the higher dose (control, 5/22; low dose, 5/18; high dose, 11/19). Increased incidences of vascular tumors occurred in dosed male mice receiving 2,5-xylidine hydrochloride (2/16; 5/18; 7/19); this increase was significant only when compared with the incidence in pooled controls (5/99).

Some monocyclic aniline compounds tested by the Carcinogenesis Testing Program (aniline, *o*-toluidine hydrochloride, and 2,4,5-trimethylaniline) were carcinogenic but did not cause nasal cavity tumors (Table 17). 2,4-Dimethoxyaniline hydrochloride and *p*-chloroaniline caused dose-related increases in tumor incidence, but the evidence was not considered to be sufficient to classify these chemicals as carcinogens. Aniline and a number of aniline derivatives have been shown to produce neoplasms in the spleen: dapsone (NCI, 1977), *o*-toluidine hydrochloride (NCI, 1979a), *p*-chloroaniline (NCI, 1979b), azobenzene (NCI, 1979c), and D & C Red No. 9 (NTP, 1982). In the current studies of 2,6-xylidine, hemosiderosis and hematopoiesis of the spleen were equally severe in dosed and control animals.

Under the conditions of these studies, 2,6-xylidine was *clearly carcinogenic** for male and female Charles River CD rats, causing significant increases in the incidences of adenomas and carcinomas of the nasal cavity. Rhabdomyosarcomas, rare tumors of the nasal cavity, were observed in dosed rats of each sex. In addition, the increased incidences of subcutaneous fibromas and fibrosarcomas in male and female rats and the increased incidence of neoplastic nodules of the liver in female rats may have been related to the administration of 2,6-xylidine.

*A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 7-8.

TABLE 17. COMPARISON OF RESULTS OF TWO-YEAR FEED STUDIES OF MONOCYCLIC ANILINE COMPOUNDS

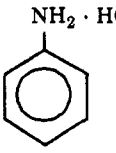
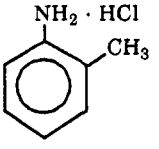
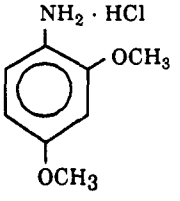
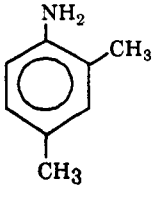
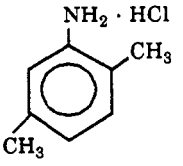
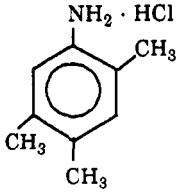
Substance	Structure	Species	Sex	High Dose (ppm)	Neoplasm
Aniline hydrochloride (NCI, 1978)		Rat	M	6,000	Splenic fibrosarcomas or sarcomas (combined)
			F	6,000	Splenic hemangiosarcomas Splenic fibrosarcomas or sarcomas (combined)
		Mouse ($B6C3F_1$)	M	12,000	None
			F	12,000	None
p-Chloroaniline (NCI, 1979b)		Rat (F344/N)	M	500	Splenic fibromas, fibrosarcomas, hemangiosarcomas, osteosarcomas or sarcomas (combined)
			F	500	None
		Mouse ($B6C3F_1$)	M	5,000	Hemangiosarcomas and hemangiomas (a)
			F	5,000	Hemangiosarcomas and hemangiomas (a)
o-Toluidine hydrochloride (NCI, 1979a)		Rat (F344/N)	M	6,000	Sarcomas, fibrosarcomas, and mesotheliomas of multiple organs, subcutaneous fibromas, and splenic fibromas and sarcomas
			F	6,000	Osteosarcomas, mammary fibroadenomas, urinary bladder transitional cell carcinomas, and splenic hemangiosarcomas
		Mouse ($B6C3F_1$)	M	3,000	Hemangiosarcomas
			F	3,000	Hepatocellular carcinomas or adenomas (combined)
2,4-Dimethoxyaniline hydrochloride (NCI, 1979d)		Rat (F344/N)	M	3,000	None
			F	3,000	None
		Mouse ($B6C3F_1$)	M	5,000	None
			F	5,000	None
2,4,5-Trimethylaniline (NCI, 1979e)		Rat (F344/N)	M	800	Hepatocellular carcinomas
			F	800	Hepatocellular carcinomas or adenomas (combined)
		Mouse ($B6C3F_1$)	M	100	Hepatocellular carcinomas
			F	100	Hepatocellular carcinomas

TABLE 17. COMPARISON OF RESULTS OF TWO-YEAR FEED STUDIES OF MONOCYCLIC ANILINE COMPOUNDS (Continued)

Substance	Structure	Species	Sex	High Dose (ppm)	Neoplasm
2,4-Xylidine hydrochloride (Weisburger et al., 1978)		Rat (Charles River)	M	1,000	None
			--		
		Mouse (HaM/ICR)	M	250	None
			F	250	Lung neoplasms
2,5-Xylidine hydrochloride (Weisburger et al., 1978)		Rat (Charles River)	M	6,000	None
			--		
		Mouse (HaM/ICR)	M	12,000	Vascular neoplasms (b)
			F	12,000	None

(a) Dose related but not statistically significant in high dose group
(b) Statistically significant compared with pooled controls only

V. REFERENCES

V. REFERENCES

1. Acheson, E. (1976) Nasal cancer in the furniture and boot and shoe manufacturing industries. Prev. Med. 5:295-315.

2. Aldrich Handbook (1978) Milwaukee: Aldrich Chemical Co.

3. Ames, B.N.; McCann, J.; Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

4. Armitage, P. (1971) Statistical Methods in Medical Research. New York: John Wiley & Sons, Inc., pp. 362-365.

5. Berenblum, I., Ed. (1969) Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2. Geneva: International Union Against Cancer.

6. Bollag, J.; Blattmann, P.; Laanio, T. (1978) Adsorption and transformation of four substituted anilines in soil. J. Agric. Food Chem. 26:1302-1306.

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

8. Bulay, O.; Mirvish, S. (1979) Carcinogenesis in rat esophagus by intraperitoneal injection of different doses of methyl-*n*-amylnitrosamine. Cancer Res. 39:3644-3646.

9. Chung, K.-T.; Fulk, G.; Egan, M. (1978) Reduction of azo dyes by intestinal anaerobes. Appl. Environ. Microbiol. 35:558-562.

10. Colour Index (1977) 3rd ed., Yorkshire, England: Society of Dyers and Colourists, 4:4863.

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

12. Dunnett, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50:1096-1122.

13. Ethyl Corporation (1982a) Unpublished Laboratory Report.

14. Ethyl Corporation (1982b) Unpublished Laboratory Report.

15. Florin, I.; Ritberg, L.; Curvall, M.; Enzell, C. (1980) Screening of tobacco smoke constitutents for mutagenicity using the Ames' test. Toxicology 18:219-232.

16. Formbeur, J. (1972) Recent cases of ethmoid-maxillary tumors in wood-workers. Arch. Mal. Prof. Med. Trav. Secur. Soc. 33:454-455.

17. Garcia, H.; Lijinsky, W. (1972) Tumorigenicity of five cyclic nitrosamines in MRC rats. Z. Krebsforschung 77:257-261.

18. Gart, J.J.; Chu, K.C.; Tarone, R.E. (1979) Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62:957-974.

19. Goodall, C.; Lijinsky, W.; Tomatis, L. (1968) Tumorigenicity of N-nitrosohexamethyleneimine. Cancer Res. 28:1217-1222.

20. Gosselin, R.E.; Hodge, H.C.; Smith, R.P.; Gleason, M.N. (1976) Clinical Toxicology of Commercial Products. Baltimore: Williams & Wilkins Co.

21. Hadfield, E. (1972) Cancer hazard from wood dust and in the boot and shoe industry. Ann. Occup. Hyg. 15:39-41.

22. Hartman, C.; Andrews, A.; Chung, K. (1979) Production of a mutagen from Ponceau 3R by a human intestinal anaerobe. Infect. Immun. 23:686-689.

23. Haseman, J.K. (1984) Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58:385-392.

24. Haseman, J.K.; Huff, J.; Boorman, G.A. (1984) Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126-135.

25. Hecht, S.; Chen, C.; Ohmori, T.; Hoffmann, D. (1980) Comparative carcinogenicity in F344 rats of the tobacco-specific nitrosamines, N'-nitrosonornicotine and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res. 40:298-302.

26. Hoch-Ligeti, C.; Argus, M.F.; Arcos, J.C. (1970). Induction of carcinomas in the nasal cavity of rats by dioxane. Br. J. Cancer 24:164-167.

27. Horn, H. (1956) Simplified LD_{50} (or ED_{50}) calculations. Biometrics 12:311-322.

28. International Agency for Research on Cancer (IARC) (1978) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 16. Some Aromatic Amines and Related Nitro Compounds--Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals. Lyon: IARC, World Health Organization.

29. Isaka, H.; Yoshii, H.; Otsuji, A.; Koike, M.; Nagai, Y.; Koura, M.; Sugiyasu, K.; Kanabayashi, T. (1979) Tumors of Sprague-Dawley rats induced by long-term feeding of phenacetin. Gann 70:29-36.

30. Jacobson, K. (1972) Acute oral toxicity of mono- and di-alkyl ring-substituted derivatives of aniline. Toxicol. Appl. Pharmacol. 22:153-154.

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

32. Keenaghan, J.; Boyes, R. (1972) The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man. J. Pharmacol. Exp. Ther. 180:454-463.

33. Konetzke, G. (1974) The carcinogenic action of arsenic and nickel. Arch. Geschwulstforsch. 44:16-22.

34. Lijinsky, W.; Taylor, H. (1975) Carcinogenicity of methylated nitrosopiperidines. Int. J. Cancer 167:318-322.

35. Lijinsky, W.; Taylor, H. (1979) Carcinogenicity of methylated derivatives of N-nitrosodiethylamine and related compounds in Sprague-Dawley rats. J. Natl. Cancer Inst. 62:407.

36. Lindstrom, H. (1961) The metabolism of FD & C Red No. 1. I. The fate of 2,4-metaxylidine in rats. J. Pharmacol. Exp. Ther. 132:306-310.

37. Lindstrom, H.; Hansen, W.; Nelson, A.; Fitzhugh, O. (1963) The metabolism of FD & C Red No. 1. II. The fate of 2,5-para-xylidine and 2,6-meta-xylidine in rats and observations on the toxicity of xylidine isomers. J. Pharmacol. Exp. Ther. 12:257-264.

38. Lindstrom, H.; Bowie, W.; Wallace, W.; Nelson, A.; Fitzhugh, O. (1969) The toxicity and metabolism of mesidine and pseudocumidine in rats. J. Pharmacol. Exp. Ther. 167:223-234.

39. Linhart, M.S.; Cooper, J.; Martin, R.L.; Page, N.; Peters, J. (1974) Carcinogenesis Bioassay Data System. Comput. Biomed. Res. 7:230-248.

40. Magnusson, G.; Bodin, N.; Hansson, E. (1971) Hepatic changes in dogs and rats induced by xylidine isomers. Acta. Pathol. Microbiol. Scand. Sect. A. 79:639-648.

41. Magnusson, G.; Majeed, S.; Down, W.; Sacharin, R.; Jorgeson, W. (1979) Hepatic effects of xylidine isomers in rats. Toxicol. 12:63-74.

42. Mannell, W. (1964) Further investigations on production of liver tumors in rats by Ponceau 3R. J. Cosmet. Toxicol. 2:169-174.

43. Mantel, N.; Haenszel, W. (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. 22:719-748.

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

V. REFERENCES

45. McConnell, E.E.; Solleveld, H.A.; Swenberg, J.A.; Boorman, G.A. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76: 283-289.

46. McLean, S.; Murphy, B.; Starmer, G.; Thomas, J. (1967) Methaemoglobin formation induced by aromatic amines and amides. J. Pharm. Pharmacol. 19:146-154.

47. McLean, S.; Starmer, G.; Thomas, J. (1969) Methaemoglobin formation by aromatic amines. J. Pharm. Pharmacol. 21:441-450.

48. Mohr, U.; Reznik, G.; Pour, P. (1977) Carcinogenic effects of diisopropanolnitrosamine in Sprague-Dawley rats. J. Natl. Cancer Inst. 58: 361-364.

49. Mosbech, J.; Acheson, E. (1971) Nasal cancer in furniture makers in Denmark. Dan. Med. Bull. 19:34-35.

50. National Cancer Institute (NCI) (1977) Bioassay of Dapsone for Possible Carcinogenicity. NCI Technical Report 20. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 97 p.

51. National Cancer Institute (NCI) (1978) Bioassay of Aniline Hydrochloride for Possible Carcinogenicity. NCI Technical Report 130. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 95 p.

52. National Cancer Institute (NCI) (1979a) Bioassay of o-Toluidine Hydrochloride for Possible Carcinogenicity. NCI Technical Report 153. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 129 p.

53. National Cancer Institute (NCI) (1979b) Bioassay of p-Chloroaniline for Possible Carcinogenicity. NCI Technical Report 189. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 53 p.

54. National Cancer Institute (NCI) (1979c) Bioassay of Azobenzene for Possible Carcinogenicity. NCI Technical Report 154. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 109 p.

55. National Cancer Institute (NCI) (1979d) Bioassay of 2,4-Dimethoxyaniline Hydrochloride for Possible Carcinogenicity. NCI Technical Report 171. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

56. National Cancer Institute (NCI) (1979e) Bioassay of 2,4,5-Trimethylaniline for Possible Carcinogenicity. NCI Technical Report 160. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD. 109 p.

57. National Toxicology Program (NTP) (1982) Carcinogenesis Bioassay of D & C Red No. 9 in F344 Rats and B6C3F_1 Mice. NTP Technical Report No. 225. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD. 168 p.

58. Patrianakos, C.; Hoffman, D. (1979) Chemical studies on tobacco smoke LXIV. On the analysis of aromatic amines in cigarette smoke. J. Anal. Toxicol. 3:150-154.

59. Pedersen, E.; Hogetviet, A.; Andersen, A. (1973) Cancer of respiratory organs among workers at a nickel refinery in Norway. Int. J. Cancer 12:32-41.

60. Pelfrene, A.; Garcia, H. (1976) Chemically induced esthesioneuroblastomas in rats. Z. Krebsforsch. 86:113-119.

61. Petterson, B.; Curvall, M.; Enzell, C. (1980) Effects of tobacco smoke compounds on the noradrenaline induced oxidative metabolism in isolated brown fat cells. Toxicology 18:1-15.

62. Proctor, N.H.; Hughes, J.P. (1978) Chemical Hazards in the Workplace. Philadelphia: Lippincott, pp. 510-511.

63. Reznik, G.; Mohr, U.; Kruger, F. (1975) Carcinogenic effects of di-*n*-propylnitrosamine, β-hydroxypropyl-*n*-propylnitrosamine, and methyl-*n*-propylnitrosamine on Sprague-Dawley rats. J. Natl. Cancer Inst. 54:937-943.

64. Sadtler Standard Spectra. Philadelphia: Sadtler Research Laboratories.

65. Singer, G.M.; Taylor, H.W. (1976) Carcinogenicity of N′-nitrosonornicotine in Sprague-Dawley rats. J. Natl. Cancer Inst. 57:1275-1276.

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

67. Thelestam, M.; Curvall, M.; Enzell, C. (1980) Effects of tobacco smoke compounds on the plasma membranes of cultured human lung fibroblasts. Toxicology 15:203-217.

68. Thomas, J.; Morgan, D.; Vine, J. (1976) Metabolism of etidocaine in man. Xenobiotica 6:39-48.

69. Tucker, M. (1975) Carcinogenic action of quinoxaline 1,4-dioxide in rats. J. Natl. Cancer Inst. 55:137.

70. U.S. International Trade Commission (USITC) (1977a) Imports of Benzenoid Chemicals and Products. Washington, DC: Government Printing Office.

71. U.S. International Trade Commission (USITC) (1977b) Synthetic Organic Chemicals, United States Production and Sales 1975. USITC Publication No. 804, Washington, DC: Government Printing Office.

72. Vernot, E.; MacEwen, J.; Haun, C.; Kinkead, E. (1977) Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol. Appl. Pharmacol. 42:417-423.

73. Weast, R., Ed. (1976) Handbook of Chemistry and Physics, 57th ed. Cleveland: CRC Press, pp. C-146, h-200.

74. Weisburger, E.; Russfield, A.; Homburger, F.; Weisburger, J.; Boger, E.; Van Dongen, C.; Chu, K. (1978) Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J. Environ. Pathol. Toxicol. 2:325-356.

75. 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.

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

77. Yahagi, T.; Degawa, M.; Seino, Y.; Matsushima, T.; Nagao, M.; Sugimura, T.; Hashimoto, Y. (1975) Mutagenicity of carcinogenic azo dyes and their derivatives. Cancer Lett. 1:91-96.

78. Zalipsky, J.; Won, C.; Patel, D. (1978) Analytical-physical profile of lidamidine hydrochloride (WHR-1142A), a novel antidiarrheal agent. Arzneim.-Forsch. 28:1441-1447.

79. Zimmer, D.; Mazurek, J.; Petzold, G.; Bhuyan, B.K. (1980) Bacterial mutagenicity and mammalian cell damage by several substituted anilines. Mutat. Res. 77:317-326.

APPENDIX A

SUMMARY OF LESIONS IN MALE CD RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
Animals initially in study	56	56	56	56
Animals necropsied	56	56	56	56
Animals examined histopathologically	56	56	56	56
INTEGUMENTARY SYSTEM				
*Multiple organs	(56)	(56)	(56)	(56)
Fibrous histiocytoma, malignant		1 (2%)	1 (2%)	
*Skin	(56)	(56)	(56)	(56)
Keratoacanthoma			1 (2%)	
*Subcutaneous tissue	(56)	(56)	(56)	(56)
Sarcoma, NOS		1 (2%)		
Fibroma		1 (2%)	2 (4%)	4 (7%)
Fibrosarcoma		1 (2%)		1 (2%)
Fibrous histiocytoma, malignant	1 (2%)			
Lipoma	1 (2%)			
Neurofibroma		2 (4%)		
RESPIRATORY SYSTEM				
*Nasal cavity	(56)	(56)	(56)	(56)
Carcinoma, NOS				26 (46%)
Adenocarcinoma, NOS				2 (4%)
Papillary adenoma			2 (4%)	10 (18%)
Rhabdomyosarcoma				2 (4%)
Mixed tumor, malignant				1 (2%)
#Lung	(56)	(56)	(55)	(56)
Squamous cell carcinoma, metastatic			1 (2%)	
Alveolar/bronchiolar adenoma			1 (2%)	
Cortical carcinoma, metastatic		2 (4%)		
Fibrosarcoma, metastatic			1 (2%)	
HEMATOPOIETIC SYSTEM				
#Brain	(56)	(55)	(56)	(56)
Malignant reticulosis			1 (2%)	
*Multiple organs	(56)	(56)	(56)	(56)
Malignant lymphoma, lymphocytic type				1 (2%)
Malignant lymphoma, histiocytic type				2 (4%)
Lymphocytic leukemia	1 (2%)	1 (2%)		
Monocytic leukemia				1 (2%)
#Mandibular lymph node	(54)	(55)	(53)	(54)
Sarcoma, NOS, metastatic				1 (2%)
Malignant lymphoma, histiocytic type		1 (2%)		
#Mediastinal lymph node	(54)	(55)	(53)	(54)
Mesothelioma, metastatic		1 (2%)		
#Thymus	(36)	(36)	(37)	(32)
Thymoma, malignant				1 (3%)
CIRCULATORY SYSTEM				
*Subcutaneous tissue	(56)	(56)	(56)	(56)
Hemangiosarcoma			1 (2%)	
#Spleen	(56)	(56)	(56)	(56)
Hemangiosarcoma				1 (2%)
#Mesenteric lymph node	(54)	(55)	(53)	(54)
Hemangioma			1 (2%)	
#Heart	(56)	(56)	(55)	(56)
Mesothelioma, malignant		1 (2%)		
#Urinary bladder	(56)	(56)	(55)	(51)
Hemangiosarcoma				1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
DIGESTIVE SYSTEM				
*Periodontal tissues	(56)	(56)	(56)	(56)
Sarcoma, NOS				1 (2%)
#Liver	(56)	(56)	(56)	(56)
Neoplastic nodule				1 (2%)
Hepatocellular carcinoma	1 (2%)		1 (2%)	
Fibrosarcoma, metastatic			1 (2%)	
#Pancreas	(56)	(55)	(56)	(52)
Acinar cell adenoma	1 (2%)	2 (4%)		
#Duodenum	(55)	(56)	(54)	(56)
Adenocarcinoma, NOS				1 (2%)
#Jejunum	(55)	(56)	(54)	(56)
Cystadenoma, NOS			1 (2%)	
URINARY SYSTEM				
#Kidney	(56)	(56)	(56)	(56)
Transitional cell carcinoma				1 (2%)
Tubular cell adenoma	1 (2%)			
Sarcoma, NOS			1 (2%)	
Lipoma		1 (2%)		
ENDOCRINE SYSTEM				
#Pituitary	(55)	(54)	(54)	(54)
Carcinoma, NOS	2 (4%)	2 (4%)	2 (4%)	
Adenoma, NOS	9 (16%)	18 (33%)	13 (24%)	9 (17%)
#Adrenal	(56)	(55)	(56)	(56)
Cortical adenoma	1 (2%)	2 (4%)	3 (5%)	
Cortical carcinoma		3 (5%)		
Pheochromocytoma	3 (5%)	4 (7%)	6 (11%)	3 (5%)
Neuroblastoma			1 (2%)	
#Thyroid	(55)	(55)	(55)	(55)
Follicular cell adenoma				2 (4%)
C-cell adenoma		1 (2%)	1 (2%)	2 (4%)
#Parathyroid	(52)	(54)	(52)	(53)
Adenoma, NOS	1 (2%)			2 (4%)
#Pancreatic islets	(56)	(55)	(56)	(52)
Islet cell adenoma		1 (2%)	1 (2%)	
Islet cell carcinoma		1 (2%)		
REPRODUCTIVE SYSTEM				
*Mammary gland	(56)	(56)	(56)	(56)
Adenoma, NOS			1 (2%)	
Adenocarcinoma, NOS			1 (2%)	
#Testis	(56)	(56)	(56)	(54)
Interstitial cell tumor	5 (9%)	2 (4%)	3 (5%)	1 (2%)
NERVOUS SYSTEM				
#Brain	(56)	(55)	(56)	(56)
Carcinoma, NOS, metastatic				5 (9%)
Granular cell tumor, NOS		1 (2%)		
SPECIAL SENSE ORGANS				
*Eyelid	(56)	(56)	(56)	(56)
Fibrosarcoma				1 (2%)
*Ear	(56)	(56)	(56)	(56)
Sarcoma, NOS			1 (2%)	1 (2%)
Fibroma	1 (2%)			

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
SPECIAL SENSE ORGANS (Continued)				
*Zymbal gland	(56)	(56)	(56)	(56)
Carcinoma, NOS		1 (2%)		1 (2%)
Squamous cell carcinoma		1 (2%)	2 (4%)	
MUSCULOSKELETAL SYSTEM				
None				
BODY CAVITIES				
*Abdominal cavity	(56)	(56)	(56)	(56)
Fibroma				1 (2%)
Fibrosarcoma			1 (2%)	
Osteosarcoma			1 (2%)	
ALL OTHER SYSTEMS				
None				
ANIMAL DISPOSITION SUMMARY				
Animals initially in study	56	56	56	56
Natural death@	10	10	14	28
Moribund sacrifice	3	7	9	14
Terminal sacrifice	43	39	33	14
TUMOR SUMMARY				
Total animals with primary tumors**	22	35	35	49
Total primary tumors	28	49	50	80
Total animals with benign tumors	20	27	27	24
Total benign tumors	23	34	36	34
Total animals with malignant tumors	5	13	13	42
Total malignant tumors	5	14	14	45
Total animals with secondary tumors##		3	2	6
Total secondary tumors		3	3	6
Total animals with tumors-- uncertain benign or malignant		1		1
Total uncertain tumors		1		1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ
@ Includes autolyzed animals

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: UNTREATED CONTROL

ANIMAL NUMBER	48 85	48 86	48 87	48 88	48 89	49 00	49 01	49 02	49 03	49 04	49 05	49 06	49 07	49 08	49 09	50 00	50 01	50 02	50 03	50 04	50 05	50 06	50 07	50 08	50 09
WEEKS ON STUDY	09 8	10 2	09 3	08 7	10 2	09 2	06 7	10 2	10 2	10 2	10 2	10 2	10 2	08 7	04 6	10 2	10 2	10 2	10 2	10 2	10 2	08 9	10 2	10 2	10 2
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	+	N	N	N	N	N	+	N	N	N	+	+	N	N	N	N	N	N	+	N	N	N
Fibrous histiocytoma, malignant																									
Lipoma														X											
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	−	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−	+	+	+	−	−	+	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma													X												
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma											X														
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma									X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS														X											
Adenoma, NOS	X						X															X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																									
Pheochromocytoma																	X								
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
Adenoma, NOS																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	N	+	+	+	+	+	N	+	N	+	+	+	+	+	+	+	+	+	+
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor													X						X		X				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Ear	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	+	N	N
Fibroma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lymphocytic leukemia			X																						

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS; UNTREATED CONTROL (Continued)

ANIMAL NUMBER	5110	5111	5112	5113	5114	5115	5116	5117	5118	5119	5220	5221	5222	5223	5224	5225	5226	5227	5228	5229	5230	5231	5232	5233	5234
WEEKS ON STUDY	1002	0788	1002	1002	1002	1002	1002	1002	1002	0991	1002	1002	1002	1002	0867	1002	1002	1002	1002	1002	1002	1002	1002	0994	1002
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant							X																		
Lipoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	−	+	+	−	+	+	−	+	+	−	−	+	+	−	+	+	−	+	+	+	+	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS		X								X					X									X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																									
Pheochromocytoma			X																		X				
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		X																							
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	+	+	N	N	+	+	+	+	+	+	+	N	+	+	+	N	+	+	+	+	+
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X																							
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Ear	N	N	N	N	N	N	+	N	N	N	+	N	N	N	N	N	N	N	N	+	N	N	N	N	+
Fibroma							X																		
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lymphocytic leukemia																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	53 53 53 53 53 54	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	10 10 10 10 10 07	
	02 02 02 02 02 08	
INTEGUMENTARY SYSTEM		
Subcutaneous tissue	N N N N N N	*56
Fibrous histiocytoma, malignant		1
Lipoma		1
RESPIRATORY SYSTEM		
Lungs and bronchi	+ + + + + +	56
Trachea	+ + + + + +	56
HEMATOPOIETIC SYSTEM		
Bone marrow	+ + + + + +	56
Spleen	+ + + + + +	56
Lymph nodes	+ + + + + +	54
Thymus	+ − + + − +	36
CIRCULATORY SYSTEM		
Heart	+ + + + + +	56
DIGESTIVE SYSTEM		
Salivary gland	+ + + + + +	56
Liver	+ + + + + +	56
Hepatocellular carcinoma		1
Bile duct	+ + + + + +	56
Gallbladder & common bile duct	N N N N N N	*56
Pancreas	+ + + + + +	56
Acinar cell adenoma		1
Esophagus	+ + + + + +	56
Stomach	+ + + + + +	56
Small intestine	+ + + + + +	55
Large intestine	+ + + + + +	56
URINARY SYSTEM		
Kidney	+ + + + + +	56
Tubular cell adenoma		1
Urinary bladder	+ + + + + +	56
ENDOCRINE SYSTEM		
Pituitary	+ + + + + +	55
Carcinoma, NOS		2
Adenoma, NOS	X	9
Adrenal	+ + + + + +	56
Cortical adenoma	X	1
Pheochromocytoma		3
Thyroid	+ + + + − +	55
Parathyroid	+ + + + − +	52
Adenoma, NOS		1
REPRODUCTIVE SYSTEM		
Mammary gland	+ + + + + +	*56
Testis	+ + + + + +	56
Interstitial cell tumor	X	5
Prostate	+ + + + + +	55
NERVOUS SYSTEM		
Brain	+ + + + + +	56
SPECIAL SENSE ORGANS		
Ear	N N N N N +	*56
Fibroma		1
ALL OTHER SYSTEMS		
Multiple organs, NOS	N N N N N N	*56
Lymphocytic leukemia		1

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: 300 ppm

ANIMAL NUMBER	59 97	59 98	59 99	60 00	60 01	60 02	60 03	60 04	60 05	60 06	60 07	60 08	60 09	61 10	61 11	61 12	61 13	61 14	61 15	61 16	61 17	61 18	61 19	62 20	62 21
WEEKS ON STUDY	102	102	100	102	089	076	102	102	058	102	102	102	102	102	102	085	074	102	082	102	102	102	092	066	088
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	+	N	N	+	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	+
Sarcoma, NOS																									X
Fibroma																									X
Fibrosarcoma					X																				
Neurofibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical carcinoma, metastatic									X																
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, metastatic				X																					
Malignant lymphoma, histiocytic type			X																						
Thymus	+	−	+	+	+	+	+	−	−	−	−	−	+	+	−	+	−	+	+	−	+	+	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, malignant				X																					
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Acinar cell adenoma							X															X			
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma			X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS	X			X			X			X	X		X	X	X	X	X			X		X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Cortical adenoma	X																								
Cortical carcinoma									X																
Pheochromocytoma						X																			
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
C-cell adenoma																X									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Islet cell adenoma																									
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	+	+	+	+	+	+	+	+	+	+	+	N	N	+	+	+	N	+	+	+	+	N	+
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																						X			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Granular cell tumor, NOS																									
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS			X																						
Squamous cell carcinoma														X											
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant																									
Lymphocytic leukemia																								X	

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 300 ppm
(Continued)

ANIMAL NUMBER	62 2	62 3	62 4	62 5	62 6	62 7	62 8	62 9	63 0	63 1	63 2	63 3	63 4	63 5	63 6	63 7	63 8	63 9	64 0	64 1	64 2	64 3	64 4	64 5	64 6
WEEKS ON STUDY	100 2	100 2	100 2	100 2	100 2	100 2	100 2	100 2	100 2	080 0	100 2	100 2	100 2	100 2	083	100 2	100 2	069	100 2	100 2	100 2	088	077 0	100 2	102
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N
Sarcoma, NOS																									
Fibroma																									
Fibrosarcoma																									
Neurofibroma								X																	
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical carcinoma, metastatic														X											
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, metastatic																									
Malignant lymphoma, histiocytic type																									
Thymus	+	+	+	+	+	−	+	−	−	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, malignant																									
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS										X					X			X		X		X	X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																					X				
Cortical carcinoma						X								X											
Pheochromocytoma					X				X															X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma								X																	
Islet cell carcinoma												X													
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	N	+	+	N	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor, NOS								X																	
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Squamous cell carcinoma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant											X														
Lymphocytic leukemia																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 300 ppm (Continued)

ANIMAL NUMBER	64 7	64 8	64 9	65 0	65 1	65 2	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	102	102	090	102	102	102	
INTEGUMENTARY SYSTEM							
Subcutaneous tissue	N	N	+	N	N	N	*56
Sarcoma, NOS							1
Fibroma							1
Fibrosarcoma							1
Neurofibroma			X				2
RESPIRATORY SYSTEM							
Lungs and bronchi	+	+	+	+	+	+	56
Cortical carcinoma, metastatic							2
Trachea	+	+	+	+	+	+	56
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	56
Spleen	+	+	+	+	+	+	56
Lymph nodes	+	+	+	+	+	+	55
Mesothelioma, metastatic							1
Malignant lymphoma, histiocytic type							1
Thymus	+	+	+	+	+	+	36
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	56
Mesothelioma, malignant							1
DIGESTIVE SYSTEM							
Salivary gland	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	56
Bile duct	+	+	+	+	+	+	56
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	55
Acinar cell adenoma							2
Esophagus	+	+	+	+	+	+	56
Stomach	+	+	+	+	+	+	56
Small intestine	+	+	+	+	+	+	56
Large intestine	+	+	+	+	+	+	56
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	56
Lipoma							1
Urinary bladder	+	+	+	+	+	+	56
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	54
Carcinoma, NOS			X			X	2
Adenoma, NOS							18
Adrenal	+	+	+	+	+	+	55
Cortical adenoma							2
Cortical carcinoma							3
Pheochromocytoma							4
Thyroid	+	+	+	+	+	+	55
C-cell adenoma							1
Parathyroid	+	+	−	+	+	+	54
Pancreatic islets	+	+	+	+	+	+	55
Islet cell adenoma							1
Islet cell carcinoma							1
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	+	+	+	*56
Testis	+	+	+	+	+	+	56
Interstitial cell tumor			X				2
Prostate	+	+	−	+	+	+	55
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	55
Granular cell tumor, NOS							1
SPECIAL SENSE ORGANS							
Zymbal gland	N	N	N	N	N	N	*56
Carcinoma, NOS							1
Squamous cell carcinoma							1
ALL OTHER SYSTEMS							
Multiple organs, NOS	N	N	N	N	N	N	*56
Fibrous histiocytoma, malignant							1
Lymphocytic leukemia							1

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: 1,000 ppm

ANIMAL NUMBER	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733
WEEKS ON STUDY	075	102	071	102	102	102	102	102	100	102	102	061	101	066	102	102	076	102	102	102	102	070	079	073	102
INTEGUMENTARY SYSTEM																									
Skin	N	+	N	N	N	+	N	N	N	N	N	N	+	+	N	N	N	N	N	N	N	N	+	N	N
Keratoacanthoma						X																			
Subcutaneous tissue	N	+	N	N	N	+	N	N	N	N	N	N	+	+	N	N	N	N	N	N	N	N	+	N	N
Fibroma																									
Hemangiosarcoma														X											
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Squamous cell carcinoma, metastatic																									
Alveolar/bronchiolar adenoma				X																					
Fibrosarcoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary adenoma																						X			
HEMATOPOIETIC SYSTEM																									
Bone marrow	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Hemangioma																									
Thymus	−	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+	−	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma						X																			
Fibrosarcoma, metastatic																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Cystadenoma, NOS																				X					
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																X									
Adenoma, NOS			X						X			X	X					X							
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma		X			X																				
Pheochromocytoma		X			X	X																			
Neuroblastoma					X																				
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
C-cell adenoma												X													
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	−	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	N	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																								X	
Adenocarcinoma, NOS													X												
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant reticulosis																							X		
SPECIAL SENSE ORGANS																									
Ear	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N
Sarcoma, NOS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N
Squamous cell carcinoma																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma																									
Osteosarcoma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant																	X								

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 1,000 ppm (Continued)

ANIMAL NUMBER	7 3 4	7 3 5	7 3 6	7 3 7	7 3 8	7 3 9	7 4 0	7 4 1	7 4 2	7 4 3	7 4 4	7 4 5	7 4 6	7 4 7	7 4 8	7 4 9	7 5 0	7 5 1	7 5 2	7 5 3	7 5 4	7 5 5	7 5 6	7 5 7	7 5 8
WEEKS ON STUDY	1 0 2	0 6 9	1 0 2	0 9 9	0 3 6	1 0 2	1 0 2	1 0 2	0 7 1	1 0 2	0 8 4	1 0 2	0 7 5	0 7 4	1 0 2	1 0 2	0 7 5	1 0 2	0 8 1	1 0 2	1 0 2	1 0 2	0 9 9	0 9 3	1 0 2
INTEGUMENTARY SYSTEM																									
Skin	N	+	N	N	N	N	N	N	N	N	N	N	+	+	N	N	N	N	N	+	N	N	N	N	N
Keratoacanthoma																									
Subcutaneous tissue	N	+	N	N	N	N	N	N	N	N	N	N	+	+	N	N	N	N	N	+	N	N	N	N	N
Fibroma		X																		X					
Hemangiosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic																									
Alveolar/bronchiolar adenoma																									
Fibrosarcoma, metastatic																			X						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary adenoma																				X					
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									X
Thymus	−	−	+	+	−	+	−	+	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Fibrosarcoma, metastatic																			X						
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma, NOS																									
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Carcinoma, NOS	X																								
Adenoma, NOS											X				X	X							X	X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma							X																		
Pheochromocytoma															X					X					X
Neuroblastoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma	X																								
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+
Adenoma, NOS																									
Adenocarcinoma, NOS																									
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor			X							X						X									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant reticulosis																									
SPECIAL SENSE ORGANS																									
Ear	+	+	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS		X																							
Zymbal gland	+	+	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma									X																
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma																			X						
Osteosarcoma				X																					
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 1,000 ppm (Continued)

ANIMAL NUMBER	75 9	76 0	76 1	76 2	76 3	76 4	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	10 2	10 2	10 2	10 2	09 1	07 1	
INTEGUMENTARY SYSTEM							
Skin	N	N	N	N	N	+	*56
Keratoacanthoma							1
Subcutaneous tissue	N	N	N	N	N	+	*56
Fibroma							2
Hemangiosarcoma							1
RESPIRATORY SYSTEM							
Lungs and bronchi	+	+	+	+	+	+	55
Squamous cell carcinoma, metastatic						X	1
Alveolar/bronchiolar adenoma							1
Fibrosarcoma, metastatic							1
Trachea	+	+	+	+	+	+	55
Nasal cavity	+	+	+	+	+	+	*56
Papillary adenoma							2
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	54
Spleen	+	+	+	+	+	+	56
Lymph nodes	+	+	+	+	+	+	53
Hemangioma							1
Thymus	−	+	+	+	+	−	37
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	55
DIGESTIVE SYSTEM							
Salivary gland	+	+	+	+	+	−	55
Liver	+	+	+	+	+	+	56
Hepatocellular carcinoma							1
Fibrosarcoma, metastatic							1
Bile duct	+	+	+	+	+	+	56
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	56
Esophagus	+	+	+	+	+	+	54
Stomach	+	+	+	+	+	+	56
Small intestine	+	+	+	+	+	+	54
Cystadenoma, NOS							1
Large intestine	+	+	+	+	+	+	55
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	56
Sarcoma, NOS						X	1
Urinary bladder	+	+	+	+	+	+	55
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	54
Carcinoma, NOS							2
Adenoma, NOS		X			X		13
Adrenal	+	+	+	+	+	+	56
Cortical adenoma							3
Pheochromocytoma							6
Neuroblastoma							1
Thyroid	+	+	+	+	+	+	55
C-cell adenoma							1
Parathyroid	+	+	+	+	+	+	52
Pancreatic islets	+	+	+	+	+	+	56
Islet cell adenoma							1
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	+	+	+	*56
Adenoma, NOS							1
Adenocarcinoma, NOS							1
Testis	+	+	+	+	+	+	56
Interstitial cell tumor							3
Prostate	+	+	+	+	+	+	56
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	56
Malignant reticulosis							1
SPECIAL SENSE ORGANS							
Ear	N	N	N	N	N	+	*56
Sarcoma, NOS							1
Zymbal gland	N	N	N	N	N	+	*56
Squamous cell carcinoma						X	2
BODY CAVITIES							
Peritoneum	N	N	N	N	N	N	*56
Fibrosarcoma							1
Osteosarcoma							1
ALL OTHER SYSTEMS							
Multiple organs, NOS	N	N	N	N	N	N	*56
Fibrous histiocytoma, malignant							1

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: 3,000 ppm

ANIMAL NUMBER	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845
WEEKS ON STUDY	099	075	065	074	067	082	042	102	058	098	102	097	066	088	102	102	098	102	076	088	102	084	102	087	087
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	N	+	N	+	+	N	N	+	N	+	+	N	N	N	N	+	N	N	N	N	+	N	+	+
Fibroma																						X			
Fibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS	X		X		X	X	X						X				X	X		X		X		X	
Adenocarcinoma, NOS																									
Papillary adenoma									X						X				X		X				
Rhabdomyosarcoma																									X
Mixed tumor, malignant										X															
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic																									
Thymus	+	+	+	−	+	−	+	+	+	−	+	−	−	−	+	+	+	+	+	+	+	+	−	−	+
Thymoma, malignant																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Large intestine	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell carcinoma													X												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+
Hemangiosarcoma																		X							
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS												X			X				X						X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma	X							X																	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									X
C-cell adenoma								X																	
Parathyroid	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																X									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic					X									X						X					
SPECIAL SENSE ORGANS																									
Eye appendages	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma																						X			
Ear	N	N	N	+	N	N	N	N	N	N	N	N	+	+	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS																									
Zymbal gland	N	N	N	+	N	N	N	N	N	N	N	N	+	+	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS													X												
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type																									
Malignant lymphoma, histiocytic type									X																
Monocytic leukemia																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 3,000 ppm (Continued)

ANIMAL NUMBER	84 46	84 47	84 48	84 49	85 50	85 51	85 52	85 53	85 54	85 55	85 56	85 57	85 58	85 59	86 60	86 61	86 62	86 63	86 64	86 65	86 66	86 67	86 68	86 69	87 70
WEEKS ON STUDY	10 02	10 02	10 02	08 86	02 02	10 02	07 75	09 97	08 81	08 81	09 94	10 02	09 99	07 78	10 02	10 02	08 86	07 76	09 90	09 90	08 82	09 90	08 85	08 85	10 00
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	+	N	N	N	N	N	N	N	N	N	+	+	N	+	N	+	+	+	N	+	N	N	+
Fibroma			X													X									
Fibrosarcoma													X												
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS	X	X	X	X			X		X			X		X							X	X	X	X	X
Adenocarcinoma, NOS																X				X					
Papillary adenoma			X									X	X							X		X			
Rhabdomyosarcoma																									
Mixed tumor, malignant																									
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X																			
Lymph nodes	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Sarcoma, NOS, metastatic																									
Thymus	–	+	–	–	+	+	–	–	+	+	–	+	–	–	+	–	–	–	+	+	+	–	–	–	–
Thymoma, malignant															X										
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS											X														
Salivary gland	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule						X																			
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Adenoma, NOS									X	X	X									X					
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																				X					
C-cell adenoma											X														
Parathyroid	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																							X		
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	N	+	+	+	N	N	N	+	N	+	+	N	N	+	+	+	+	+	+	N	N	+	+
Testis	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic												X													
SPECIAL SENSE ORGANS																									
Eye appendages	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma																									
Ear	N	+	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS																									
Zymbal gland	N	+	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma								X																	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type																									
Malignant lymphoma, histiocytic type								X																	
Monocytic leukemia					X																				

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 3,000 ppm (Continued)

ANIMAL NUMBER	87171	87172	87173	87174	87175	87176	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	084	095	101	051	080	088	
INTEGUMENTARY SYSTEM							
Subcutaneous tissue	N	+	+	+	N	+	*56
Fibroma			X				4
Fibrosarcoma							1
RESPIRATORY SYSTEM							
Lungs and bronchi	+	+	+	+	+	+	56
Trachea	+	+	+	+	+	+	56
Nasal cavity	+	+	+	+	+	+	*56
Carcinoma, NOS		X	X				26
Adenocarcinoma, NOS							2
Papillary adenoma		X					10
Rhabdomyosarcoma					X		2
Mixed tumor, malignant							1
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	56
Spleen	+	+	+	+	+	+	56
Hemangiosarcoma							1
Lymph nodes	+	+	+	+	+	+	54
Sarcoma, NOS, metastatic	X						1
Thymus	+	+	+	+	–	+	32
Thymoma, malignant							1
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	56
DIGESTIVE SYSTEM							
Oral cavity	N	N	N	N	N	N	*56
Sarcoma, NOS							1
Salivary gland	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	56
Neoplastic nodule							1
Bile duct	+	+	+	+	+	+	56
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	52
Esophagus	+	+	+	+	–	+	54
Stomach	+	+	+	+	+	+	56
Small intestine	+	+	+	+	+	+	56
Adenocarcinoma, NOS						X	1
Large intestine	+	+	+	+	+	+	55
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	56
Transitional cell carcinoma							1
Urinary bladder	+	+	+	+	+	+	51
Hemangiosarcoma							1
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	54
Adenoma, NOS	X						9
Adrenal	+	+	+	+	+	+	56
Pheochromocytoma			X				3
Thyroid	+	+	+	+	+	+	55
Follicular cell adenoma							2
C-cell adenoma							2
Parathyroid	+	+	+	+	+	+	53
Adenoma, NOS							2
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	N	+	+	*56
Testis	+	+	+	+	+	+	54
Interstitial cell tumor		X					1
Prostate	+	+	+	+	+	+	51
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	56
Carcinoma, NOS, metastatic			X				5
SPECIAL SENSE ORGANS							
Eye appendages	N	N	N	N	N	N	*56
Fibrosarcoma							1
Ear	+	N	N	N	N	N	*56
Sarcoma, NOS	X						1
Zymbal gland	+	N	N	N	N	N	*56
Carcinoma, NOS							1
BODY CAVITIES							
Peritoneum	N	N	N	N	N	N	*56
Fibroma							1
ALL OTHER SYSTEMS							
Multiple organs, NOS	N	N	N	N	N	N	*56
Malignant lymphoma, lymphocytic type				X			1
Malignant lymphoma, histiocytic type							2
Monocytic leukemia							1

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

	Control	300 ppm	1,000 ppm	3,000 ppm
Subcutaneous Tissue: Fibroma				
Overall Rates (a)	0/56 (0%)	1/56 (2%)	2/56 (4%)	4/56 (7%)
Adjusted Rates (b)	0.0%	2.2%	4.9%	22.1%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	1/33 (3%)	2/14 (14%)
Life Table Tests (d)	P=0.002	P=0.483	P=0.211	P=0.005
Incidental Tumor Tests (d)	P=0.058	P=0.617	P=0.322	P=0.042
Cochran-Armitage Trend Test (d)	P=0.029			
Fisher Exact Test		P=0.500	P=0.248	P=0.059
Subcutaneous Tissue: Fibroma or Neurofibroma				
Overall Rates (a)	0/56 (0%)	3/56 (5%)	2/56 (4%)	4/56 (7%)
Adjusted Rates (b)	0.0%	6.9%	4.9%	22.1%
Terminal Rates (c)	0/43 (0%)	1/40 (3%)	1/33 (3%)	2/14 (14%)
Life Table Tests (d)	P=0.011	P=0.109	P=0.211	P=0.005
Incidental Tumor Tests (d)	P=0.196	P=0.186	P=0.322	P=0.042
Cochran-Armitage Trend Test (d)	P=0.109			
Fisher Exact Test		P=0.122	P=0.248	P=0.059
Subcutaneous Tissue: Fibroma or Fibrosarcoma				
Overall Rates (a)	0/56 (0%)	2/56 (4%)	2/56 (4%)	5/56 (9%)
Adjusted Rates (b)	0.0%	4.4%	4.9%	26.4%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	1/33 (3%)	2/14 (14%)
Life Table Tests (d)	P<0.001	P=0.219	P=0.211	P=0.001
Incidental Tumor Tests (d)	P=0.101	P=0.371	P=0.322	P=0.035
Cochran-Armitage Trend Test (d)	P=0.022			
Fisher Exact Test		P=0.248	P=0.248	P=0.028
Subcutaneous Tissue: Fibromas, Neurofibroma, or Fibrosarcoma				
Overall Rates (a)	0/56 (0%)	4/56 (7%)	2/56 (4%)	5/56 (9%)
Adjusted Rates (b)	0.0%	9.0%	4.9%	26.4%
Terminal Rates (c)	0/43 (0%)	1/40 (3%)	1/33 (3%)	2/14 (14%)
Life Table Tests (d)	P=0.005	P=0.055	P=0.211	P=0.001
Incidental Tumor Tests (d)	P=0.246	P=0.116	P=0.322	P=0.035
Cochran-Armitage Trend Test (d)	P=0.078			
Fisher Exact Test		P=0.059	P=0.248	P=0.028
Nasal Cavity: Papillary Adenoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	2/56 (4%)	10/56 (18%)
Adjusted Rates (b)	0.0%	0.0%	4.9%	42.7%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	1/33 (3%)	4/14 (29%)
Life Table Tests (d)	P<0.001	(e)	P=0.209	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	P=0.322	P=0.001
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	P=0.248	P<0.001
Nasal Cavity: Carcinoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	0/56 (0%)	26/56 (46%)
Adjusted Rates (b)	0.0%	0.0%	0.0%	68.6%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	0/33 (0%)	5/14 (36%)
Life Table Tests (d)	P<0.001	(e)	(e)	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	(e)	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	(e)	P<0.001
Nasal Cavity: Adenocarcinoma or Carcinoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	0/56 (0%)	28/56 (50%)
Adjusted Rates (b)	0.0%	0.0%	0.0%	73.1%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	0/33 (0%)	6/14 (43%)
Life Table Tests (d)	P<0.001	(e)	(e)	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	(e)	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	(e)	P<0.001

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Control	300 ppm	1,000 ppm	3,000 ppm
Nasal Cavity: Adenoma, Adenocarcinoma, or Carcinoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	2/56 (4%)	33/56 (59%)
Adjusted Rates (b)	0.0%	0.0%	4.9%	81.8%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	1/33 (3%)	8/14 (57%)
Life Table Tests (d)	P<0.001	(e)	P=0.209	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	P=0.322	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	P=0.248	P<0.001
Hematopoietic System: Lymphoma				
Overall Rates (a)	0/56 (0%)	1/56 (2%)	0/56 (0%)	3/56 (5%)
Adjusted Rates (b)	0.0%	2.4%	0.0%	8.1%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	0/33 (0%)	0/14 (0%)
Life Table Tests (d)	P=0.014	P=0.490	(e)	P=0.082
Incidental Tumor Tests (d)	P=0.306	P=0.282	(e)	P=0.362
Cochran-Armitage Trend Test (d)	P=0.042			
Fisher Exact Test		P=0.500	(e)	P=0.122
Hematopoietic System: Lymphoma or Leukemia				
Overall Rates (a)	1/56 (2%)	2/56 (4%)	0/56 (0%)	4/56 (7%)
Adjusted Rates (b)	2.2%	4.2%	0.0%	9.7%
Terminal Rates (c)	0/43 (0%)	0/40 (0%)	0/33 (0%)	0/14 (0%)
Life Table Tests (d)	P=0.040	P=0.481	P=0.538N	P=0.111
Incidental Tumor Tests (d)	P=0.591	P=0.284	P=0.398N	P=0.613
Cochran-Armitage Trend Test (d)	P=0.090			
Fisher Exact Test		P=0.500	P=0.500N	P=0.182
Pituitary Gland: Adenoma				
Overall Rates (a)	9/55 (16%)	18/54 (33%)	13/54 (24%)	9/54 (17%)
Adjusted Rates (b)	17.5%	38.3%	31.1%	28.1%
Terminal Rates (c)	2/42 (5%)	11/38 (29%)	5/32 (16%)	1/14 (7%)
Life Table Tests (d)	P=0.274	P=0.035	P=0.132	P=0.190
Incidental Tumor Tests (d)	P=0.010N	P=0.056	P=0.457	P=0.049N
Cochran-Armitage Trend Test (d)	P=0.201N			
Fisher Exact Test		P=0.033	P=0.223	P=0.585
Pituitary Gland: Adenoma or Carcinoma				
Overall Rates (a)	11/55 (20%)	20/54 (37%)	15/54 (28%)	9/54 (17%)
Adjusted Rates (b)	20.6%	42.0%	36.2%	28.1%
Terminal Rates (c)	2/42 (5%)	12/38 (32%)	7/32 (22%)	1/14 (7%)
Life Table Tests (d)	P=0.392	P=0.042	P=0.127	P=0.331
Incidental Tumor Tests (d)	P=0.002N	P=0.080	P=0.472	P=0.006N
Cochran-Armitage Trend Test (d)	P=0.094N			
Fisher Exact Test		P=0.039	P=0.234	P=0.420N
Adrenal Gland: Cortical Adenoma				
Overall Rates (a)	1/56 (2%)	2/55 (4%)	3/56 (5%)	0/56 (0%)
Adjusted Rates (b)	2.3%	5.0%	9.1%	0.0%
Terminal Rates (c)	1/43 (2%)	2/40 (5%)	3/33 (9%)	0/14 (0%)
Life Table Tests (d)	P=0.607N	P=0.475	P=0.216	P=0.723N
Incidental Tumor Tests (d)	P=0.607N	P=0.475	P=0.216	P=0.723N
Cochran-Armitage Trend Test (d)	P=0.254N			
Fisher Exact Test		P=0.493	P=0.309	P=0.500N
Adrenal Gland: Cortical Carcinoma				
Overall Rates (a)	0/56 (0%)	3/55 (5%)	0/56 (0%)	0/56 (0%)
Adjusted Rates (b)	0.0%	6.7%	0.0%	0.0%
Terminal Rates (c)	0/43 (0%)	2/40 (5%)	0/33 (0%)	0/14 (0%)
Life Table Tests (d)	P=0.387N	P=0.116	(e)	(e)
Incidental Tumor Tests (d)	P=0.320N	P=0.137	(e)	(e)
Cochran-Armitage Trend Test (d)	P=0.254N			
Fisher Exact Test		P=0.118	(e)	(e)

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Control	300 ppm	1,000 ppm	3,000 ppm
Adrenal Gland: Cortical Adenoma or Carcinoma				
Overall Rates (a)	1/56 (2%)	5/55 (9%)	3/56 (5%)	0/56 (0%)
Adjusted Rates (b)	2.3%	11.6%	9.1%	0.0%
Terminal Rates (c)	1/43 (2%)	4/40 (10%)	3/33 (9%)	0/14 (0%)
Life Table Tests (d)	P=0.369N	P=0.093	P=0.216	P=0.723N
Incidental Tumor Tests (d)	P=0.328N	P=0.106	P=0.216	P=0.723N
Cochran-Armitage Trend Test (d)	P=0.111N			
Fisher Exact Test		P=0.099	P=0.309	P=0.500N
Adrenal Gland: Pheochromocytoma				
Overall Rates (a)	3/56 (5%)	4/55 (7%)	6/56 (11%)	3/56 (5%)
Adjusted Rates (b)	7.0%	9.3%	18.2%	18.1%
Terminal Rates (c)	3/43 (7%)	3/40 (7%)	6/33 (18%)	1/14 (7%)
Life Table Tests (d)	P=0.127	P=0.463	P=0.129	P=0.179
Incidental Tumor Tests (d)	P=0.350	P=0.497	P=0.129	P=0.508
Cochran-Armitage Trend Test (d)	P=0.495N			
Fisher Exact Test		P=0.490	P=0.245	P=0.661
Testis: Interstitial Cell Tumor				
Overall Rates (a)	5/56 (9%)	2/56 (4%)	3/56 (5%)	1/54 (2%)
Adjusted Rates (b)	11.0%	4.8%	9.1%	4.3%
Terminal Rates (c)	4/43 (9%)	1/40 (3%)	3/33 (9%)	0/14 (0%)
Life Table Tests (d)	P=0.446N	P=0.252N	P=0.505N	P=0.398N
Incidental Tumor Tests (d)	P=0.212N	P=0.185N	P=0.409N	P=0.177N
Cochran-Armitage Trend Test (d)	P=0.141N			
Fisher Exact Test		P=0.219N	P=0.358N	P=0.111N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatalymph The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or a lower incidence in a dosed group than in controls is indicated by (N).
(e) No P value is reported because no tumors were observed in the dosed and control groups.

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
Animals initially in study	56	56	56	56
Animals necropsied	56	56	56	56
Animals examined histopathologically	56	56	56	56
INTEGUMENTARY SYSTEM				
*Skin	(56)	(56)	(56)	(56)
Epidermal inclusion cyst			1 (2%)	
Inflammation, acute	2 (4%)	1 (2%)	3 (5%)	†5 (9%)
Inflammation, chronic			1 (2%)	1 (2%)
Hyperkeratosis			1 (2%)	†8 (14%)
*Subcutaneous tissue	(56)	(56)	(56)	(56)
Inflammation, NOS	1 (2%)			
Inflammation, acute				1 (2%)
Abscess, NOS	1 (2%)			1 (2%)
RESPIRATORY SYSTEM				
*Nasal cavity	(56)	(56)	(56)	(56)
Inflammation, acute	12 (21%)	21 (38%)	32 (57%)	42 (75%)
Inflammation, acute/chronic		1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic			5 (9%)	
Postmortem change		1 (2%)	2 (4%)	2 (4%)
Hyperplasia, epithelial			1 (2%)	4 (7%)
Metaplasia, squamous				5 (9%)
#Trachea	(56)	(56)	(55)	(56)
Inflammation, acute				2 (4%)
Inflammation, chronic	1 (2%)			1 (2%)
Postmortem change			1 (2%)	2 (4%)
#Lung	(56)	(56)	(55)	(56)
Mineralization	1 (2%)		1 (2%)	
Atelectasis		1 (2%)		
Congestion, NOS	15 (27%)	14 (25%)	16 (29%)	21 (38%)
Hemorrhage		1 (2%)	6 (11%)	7 (13%)
Bronchopneumonia, acute			1 (2%)	1 (2%)
Inflammation, acute focal				2 (4%)
Inflammation, chronic		2 (4%)	1 (2%)	3 (5%)
Inflammation, chronic focal	1 (2%)	1 (2%)		
Alveolar macrophages	30 (54%)	22 (39%)	21 (38%)	19 (34%)
Hyperplasia, adenomatous	1 (2%)	2 (4%)	4 (7%)	1 (2%)
HEMATOPOIETIC SYSTEM				
#Spleen	(56)	(56)	(56)	(56)
Congestion, NOS		1 (2%)		
Postmortem change			1 (2%)	1 (2%)
Infarct, NOS				1 (2%)
Hemosiderosis	50 (89%)	48 (86%)	48 (86%)	46 (82%)
Lymphoid depletion	1 (2%)	2 (4%)	1 (2%)	3 (5%)
Angiectasis				1 (2%)
Hematopoiesis	50 (89%)	48 (86%)	48 (86%)	39 (70%)
#Lymph node	(54)	(55)	(53)	(54)
Congestion, NOS		1 (2%)		
Pigmentation, NOS		1 (2%)		
Hyperplasia, lymphoid		1 (2%)		
#Mandibular lymph node	(54)	(55)	(53)	(54)
Congestion, NOS				1 (2%)
Inflammation, chronic			1 (2%)	
Hyperplasia, lymphoid	8 (15%)		2 (4%)	2 (4%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
HEMATOPOIETIC SYSTEM (Continued)				
#Mediastinal lymph node	(54)	(55)	(53)	(54)
Congestion, NOS		1 (2%)		
Hemorrhage		1 (2%)		
Pigmentation, NOS		1 (2%)		
Hyperplasia, lymphoid		1 (2%)		
#Mesenteric lymph node	(54)	(55)	(53)	(54)
Congestion, NOS		1 (2%)	3 (6%)	1 (2%)
Inflammation, acute		1 (2%)		
Pigmentation, NOS	4 (7%)	2 (4%)		3 (6%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
#Lung	(56)	(56)	(55)	(56)
Leukemoid reaction			1 (2%)	
#Liver	(56)	(56)	(56)	(56)
Hematopoiesis			1 (2%)	1 (2%)
#Ileum	(55)	(56)	(54)	(56)
Hyperplasia, lymphoid			1 (2%)	
#Thymus	(36)	(36)	(37)	(32)
Involution, NOS	32 (89%)	35 (97%)	37 (100%)	26 (81%)
CIRCULATORY SYSTEM				
*Abdominal cavity	(56)	(56)	(56)	(56)
Periarteritis			1 (2%)	
#Lymph node	(54)	(55)	(53)	(54)
Lymphangiectasis				1 (2%)
#Mandibular lymph node	(54)	(55)	(53)	(54)
Lymphangiectasis			1 (2%)	1 (2%)
#Mediastinal lymph node	(54)	(55)	(53)	(54)
Lymphangiectasis	1 (2%)	1 (2%)		1 (2%)
#Mesenteric lymph node	(54)	(55)	(53)	(54)
Lymphangiectasis	7 (13%)	1 (2%)	1 (2%)	3 (6%)
#Heart	(56)	(56)	(55)	(56)
Mineralization	1 (2%)	1 (2%)		1 (2%)
Fibrosis, focal	4 (7%)	5 (9%)	9 (16%)	3 (5%)
#Myocardium	(56)	(56)	(55)	(56)
Inflammation, chronic focal	6 (11%)	5 (9%)	5 (9%)	7 (13%)
#Endocardium	(56)	(56)	(55)	(56)
Inflammation, NOS				1 (2%)
#Pancreas	(56)	(55)	(56)	(52)
Periarteritis			2 (4%)	
#Colon	(56)	(56)	(55)	(55)
Periarteritis		1 (2%)		1 (2%)
#Testis	(56)	(56)	(56)	(54)
Periarteritis	1 (2%)		3 (5%)	1 (2%)
#Adrenal	(56)	(55)	(56)	(56)
Thrombosis, NOS			1 (2%)	
DIGESTIVE SYSTEM				
*Intestinal tract	(56)	(56)	(56)	(56)
Inflammation, NOS				1 (2%)
Postmortem change				1 (2%)
#Liver	(56)	(56)	(56)	(56)
Congestion, NOS	10 (18%)	1 (2%)	1 (2%)	14 (25%)
Inflammation, acute focal				1 (2%)
Inflammation, chronic focal	1 (2%)	3 (5%)	4 (7%)	
Inflammation, chronic diffuse			1 (2%)	
Degeneration, cystic	2 (4%)	5 (9%)	3 (5%)	3 (5%)
Necrosis, focal	2 (4%)	2 (4%)	1 (2%)	3 (5%)
Postmortem change	2 (4%)			

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
DIGESTIVE SYSTEM				
#Liver (Continued)				
Cytoplasmic vacuolization	1 (2%)	9 (16%)	7 (13%)	4 (7%)
Basophilic cyto change	2 (4%)	2 (4%)	3 (5%)	6 (11%)
Eosinophilic cyto change	2 (4%)	4 (7%)	8 (14%)	8 (14%)
Clear cell change	3 (5%)	8 (14%)	2 (4%)	1 (2%)
Angiectasis		3 (5%)	2 (4%)	2 (4%)
#Hepatic capsule	(56)	(56)	(56)	(56)
Inflammation, chronic		1 (2%)		
#Bile duct	(56)	(56)	(56)	(56)
Cyst, NOS	2 (4%)	1 (2%)		
Hyperplasia, NOS	14 (25%)	17 (30%)	16 (29%)	12 (21%)
#Pancreas	(56)	(55)	(56)	(52)
Inflammation, acute focal		1 (2%)		
Inflammation, chronic		2 (4%)		
Postmortem change				1 (2%)
#Pancreatic acinus	(56)	(55)	(56)	(52)
Degeneration, NOS				1 (2%)
Atrophy, NOS	5 (9%)	2 (4%)	2 (4%)	8 (15%)
Hyperplasia, focal				1 (2%)
#Esophageal musculari	(56)	(56)	(54)	(54)
Inflammation, chronic		1 (2%)		
#Stomach	(56)	(56)	(56)	(56)
Mineralization	1 (2%)		2 (4%)	
Epidermal inclusion cyst		1 (2%)	1 (2%)	
Ulcer, NOS			1 (2%)	1 (2%)
Inflammation, acute		4 (7%)	2 (4%)	6 (11%)
Inflammation, acute/chronic	2 (4%)			
Inflammation, chronic	2 (4%)		3 (5%)	
Erosion			1 (2%)	
Hyperkeratosis	2 (4%)	3 (5%)	6 (11%)	8 (14%)
Acanthosis	4 (7%)	3 (5%)	6 (11%)	8 (14%)
#Gastric mucosa	(56)	(56)	(56)	(56)
Cyst, NOS	1 (2%)			
#Duodenum	(55)	(56)	(54)	(56)
Congestion, NOS				1 (2%)
Postmortem change	1 (2%)	2 (4%)	4 (7%)	5 (9%)
#Jejunum	(55)	(56)	(54)	(56)
Postmortem change	1 (2%)			
#Ileum	(55)	(56)	(54)	(56)
Cyst, NOS	1 (2%)			
Inflammation, acute	1 (2%)			
Postmortem change	1 (2%)			
#Colon	(56)	(56)	(55)	(55)
Parasitism	1 (2%)	2 (4%)	2 (4%)	
Postmortem change			4 (7%)	1 (2%)
#Colonic serosa	(56)	(56)	(55)	(55)
Granuloma, NOS			1 (2%)	
Inflammation, pyogranulomatous	1 (2%)			
#Cecum	(56)	(56)	(55)	(55)
Postmortem change	1 (2%)			
URINARY SYSTEM				
#Kidney	(56)	(56)	(56)	(56)
Hydronephrosis	1 (2%)			
Congestion, NOS	1 (2%)			
Abscess, NOS			2 (4%)	
Nephropathy	42 (75%)	46 (82%)	40 (71%)	42 (75%)
Degeneration, hyaline		2 (4%)	2 (4%)	2 (4%)
Postmortem change			1 (2%)	2 (4%)
Infarct, healed	1 (2%)		2 (4%)	
Hyperplasia, tubular cell	1 (2%)		1 (2%)	

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
URINARY SYSTEM (Continued)				
#Kidney/cortex	(56)	(56)	(56)	(56)
Mineralization		1 (2%)		1 (2%)
Cyst, NOS	1 (2%)	4 (7%)	2 (4%)	
#Kidney/pelvis	(56)	(56)	(56)	(56)
Mineralization	2 (4%)	2 (4%)	3 (5%)	3 (5%)
Hemorrhage	1 (2%)			
Inflammation, acute	3 (5%)	1 (2%)	3 (5%)	
Inflammation, acute/chronic			1 (2%)	
Inflammation, chronic		1 (2%)	1 (2%)	1 (2%)
#Urinary bladder	(56)	(56)	(55)	(51)
Congestion, NOS				1 (2%)
Inflammation, acute		1 (2%)	1 (2%)	2 (4%)
Inflammation, acute/chronic			1 (2%)	
Inflammation, chronic		1 (2%)	1 (2%)	1 (2%)
Postmortem change		1 (2%)		
Hyperplasia, epithelial				1 (2%)
Polyp, inflammatory		1 (2%)		
ENDOCRINE SYSTEM				
#Pituitary	(55)	(54)	(54)	(54)
Cyst, NOS	7 (13%)	4 (7%)	1 (2%)	1 (2%)
Hemorrhage	7 (13%)	5 (9%)	1 (2%)	
Hyperplasia, focal	11 (20%)	5 (9%)	5 (9%)	5 (9%)
#Adrenal cortex	(56)	(55)	(56)	(56)
Cyst, NOS		1 (2%)		
Cytoplasmic vacuolization	25 (45%)	13 (24%)	11 (20%)	8 (14%)
Atrophy, NOS		2 (4%)		
Hyperplasia, focal	13 (23%)	11 (20%)	11 (20%)	7 (13%)
#Adrenal medulla	(56)	(55)	(56)	(56)
Hyperplasia, epithelial	1 (2%)			
Hyperplasia, focal	6 (11%)	2 (4%)	4 (7%)	3 (5%)
#Thyroid	(55)	(55)	(55)	(55)
Ultimobranchial cyst	2 (4%)	4 (7%)	2 (4%)	2 (4%)
Cystic follicles			1 (2%)	
Inflammation, chronic		1 (2%)		1 (2%)
Hyperplasia, C-cell		4 (7%)	5 (9%)	1 (2%)
Hyperplasia, follicular cell	1 (2%)		2 (4%)	
#Parathyroid	(52)	(54)	(52)	(53)
Hyperplasia, focal	2 (4%)	2 (4%)		
Hyperplasia, diffuse	4 (8%)	1 (2%)	2 (4%)	
REPRODUCTIVE SYSTEM				
*Mammary gland	(56)	(56)	(56)	(56)
Galactocele	1 (2%)			
Hyperplasia, cystic	1 (2%)	1 (2%)	2 (4%)	1 (2%)
*Prepuce	(56)	(56)	(56)	(56)
Inflammation, acute				1 (2%)
*Preputial gland	(56)	(56)	(56)	(56)
Cystic ducts	10 (18%)	4 (7%)		
Inflammation, acute	1 (2%)			
Abscess, NOS	2 (4%)			
Inflammation, chronic	3 (5%)			
#Prostate	(55)	(55)	(56)	(51)
Inflammation, acute	8 (15%)	13 (24%)	7 (13%)	9 (18%)
Inflammation, acute/chronic	1 (2%)	2 (4%)	8 (14%)	3 (6%)
Inflammation, chronic	2 (4%)		2 (4%)	3 (6%)
Hyperplasia, focal				1 (2%)
*Seminal vesicle	(56)	(56)	(56)	(56)
Distention			1 (2%)	

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
REPRODUCTIVE SYSTEM (Continued)				
#Testis	(56)	(56)	(56)	(54)
Infarct, acute				1 (2%)
Atrophy, NOS	12 (21%)	7 (13%)	12 (21%)	5 (9%)
Hyperplasia, interstitial cell		1 (2%)	1 (2%)	
*Epididymis	(56)	(56)	(56)	(56)
Necrosis, fat			1 (2%)	
NERVOUS SYSTEM				
#Brain	(56)	(55)	(56)	(56)
Mineralization	1 (2%)		1 (2%)	
Hemorrhage			1 (2%)	
Gliosis			1 (2%)	1 (2%)
SPECIAL SENSE ORGANS				
*Eye	(56)	(56)	(56)	(56)
Inflammation, NOS			1 (2%)	
Cataract	1 (2%)			
Postmortem change				1 (2%)
*Eye anterior chamber	(56)	(56)	(56)	(56)
Empyema				1 (2%)
*Eye/cornea	(56)	(56)	(56)	(56)
Inflammation, acute	2 (4%)			
*Eyelid	(56)	(56)	(56)	(56)
Abscess, NOS	1 (2%)			
Hyperkeratosis	1 (2%)			4 (7%)
*Nasolacrimal duct	(56)	(56)	(56)	(56)
Inflammation, acute	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic	2 (4%)	3 (5%)	13 (23%)	4 (7%)
Metaplasia, squamous		1 (2%)		
*Ear	(56)	(56)	(56)	(56)
Abscess, NOS	1 (2%)			
Inflammation, chronic				1 (2%)
Inflammation, pyogranulomatous	1 (2%)		1 (2%)	
Fibrosis	1 (2%)			
Osteochondritis dissecans	1 (2%)			
Hyperkeratosis				1 (2%)
Metaplasia, osseous	1 (2%)			1 (2%)
*Auricular cartilage	(56)	(56)	(56)	(56)
Degeneration, NOS			1 (2%)	
Necrosis, NOS	1 (2%)			
Hyperplasia, NOS	2 (4%)			2 (4%)
MUSCULOSKELETAL SYSTEM				
*Maxilla	(56)	(56)	(56)	(56)
Fibrous osteodystrophy	2 (4%)			
*Sternum	(56)	(56)	(56)	(56)
Fibrous osteodystrophy	2 (4%)			
*Cartilage, NOS	(56)	(56)	(56)	(56)
Necrosis, fat				1 (2%)
BODY CAVITIES				
*Abdominal cavity	(56)	(56)	(56)	(56)
Hemorrhage		1 (2%)		
Hematoma, NOS			1 (2%)	
Abscess, NOS	1 (2%)			
Necrosis, fat			1 (2%)	

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
BODY CAVITIES (Continued)				
*Peritoneum	(56)	(56)	(56)	(56)
Inflammation, chronic		1 (2%)		
Inflammation, pyogranulomatous	1 (2%)			
ALL OTHER SYSTEMS				
*Multiple organs	(56)	(56)	(56)	(56)
Mineralization	1 (2%)			
Postmortem change		5 (9%)	7 (13%)	12 (21%)
SPECIAL MORPHOLOGY SUMMARY				
None				

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site
† Multiple occurrence of morphology in the same organ; tissue is counted only once.

APPENDIX B

SUMMARY OF LESIONS IN FEMALE CD RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
Animals initially in study	56	56	56	56
Animals necropsied	56	56	56	56
Animals examined histopathologically	56	56	56	56
INTEGUMENTARY SYSTEM				
*Multiple organs	(56)	(56)	(56)	(56)
Fibrous histiocytoma, malignant	1 (2%)			
*Subcutaneous tissue	(56)	(56)	(56)	(56)
Fibroma		2 (4%)	1 (2%)	4 (7%)
Fibrosarcoma	1 (2%)	1 (2%)	1 (2%)	3 (5%)
Fibrous histiocytoma, malignant		1 (2%)		
Lipoma				1 (2%)
#Lung	(56)	(56)	(55)	(56)
Fibrous histiocytoma, malignant		1 (2%)		
RESPIRATORY SYSTEM				
*Nasal cavity	(56)	(56)	(56)	(56)
Carcinoma, NOS			1 (2%)	24 (43%)
Adenoma, NOS			1 (2%)	
Papillary adenoma				6 (11%)
Sarcoma, NOS				1 (2%)
Rhabdomyosarcoma				2 (4%)
Mixed tumor, malignant				1 (2%)
#Lung	(56)	(56)	(55)	(56)
Carcinoma, NOS, metastatic	2 (4%)		1 (2%)	
Undifferentiated carcinoma metastatic			1 (2%)	
Adenocarcinoma, NOS, metastatic	1 (2%)			
Mixed tumor, metastatic			1 (2%)	
HEMATOPOIETIC SYSTEM				
*Multiple organs	(56)	(56)	(56)	(56)
Malignant lymphoma, histiocytic type		1 (2%)		
#Mandibular lymph node	(55)	(52)	(56)	(54)
Carcinoma, NOS, metastatic				1 (2%)
#Thymus	(31)	(42)	(37)	(29)
Undifferentiated carcinoma			1 (3%)	
CIRCULATORY SYSTEM				
None				
DIGESTIVE SYSTEM				
#Liver	(56)	(56)	(56)	(55)
Neoplastic nodule		1 (2%)	2 (4%)	4 (7%)
Hepatocellular carcinoma	1 (2%)		1 (2%)	1 (2%)
URINARY SYSTEM				
None				
ENDOCRINE SYSTEM				
#Pituitary	(56)	(55)	(56)	(56)
Carcinoma, NOS	4 (7%)	1 (2%)	1 (2%)	2 (4%)
Adenoma, NOS	29 (52%)	32 (58%)	30 (54%)	20 (36%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
ENDOCRINE SYSTEM (Continued)				
#Adrenal	(56)	(56)	(55)	(55)
Cortical adenoma	7 (13%)	9 (16%)	6 (11%)	12 (22%)
Cortical carcinoma	1 (2%)			
Pheochromocytoma	2 (4%)	1 (2%)		
#Thyroid	(54)	(54)	(55)	(54)
Follicular cell adenoma	2 (4%)			
Follicular cell carcinoma				1 (2%)
C-cell adenoma	1 (2%)	1 (2%)	3 (5%)	3 (6%)
C-cell carcinoma		3 (6%)	2 (4%)	
#Pancreatic islets	(56)	(56)	(56)	(54)
Islet cell adenoma	2 (4%)			1 (2%)
Islet cell carcinoma			1 (2%)	
REPRODUCTIVE SYSTEM				
*Mammary gland	(56)	(56)	(56)	(56)
Adenocarcinoma, NOS	6 (11%)	6 (11%)	5 (9%)	3 (5%)
Cystadenoma, NOS		3 (5%)		
Mixed tumor, malignant			1 (2%)	
Fibroadenoma	19 (34%)	19 (34%)	20 (36%)	24 (43%)
#Uterus	(55)	(56)	(56)	(54)
Papillary adenoma			1 (2%)	
Endometrial stromal polyp	3 (5%)	2 (4%)	2 (4%)	1 (2%)
Endometrial stromal sarcoma	1 (2%)	1 (2%)		
#Cervix uteri	(55)	(56)	(56)	(54)
Fibroma			1 (2%)	
#Ovary	(55)	(56)	(56)	(55)
Granulosa cell tumor			1 (2%)	
NERVOUS SYSTEM				
#Brain	(56)	(56)	(56)	(56)
Carcinoma, NOS, metastatic				7 (13%)
Squamous cell carcinoma, metastatic				1 (2%)
Astrocytoma				1 (2%)
Oligodendroglioma			1 (2%)	
SPECIAL SENSE ORGANS				
*Ear	(56)	(56)	(56)	(56)
Sarcoma, NOS	1 (2%)			
Osteosarcoma	1 (2%)	1 (2%)		
MUSCULOSKELETAL SYSTEM				
None				
BODY CAVITIES				
*Abdominal cavity	(56)	(56)	(56)	(56)
Fibroma	1 (2%)			
*Mesentery	(56)	(56)	(56)	(56)
Paraganglioma, NOS		1 (2%)		
ALL OTHER SYSTEMS				
None				

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
ANIMAL DISPOSITION SUMMARY				
Animals initially in study	56	56	56	56
Natural death@	9	17	10	19
Moribund sacrifice	14	15	16	14
Terminal sacrifice	33	24	30	23
TUMOR SUMMARY				
Total animals with primary tumors**	44	50	50	53
Total primary tumors	83	86	83	115
Total animals with benign tumors	38	45	46	40
Total benign tumors	66	69	65	72
Total animals with malignant tumors	17	11	15	33
Total malignant tumors	17	15	15	39
Total animals with secondary tumors##	3		3	8
Total secondary tumors	3		3	9
Total animals with tumors-- uncertain benign or malignant		2	3	4
Total uncertain tumors		2	3	4

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ
@ Includes autolyzed animals

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: UNTREATED CONTROL

ANIMAL NUMBER	54 41	54 42	54 43	54 44	54 45	54 46	54 47	54 48	54 49	55 50	55 51	55 52	55 53	55 54	55 55	55 56	55 57	55 58	55 59	56 60	56 61	56 62	56 63	56 64	56 65
WEEKS ON STUDY	07 74	09 74	09 75	07 78	10 72	06 75	07 78	10 72	10 72	10 72	10 72	10 72	07 74	07 78	08 77	07 77	07 78	10 72	10 72	10 72	10 72	10 72	10 72	10 72	10 72
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	N	+	N	+	N	+	N	N	N	N	N	+	+	+	N	+	N	N	N	N	N	N	N	+
Fibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic				X											X										
Adenocarcinoma, NOS, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	+	+	+	−	−	+	−	+	+	+	+	+	+	−	−	−	−	−	+	−	−	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS						X									X										
Adenoma, NOS	X	X			X		X			X	X			X		X		X	X			X		X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma										X	X														
Cortical carcinoma																									
Pheochromocytoma							X																		
Thyroid	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																						X			
C-cell adenoma		X																							
Parathyroid	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									X
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+
Adenocarcinoma, NOS		X								X															
Fibroadenoma	X	X								X		X	X						X		X	X			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp															X										X
Endometrial stromal sarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Ear	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	+	N	+	N	N	+
Sarcoma, NOS																				X					
Osteosarcoma																						X			
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant																									

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	56601	56701	56801	56901	57001	57101	57201	57301	57401	57501	57601	57701	57801	57901	58001	58101	58201	58301	58401	58501	58601	58701	58801	58901	59001
WEEKS ON STUDY	10002	08009	10002	06000	10002	10002	10002	04001	10002	08002	10002	09009	10002	10002	10002	10002	07808	10002	08006	10002	10002	10002	09006	10002	10002
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma					X																				
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Adenocarcinoma, NOS, metastatic								X																	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	+	+	−	−	+	+	−	−	−	−	+	+	+	+	+	+	−	−	+	+	+	−	+	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma											X														
Bile duct	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																								X	
Adenoma, NOS	X	X	X			X				X				X		X	X	X	X		X	X	X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Cortical adenoma	X	X																X			X				
Cortical carcinoma																		X							
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																								X	
C-cell adenoma																									
Parathyroid	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																							X		
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS	X							X													X	X			
Fibroadenoma	X	X	X			X			X			X	X			X		X							
Uterus	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp												X													
Endometrial stromal sarcoma														X											
Ovary	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Ear	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N
Sarcoma, NOS																									
Osteosarcoma																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma						X																			
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant																			X						

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	59 11	59 92	59 93	59 94	59 95	59 96	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	10 02	09 05	07 02	08 09	10 02	07 08	
INTEGUMENTARY SYSTEM							
Subcutaneous tissue	N	N	N	N	N	+	*56
Fibrosarcoma							1
RESPIRATORY SYSTEM							
Lungs and bronchi	+	+	+	+	+	+	56
Carcinoma, NOS, metastatic							2
Adenocarcinoma, NOS, metastatic							1
Trachea	+	+	+	+	+	+	56
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	55
Spleen	+	+	+	+	+	+	56
Lymph nodes	+	+	+	+	+	+	55
Thymus	+	−	+	−	+	+	31
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	56
DIGESTIVE SYSTEM							
Salivary gland	−	+	+	+	+	+	55
Liver	+	+	+	+	+	+	56
Hepatocellular carcinoma							1
Bile duct	+	+	+	+	+	+	56
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	56
Esophagus	+	+	+	+	+	+	56
Stomach	+	+	+	+	+	+	56
Small intestine	+	+	+	+	+	+	56
Large intestine	+	+	+	+	+	+	56
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	56
Urinary bladder	+	+	+	+	+	+	54
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	56
Carcinoma, NOS							4
Adenoma, NOS				X	X	X	29
Adrenal	+	+	+	+	+	+	56
Cortical adenoma					X		7
Cortical carcinoma							1
Pheochromocytoma	X						2
Thyroid	+	+	+	+	+	−	54
Follicular cell adenoma							2
C-cell adenoma							1
Parathyroid	+	+	+	+	+	−	52
Pancreatic islets	+	+	+	+	+	+	56
Islet cell adenoma							2
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	+	+	+	*56
Adenocarcinoma, NOS							6
Fibroadenoma	X				X		19
Uterus	+	+	+	+	+	+	55
Endometrial stromal polyp							3
Endometrial stromal sarcoma							1
Ovary	+	+	+	+	+	+	55
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	56
SPECIAL SENSE ORGANS							
Ear	N	N	N	N	N	N	*56
Sarcoma, NOS							1
Osteosarcoma							1
BODY CAVITIES							
Peritoneum	N	N	N	N	N	N	*56
Fibroma							1
ALL OTHER SYSTEMS							
Multiple organs, NOS	N	N	N	N	N	N	*56
Fibrous histiocytoma, malignant							1

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: 300 ppm

ANIMAL NUMBER	65 3	65 4	65 5	65 6	65 7	65 8	65 9	66 0	66 1	66 2	66 3	66 4	66 5	66 6	66 7	66 8	66 9	67 0	67 1	67 2	67 3	67 4	67 5	67 6	67 7
WEEKS ON STUDY	102	091	102	102	102	091	102	071	090	093	102	096	085	073	097	102	102	102	053	073	093	091	094	102	069
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	N	N	+	N	N	N	+	N	N	+	N	N	N	N	N	N	N	+	N	N	+	
Fibroma																									
Fibrous histiocytoma, malignant																								X	
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Fibrous histiocytoma, malignant																								X	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	
Thymus	+	+	+	−	+	+	−	−	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	−	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Neoplastic nodule					X																				
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	
Stomach	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
Carcinoma, NOS																									
Adenoma, NOS	X	X	X	X	X	X	X	X				X	X	X	X		X	X	X	X		X		X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cortical adenoma				X			X								X										
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
C-cell adenoma																									
C-cell carcinoma																	X								
Parathyroid	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenocarcinoma, NOS										X		X						X						X	
Cystadenoma, NOS	X					X																			
Fibroadenoma				X					X		X							X			X		X		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Endometrial stromal polyp											X														
Endometrial stromal sarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SPECIAL SENSE ORGANS																									
Ear	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Osteosarcoma																									
BODY CAVITIES																									
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Paraganglioma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Malignant lymphoma, histiocytic type										X															

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 300 ppm (Continued)

ANIMAL NUMBER	6787	6790	6801	6811	6823	6834	6855	6866	6877	6888	6899	6901	6911	6922	6933	6944	6955	6966	6977	6988	6999	7001	7012
WEEKS ON STUDY	079	093	102	102	102	102	102	068	097	092	071	097	102	088	100	102	102	102	099	071	083	074	102
INTEGUMENTARY SYSTEM																							
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N
Fibroma											X														
Fibrous histiocytoma, malignant																									
RESPIRATORY SYSTEM																							
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, malignant																									
Trachea	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																							
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																							
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																							
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																							
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS		X	X							X			X	X			X	X	X		X	X	X		X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma	X		X					X																	X
Pheochromocytoma										X															
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
C-cell adenoma			X																						
C-cell carcinoma																			X						
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																							
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS										X															
Cystadenoma, NOS																									
Fibroadenoma	X	X				X						X				X	X			X	X		X	X	X
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp			X																						
Endometrial stromal sarcoma															X										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																							
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																							
Ear	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Osteosarcoma				X																					
BODY CAVITIES																							
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Paraganglioma, NOS																									
ALL OTHER SYSTEMS																							
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, histiocytic type																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: *300 ppm*
(Continued)

ANIMAL NUMBER	70003	70004	70005	70006	70007	70008	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	099	102	074	102	087	102	
INTEGUMENTARY SYSTEM							
Subcutaneous tissue	N	+	+	N	N	N	*56
Fibroma							2
Fibrous histiocytoma, malignant							1
RESPIRATORY SYSTEM							
Fibrous histiocytoma, malignant	+	+	+	+	+	+	56
Trachea	+	+	+	+	+	+	55
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	56
Spleen	+	+	+	+	+	+	56
Lymph nodes	+	+	+	+	+	+	52
Thymus	+	+	−	−	−	+	42
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	56
DIGESTIVE SYSTEM							
Salivary gland	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	56
Neoplastic nodule							1
Bile duct	+	+	+	+	+	+	56
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	56
Esophagus	+	+	−	+	+	+	53
Stomach	+	+	+	+	+	+	55
Small intestine	+	+	+	+	+	+	56
Large intestine	+	+	+	+	+	+	55
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	56
Urinary bladder	+	+	+	+	+	+	54
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	55
Carcinoma, NOS	X						1
Adenoma, NOS		X		X			32
Adrenal	+	+	+	+	+	+	56
Cortical adenoma		X		X			9
Pheochromocytoma							1
Thyroid	+	+	+	+	+	+	54
C-cell adenoma							1
C-cell carcinoma				X			3
Parathyroid	+	+	+	+	+	+	51
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	+	+	+	*56
Adenocarcinoma, NOS							6
Cystadenoma, NOS							3
Fibroadenoma							19
Uterus							56
Endometrial stromal polyp							2
Endometrial stromal sarcoma							1
Ovary	+	+	+	+	+	+	56
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	56
SPECIAL SENSE ORGANS							
Ear	N	N	N	N	N	N	*56
Osteosarcoma							1
BODY CAVITIES							
Mesentery	N	N	N	N	N	N	*56
Paraganglioma, NOS					X		1
ALL OTHER SYSTEMS							
Multiple organs, NOS	N	N	N	N	N	N	*56
Malignant lymphoma, histiocytic type							1

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: 1,000 ppm

ANIMAL NUMBER	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789
WEEKS ON STUDY	102	102	069	102	083	102	090	102	102	102	074	094	097	102	095	083	095	102	102	102	095	089	102	102	073
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	+	N	N	N	N	N	N	N	+	N	N	N	+	N	N	N	N	N	N	N	N	N	+
Fibroma																									
Fibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Carcinoma, NOS, metastatic					X																				
Undifferentiated carcinoma metastatic															X										
Mixed tumor, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS																									
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	−	+	+	−	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	+	+	+
Undifferentiated carcinoma															X										
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS		X			X			X	X	X		X	X	X		X		X		X				X	
Adrenal	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																	X								
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
C-cell adenoma							X		X																
C-cell carcinoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma																						X			
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Adenocarcinoma, NOS		X							X	X						X		X							
Mixed tumor, malignant																									
Fibroadenoma				X			X			X	X	X	X		X	X	X				X	X	X		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary adenoma																			X						
Fibroma																									
Endometrial stromal polyp				X																					
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma																				X					

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 1,000 ppm (Continued)

ANIMAL NUMBER	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814
WEEKS ON STUDY	102	085	102	071	102	102	102	102	090	100	102	102	090	102	102	102	102	102	071	102	102	059	071	102	102
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	+	N	N	N	N	N	N	+	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma		X																							
Fibrosarcoma													X												
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Undifferentiated carcinoma metastatic																									
Mixed tumor, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																						X			
Adenoma, NOS																		X							
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	−	+	+	−	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+
Undifferentiated carcinoma																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule												X						X							
Hepatocellular carcinoma																X									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																							X		
Adenoma, NOS	X	X		X	X	X		X	X	X		X					X	X		X	X				X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma			X													X		X		X					
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma									X																
C-cell carcinoma								X	X																
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Adenocarcinoma, NOS																									
Mixed tumor, malignant																									
Fibroadenoma					X		X							X							X			X	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary adenoma																									
Fibroma																						X			
Endometrial stromal polyp																	X								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor											X														
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 1,000 ppm (Continued)

ANIMAL NUMBER	81 15	81 16	81 17	81 18	81 19	82 20	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	09 99	09 98	09 92	09 97	10 02	10 02	
INTEGUMENTARY SYSTEM							
Subcutaneous tissue	N	+	N	+	N	N	*56
Fibroma							1
Fibrosarcoma							1
RESPIRATORY SYSTEM							
Lungs and bronchi	–	+	+	+	+	+	55
Carcinoma, NOS, metastatic							1
Undifferentiated carcinoma metastatic							1
Mixed tumor, metastatic			X				1
Trachea	+	+	+	+	+	+	54
Nasal cavity	+	+	+	+	+	+	*56
Carcinoma, NOS							1
Adenoma, NOS							1
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	56
Spleen	+	+	+	+	+	+	56
Lymph nodes	+	+	+	+	+	+	56
Thymus	–	+	+	–	–	–	37
Undifferentiated carcinoma							1
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	56
DIGESTIVE SYSTEM							
Salivary gland	+	+	+	+	+	+	54
Liver	+	+	+	+	+	+	56
Neoplastic nodule							2
Hepatocellular carcinoma							1
Bile duct	+	+	+	+	+	+	56
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	56
Esophagus	+	+	+	+	+	+	55
Stomach	+	+	+	+	+	+	56
Small intestine	+	+	+	+	+	+	56
Large intestine	+	+	+	+	+	+	56
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	56
Urinary bladder	+	+	+	+	+	+	53
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	56
Carcinoma, NOS							1
Adenoma, NOS		X		X	X	X	30
Adrenal	+	+	+	+	+	+	55
Cortical adenoma		X					6
Thyroid	+	+	+	+	+	+	55
C-cell adenoma							3
C-cell carcinoma							2
Parathyroid	+	+	+	+	+	+	55
Pancreatic islets	+	+	+	+	+	+	56
Islet cell carcinoma							1
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	+	+	+	*56
Adenocarcinoma, NOS							5
Mixed tumor, malignant			X				1
Fibroadenoma	X	X				X	20
Uterus	+	+	+	+	+	+	56
Papillary adenoma							1
Fibroma							1
Endometrial stromal polyp							2
Ovary	+	–	+	+	+	+	56
Granulosa cell tumor							1
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	56
Oligodendroglioma							1

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE: 3,000 ppm

ANIMAL NUMBER	87701	87802	87902	88001	88101	88202	88302	88404	88505	88606	88707	88808	88902	89001	89101	89202	89303	89404	89505	89606	89707	89808	89909	90002	90101
WEEKS ON STUDY	102	071	102	091	081	073	090	096	094	102	077	073	102	091	099	102	088	102	060	102	003	102	102	102	102
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	N	+	N	N	N	N	+	+	N	+	N	N	N	+	N	N	N	N	N	N	N	N	N	N
Fibroma			X					X																	
Fibrosarcoma	X		X																						
Lipoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS	X			X	X	X		X					X	X								X		X	
Papillary adenoma		X		X					X		X														
Sarcoma, NOS																									X
Rhabdomyosarcoma																			X						
Mixed tumor, malignant																									
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Carcinoma, NOS, metastatic																									
Thymus	+	+	+	−	+	−	−	−	−	−	+	+	+	−	+	+	+	+	+	+	−	−	−	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Neoplastic nodule													X					X							
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS								X																	
Adenoma, NOS		X	X	X		X	X				X	X				X									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Cortical adenoma							X			X											X		X	X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+
Adenocarcinoma, NOS							X											X				X			
Fibroadenoma	X	X		X	X		X	X	X	X	X	X												X	X
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Endometrial stromal polyp																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic					X																				
Squamous cell carcinoma, metastatic																	X								
Astrocytoma																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 3,000 ppm (Continued)

ANIMAL NUMBER	90202	90203	90204	90205	90206	90207	90208	90209	90210	90211	90212	90213	90214	90215	90216	90217	90218	90219	90220	90221	90222	90223	90224	90225	90226
WEEKS ON STUDY	10202	07209	09204	10202	10202	09205	07201	08606	10202	10202	09909	09404	06404	10202	10202	06808	09909	10202	10202	09404	06605	10202	10202	08808	09090
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	+	N	N	N	+	+	N	N	N	N	N	N	N	+	+	N	N	N	N	N	N	N
Fibroma				X														X							
Fibrosarcoma																	X								
Lipoma									X																
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS			X					X	X		X	X	X			X			X	X	X	X		X	X
Papillary adenoma					X																				
Sarcoma, NOS																									
Rhabdomyosarcoma											X														
Mixed tumor, malignant															X										
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Thymus	+	+	−	+	+	−	−	−	−	−	+	+	+	+	−	+	+	+	+	−	+	+	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule					X																	X			
Hepatocellular carcinoma																							X		
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS		X			X	X			X	X	X			X			X		X						
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Cortical adenoma					X				X		X			X					X				X		
Thyroid	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma																								X	
Parathyroid	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Pancreatic islets	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma	X																								
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Fibroadenoma					X					X	X						X	X		X				X	X
Uterus	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp																				X					
Ovary	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic								X												X	X			X	
Squamous cell carcinoma, metastatic																									
Astrocytoma											X														

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 3,000 ppm (Continued)

ANIMAL NUMBER	92727	92728	92729	93030	93031	93032	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	102	102	097	090	081	088	
INTEGUMENTARY SYSTEM							
Subcutaneous tissue	+	N	+	N	+	+	*56
Fibroma							4
Fibrosarcoma							3
Lipoma							1
RESPIRATORY SYSTEM							
Lungs and bronchi	+	+	+	+	+	+	56
Trachea	+	+	+	+	+	+	56
Nasal cavity	+	+	+	+	+	+	*56
Carcinoma, NOS				X		X	24
Papillary adenoma	X						6
Sarcoma, NOS							1
Rhabdomyosarcoma							2
Mixed tumor, malignant							1
HEMATOPOIETIC SYSTEM							
Bone marrow	+	+	+	+	+	+	56
Spleen	+	+	+	+	+	+	54
Lymph nodes	+	+	+	+	+	+	54
Carcinoma, NOS, metastatic						X	1
Thymus	−	−	+	−	−	−	29
CIRCULATORY SYSTEM							
Heart	+	+	+	+	+	+	56
DIGESTIVE SYSTEM							
Salivary gland	+	−	+	+	+	+	52
Liver	+	+	+	+	+	+	55
Neoplastic nodule							4
Hepatocellular carcinoma							1
Bile duct	+	+	+	+	+	+	55
Gallbladder & common bile duct	N	N	N	N	N	N	*56
Pancreas	+	+	+	+	+	+	54
Esophagus	+	+	+	+	+	+	53
Stomach	+	+	+	+	+	+	55
Small intestine	+	+	+	+	+	+	55
Large intestine	+	+	+	+	+	+	51
URINARY SYSTEM							
Kidney	+	+	+	+	+	+	55
Urinary bladder	+	−	+	+	+	+	53
ENDOCRINE SYSTEM							
Pituitary	+	+	+	+	+	+	56
Carcinoma, NOS		X					2
Adenoma, NOS	X		X		X		20
Adrenal	+	+	+	+	+	+	55
Cortical adenoma							12
Thyroid	+	+	+	+	+	+	54
Follicular cell carcinoma		X					1
C-cell adenoma			X	X			3
Parathyroid	+	+	+	+	+	−	51
Pancreatic islets	+	+	+	+	+	+	54
Islet cell adenoma							1
REPRODUCTIVE SYSTEM							
Mammary gland	+	+	+	+	+	+	*56
Adenocarcinoma, NOS							3
Fibroadenoma		X		X	X	X	24
Uterus	+	−	+	+	+	+	54
Endometrial stromal polyp							1
Ovary	+	+	+	+	+	+	55
NERVOUS SYSTEM							
Brain	+	+	+	+	+	+	56
Carcinoma, NOS, metastatic				X		X	7
Squamous cell carcinoma, metastatic							1
Astrocytoma							1

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

	Control	300 ppm	1,000 ppm	3,000 ppm
Subcutaneous Tissue: Fibroma				
Overall Rates (a)	0/56 (0%)	2/56 (4%)	1/56 (2%)	4/56 (7%)
Adjusted Rates (b)	0.0%	6.6%	2.1%	15.5%
Terminal Rates (c)	0/33 (0%)	1/25 (4%)	0/32 (0%)	3/24 (13%)
Life Table Tests (d)	P=0.029	P=0.207	P=0.522	P=0.034
Incidental Tumor Tests (d)	P=0.057	P=0.278	P=0.594	P=0.052
Cochran-Armitage Trend Test (d)	P=0.048			
Fisher Exact Test		P=0.248	P=0.500	P=0.059
Subcutaneous Tissue: Fibrosarcoma				
Overall Rates (a)	1/56 (2%)	0/56 (0%)	1/56 (2%)	3/56 (5%)
Adjusted Rates (b)	3.0%	0.0%	2.2%	11.7%
Terminal Rates (c)	1/33 (3%)	0/25 (0%)	0/32 (0%)	2/24 (8%)
Life Table Tests (d)	P=0.055	P=0.555N	P=0.745N	P=0.208
Incidental Tumor Tests (d)	P=0.085	P=0.555N	P=0.713N	P=0.277
Cochran-Armitage Trend Test (d)	P=0.080			
Fisher Exact Test		P=0.500N	P=0.752	P=0.309
Subcutaneous Tissue: Fibroma or Fibrosarcoma				
Overall Rates (a)	1/56 (2%)	2/56 (4%)	2/56 (4%)	6/56 (11%)
Adjusted Rates (b)	3.0%	6.6%	4.3%	22.5%
Terminal Rates (c)	1/33 (3%)	1/25 (4%)	0/32 (0%)	4/24 (17%)
Life Table Tests (d)	P=0.010	P=0.433	P=0.529	P=0.025
Incidental Tumor Tests (d)	P=0.026	P=0.514	P=0.606	P=0.049
Cochran-Armitage Trend Test (d)	P=0.020			
Fisher Exact Test		P=0.500	P=0.500	P=0.057
Nasal Cavity: Papillary Adenoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	0/56 (0%)	6/56 (11%)
Adjusted Rates (b)	0.0%	0.0%	0.0%	17.0%
Terminal Rates (c)	0/33 (0%)	0/25 (0%)	0/32 (0%)	2/24 (8%)
Life Table Tests (d)	P<0.001	(e)	(e)	P=0.013
Incidental Tumor Tests (d)	P<0.001	(e)	(e)	P=0.019
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	(e)	P=0.014
Nasal Cavity: Adenoma or Papillary Adenoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	1/56 (2%)	6/56 (11%)
Adjusted Rates (b)	0.0%	0.0%	3.1%	17.0%
Terminal Rates (c)	0/33 (0%)	0/25 (0%)	1/32 (3%)	2/24 (8%)
Life Table Tests (d)	P<0.001	(e)	P=0.494	P=0.013
Incidental Tumor Tests (d)	P<0.001	(e)	P=0.494	P=0.019
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	P=0.500	P=0.014
Nasal Cavity: Carcinoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	1/56 (2%)	24/56 (43%)
Adjusted Rates (b)	0.0%	0.0%	1.8%	53.9%
Terminal Rates (c)	0/33 (0%)	0/25 (0%)	0/32 (0%)	6/24 (25%)
Life Table Tests (d)	P<0.001	(e)	P=0.504	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	P=0.393	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	P=0.500	P<0.001
Nasal Cavity: Adenoma, Papillary Adenoma, or Carcinoma				
Overall Rates (a)	0/56 (0%)	0/56 (0%)	2/56 (4%)	29/56 (52%)
Adjusted Rates (b)	0.0%	0.0%	4.9%	62.0%
Terminal Rates (c)	0/33 (0%)	0/25 (0%)	1/32 (3%)	8/24 (33%)
Life Table Tests (d)	P<0.001	(e)	P=0.238	P<0.001
Incidental Tumor Tests (d)	P<0.001	(e)	P=0.179	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001			
Fisher Exact Test		(e)	P=0.248	P<0.001

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Control	300 ppm	1,000 ppm	3,000 ppm
Liver: Neoplastic Nodule				
Overall Rates (a)	0/56 (0%)	1/56 (2%)	2/56 (4%)	4/55 (7%)
Adjusted Rates (b)	0.0%	4.0%	6.3%	16.7%
Terminal Rates (c)	0/33 (0%)	1/25 (4%)	2/32 (6%)	4/24 (17%)
Life Table Tests (d)	P=0.012	P=0.445N	P=0.231	P=0.029
Incidental Tumor Tests (d)	P=0.012	P=0.445N	P=0.231	P=0.029
Cochran-Armitage Trend Test (d)	P=0.027			
Fisher Exact Test		P=0.500	P=0.248	P=0.057
Liver: Neoplastic Nodule or Hepatocellular Carcinoma				
Overall Rates (a)	1/56 (2%)	1/56 (2%)	3/56 (5%)	5/55 (9%)
Adjusted Rates (b)	3.0%	4.0%	9.4%	20.8%
Terminal Rates (c)	1/33 (3%)	1/25 (4%)	3/32 (9%)	5/24 (21%)
Life Table Tests (d)	P=0.013	P=0.699	P=0.293	P=0.044
Incidental Tumor Tests (d)	P=0.013	P=0.699	P=0.293	P=0.044
Cochran-Armitage Trend Test (d)	P=0.033			
Fisher Exact Test		P=0.752	P=0.309	P=0.099
Pituitary Gland: Adenoma				
Overall Rates (a)	29/56 (52%)	32/55 (58%)	30/56 (54%)	20/56 (36%)
Adjusted Rates (b)	63.3%	76.0%	70.6%	52.0%
Terminal Rates (c)	17/33 (52%)	16/25 (64%)	20/32 (63%)	8/24 (33%)
Life Table Tests (d)	P=0.130N	P=0.140	P=0.510	P=0.300N
Incidental Tumor Tests (d)	P=0.018N	P=0.328	P=0.543N	P=0.062N
Cochran-Armitage Trend Test (d)	P=0.014N			
Fisher Exact Test		P=0.313	P=0.500	P=0.064N
Pituitary Gland: Carcinoma				
Overall Rates (a)	4/56 (7%)	1/55 (2%)	1/56 (2%)	2/56 (4%)
Adjusted Rates (b)	9.0%	3.6%	1.9%	7.5%
Terminal Rates (c)	1/33 (3%)	0/25 (0%)	0/32 (0%)	1/24 (4%)
Life Table Tests (d)	P=0.511N	P=0.214N	P=0.178N	P=0.409N
Incidental Tumor Tests (d)	P=0.452N	P=0.138N	P=0.249N	P=0.313N
Cochran-Armitage Trend Test (d)	P=0.460N			
Fisher Exact Test		P=0.187N	P=0.182N	P=0.339N
Pituitary Gland: Adenoma or Carcinoma				
Overall Rates (a)	33/56 (59%)	33/55 (60%)	31/56 (55%)	22/56 (39%)
Adjusted Rates (b)	67.8%	76.8%	71.2%	56.6%
Terminal Rates (c)	18/33 (55%)	16/25 (64%)	20/32 (63%)	9/24 (38%)
Life Table Tests (d)	P=0.120N	P=0.266	P=0.425N	P=0.230N
Incidental Tumor Tests (d)	P=0.012N	P=0.575N	P=0.360N	P=0.026N
Cochran-Armitage Trend Test (d)	P=0.010N			
Fisher Exact Test		P=0.531	P=0.424N	P=0.029N
Adrenal Gland: Cortical Adenoma				
Overall Rates (a)	7/56 (13%)(f)	9/56 (16%)	6/55 (11%)	12/55 (22%)
Adjusted Rates (b)	20.2%	31.8%	17.1%	45.3%
Terminal Rates (c)	6/33 (18%)	7/25 (28%)	4/32 (13%)	10/24 (42%)
Life Table Tests (d)	P=0.044	P=0.220	P=0.504N	P=0.040
Incidental Tumor Tests (d)	P=0.078	P=0.311	P=0.423N	P=0.075
Cochran-Armitage Trend Test (d)	P=0.118			
Fisher Exact Test		P=0.394	P=0.514N	P=0.147
Thyroid Gland: C-Cell Adenoma				
Overall Rates (a)	1/54 (2%)	1/54 (2%)	3/55 (5%)	3/54 (6%)
Adjusted Rates (b)	2.6%	4.0%	7.4%	8.3%
Terminal Rates (c)	0/32 (0%)	1/25 (4%)	1/32 (3%)	0/24 (0%)
Life Table Tests (d)	P=0.176	P=0.713	P=0.345	P=0.288
Incidental Tumor Tests (d)	P=0.345	P=0.718N	P=0.421	P=0.608
Cochran-Armitage Trend Test (d)	P=0.202			
Fisher Exact Test		P=0.752	P=0.316	P=0.309

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Control	300 ppm	1,000 ppm	3,000 ppm
Thyroid Gland: C-Cell Carcinoma				
Overall Rates (a)	0/54 (0%)	3/54 (6%)	2/55 (4%)	0/54 (0%)
Adjusted Rates (b)	0.0%	12.0%	5.3%	0.0%
Terminal Rates (c)	0/32 (0%)	3/25 (12%)	1/32 (3%)	0/24 (0%)
Life Table Tests (d)	P=0.326N	P=0.080	P=0.258	(e)
Incidental Tumor Tests (d)	P=0.286N	P=0.080	P=0.276	(e)
Cochran-Armitage Trend Test (d)	P=0.284N			
Fisher Exact Test		P=0.121	P=0.252	(e)
Thyroid Gland: C-Cell Adenoma or Carcinoma				
Overall Rates (a)	1/54 (2%)	4/54 (7%)	4/55 (7%)	3/54 (6%)
Adjusted Rates (b)	2.6%	16.0%	10.4%	8.3%
Terminal Rates (c)	0/32 (0%)	4/25 (16%)	2/32 (6%)	0/24 (0%)
Life Table Tests (d)	P=0.405	P=0.118	P=0.210	P=0.288
Incidental Tumor Tests (d)	P=0.573	P=0.158	P=0.261	P=0.608
Cochran-Armitage Trend Test (d)	P=0.469			
Fisher Exact Test		P=0.182	P=0.187	P=0.309
Mammary Gland: Cystadenoma				
Overall Rates (a)	0/56 (0%)	3/56 (5%)	0/56 (0%)	0/56 (0%)
Adjusted Rates (b)	0.0%	10.3%	0.0%	0.0%
Terminal Rates (c)	0/33 (0%)	2/25 (8%)	0/32 (0%)	0/24 (0%)
Life Table Tests (d)	P=0.286N	P=0.094	(e)	(e)
Incidental Tumor Tests (d)	P=0.242N	P=0.124	(e)	(e)
Cochran-Armitage Trend Test (d)	P=0.255N			
Fisher Exact Test		P=0.122	(e)	(e)
Mammary Gland: Adenocarcinoma				
Overall Rates (a)	6/56 (11%)	6/56 (11%)	5/56 (9%)	3/56 (5%)
Adjusted Rates (b)	16.0%	17.2%	14.3%	10.7%
Terminal Rates (c)	4/33 (12%)	1/25 (4%)	4/32 (13%)	2/24 (8%)
Life Table Tests (d)	P=0.250N	P=0.504	P=0.501N	P=0.364N
Incidental Tumor Tests (d)	P=0.152N	P=0.621N	P=0.580N	P=0.263N
Cochran-Armitage Trend Test (d)	P=0.173N			
Fisher Exact Test		P=0.619	P=0.500N	P=0.245N
Mammary Gland: Cystadenoma or Adenocarcinoma				
Overall Rates (a)	6/56 (11%)	9/56 (16%)	5/56 (9%)	3/56 (5%)
Adjusted Rates (b)	16.0%	26.0%	14.3%	10.7%
Terminal Rates (c)	4/33 (12%)	3/25 (12%)	4/32 (13%)	2/24 (8%)
Life Table Tests (d)	P=0.151N	P=0.196	P=0.501N	P=0.364N
Incidental Tumor Tests (d)	P=0.078N	P=0.290	P=0.580N	P=0.263N
Cochran-Armitage Trend Test (d)	P=0.093N			
Fisher Exact Test		P=0.290	P=0.500N	P=0.245N
Mammary Gland: Fibroadenoma				
Overall Rates (a)	19/56 (34%)	19/56 (34%)	20/56 (36%)	24/56 (43%)
Adjusted Rates (b)	49.0%	50.8%	45.4%	56.9%
Terminal Rates (c)	14/33 (42%)	9/25 (36%)	9/32 (28%)	8/24 (33%)
Life Table Tests (d)	P=0.069	P=0.312	P=0.510	P=0.081
Incidental Tumor Tests (d)	P=0.202	P=0.577	P=0.471N	P=0.332
Cochran-Armitage Trend Test (d)	P=0.155			
Fisher Exact Test		P=0.579	P=0.500	P=0.219
Uterus: Endometrial Stromal Polyp				
Overall Rates (a)	3/55 (5%)	2/56 (4%)	2/56 (4%)	1/54 (2%)
Adjusted Rates (b)	8.2%	8.0%	6.3%	2.9%
Terminal Rates (c)	1/33 (3%)	2/25 (8%)	2/32 (6%)	0/23 (0%)
Life Table Tests (d)	P=0.335N	P=0.588N	P=0.498N	P=0.369N
Incidental Tumor Tests (d)	P=0.269N	P=0.445N	P=0.415N	P=0.153N
Cochran-Armitage Trend Test (d)	P=0.278N			
Fisher Exact Test		P=0.491N	P=0.491N	P=0.316N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or a lower incidence in a dosed group than in controls is indicated by (N).
(e) No P value is reported because no tumors were observed in the dosed and control groups.

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
Animals initially in study	56	56	56	56
Animals necropsied	56	56	56	56
Animals examined histopathologically	56	56	56	56
INTEGUMENTARY SYSTEM				
*Skin	(56)	(56)	(56)	(56)
Inflammation, acute	2 (4%)	2 (4%)	1 (2%)	
Inflammation, acute/chronic			2 (4%)	1 (2%)
Inflammation, chronic	2 (4%)			1 (2%)
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)	4 (7%)
*Subcut tissue	(56)	(56)	(56)	(56)
Inflammation, acute	1 (2%)			
Inflammation, chronic				1 (2%)
RESPIRATORY SYSTEM				
*Nasal cavity	(56)	(56)	(56)	(56)
Inflammation, acute	7 (13%)	14 (25%)	15 (27%)	38 (68%)
Inflammation, chronic	1 (2%)			
Postmortem change				3 (5%)
Hyperplasia, epithelial			1 (2%)	11 (20%)
Metaplasia, squamous				4 (7%)
#Trachea	(56)	(55)	(54)	(56)
Inflammation, acute				1 (2%)
Inflammation, chronic		2 (4%)		1 (2%)
Postmortem change				2 (4%)
#Lung	(56)	(56)	(55)	(56)
Congestion, NOS	6 (11%)	10 (18%)	4 (7%)	18 (32%)
Hemorrhage	1 (2%)		1 (2%)	
Inflammation, acute		2 (4%)		3 (5%)
Alveolar macrophages	23 (41%)	20 (36%)	26 (47%)	31 (55%)
Hyperplasia, adenomatous	2 (4%)	3 (5%)		1 (2%)
HEMATOPOIETIC SYSTEM				
#Bone marrow	(55)	(56)	(56)	(56)
Myelofibrosis				1 (2%)
#Spleen	(56)	(56)	(56)	(54)
Inflammation, acute		1 (2%)		
Necrosis, diffuse		1 (2%)		
Postmortem change	1 (2%)	1 (2%)		1 (2%)
Hemosiderosis	51 (91%)	46 (82%)	54 (96%)	54 (100%)
Atrophy, NOS				2 (4%)
Lymphoid depletion	1 (2%)			
Hyperplasia, reticulum cell				1 (2%)
Hematopoiesis	51 (91%)	50 (89%)	54 (96%)	44 (81%)
#Lymph node	(55)	(52)	(56)	(54)
Pigmentation, NOS				1 (2%)
Hyperplasia, NOS				1 (2%)
Hyperplasia, lymphoid		1 (2%)		
#Mandibular lymph node	(55)	(52)	(56)	(54)
Congestion, NOS		1 (2%)		2 (4%)
Abscess, NOS				1 (2%)
Hyperplasia, lymphoid	1 (2%)		3 (5%)	1 (2%)
#Mesenteric lymph node	(55)	(52)	(56)	(54)
Congestion, NOS	2 (4%)	1 (2%)	3 (5%)	
Fibrosis, focal			1 (2%)	1 (2%)
Postmortem change				1 (2%)
Pigmentation, NOS		2 (4%)	3 (5%)	3 (6%)
Hyperplasia, NOS				1 (2%)
Hyperplasia, lymphoid	1 (2%)			2 (4%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
HEMATOPOIETIC SYSTEM (Continued)				
#Liver	(56)	(56)	(56)	(55)
Hematopoiesis	2 (4%)		2 (4%)	
#Adrenal	(56)	(56)	(55)	(55)
Hematopoiesis		1 (2%)	1 (2%)	
#Thymus	(31)	(42)	(37)	(29)
Cyst, NOS			1 (3%)	
Congestion, NOS				1 (3%)
Involution, NOS	27 (87%)	38 (90%)	33 (89%)	27 (93%)
CIRCULATORY SYSTEM				
#Mesenteric lymph node	(55)	(52)	(56)	(54)
Lymphangiectasis	3 (5%)	1 (2%)	1 (2%)	
#Heart	(56)	(56)	(56)	(56)
Fibrosis, focal	1 (2%)		1 (2%)	1 (2%)
#Myocardium	(56)	(56)	(56)	(56)
Inflammation, chronic focal	3 (5%)	5 (9%)	1 (2%)	6 (11%)
Inflammation, chronic diffuse				1 (2%)
#Pancreas	(56)	(56)	(56)	(54)
Periarteritis				1 (2%)
*Mesentery	(56)	(56)	(56)	(56)
Periarteritis		1 (2%)		
#Adrenal	(56)	(56)	(55)	(55)
Thrombosis, NOS	1 (2%)	1 (2%)		
DIGESTIVE SYSTEM				
#Salivary gland	(55)	(53)	(54)	(52)
Atrophy, NOS			1 (2%)	
#Liver	(56)	(56)	(56)	(55)
Hemorrhage			1 (2%)	
Inflammation, acute focal		1 (2%)		
Inflammation, chronic focal	2 (4%)	1 (2%)		4 (7%)
Degeneration, cystic				1 (2%)
Necrosis, focal	2 (4%)	2 (4%)	4 (7%)	1 (2%)
Postmortem change		1 (2%)		
Cytoplasmic vacuolization	9 (16%)	6 (11%)	5 (9%)	10 (18%)
Basophilic cyto change	10 (18%)	6 (11%)	3 (5%)	10 (18%)
Eosinophilic cyto change	6 (11%)	5 (9%)	6 (11%)	7 (13%)
Clear cell change	1 (2%)		2 (4%)	
Angiectasis	1 (2%)	2 (4%)	1 (2%)	5 (9%)
#Liver/centrilobular	(56)	(56)	(56)	(55)
Necrosis, NOS	1 (2%)			
#Liver/periportal	(56)	(56)	(56)	(55)
Fibrosis				1 (2%)
Hepatocytomegaly				1 (2%)
#Bile duct	(56)	(56)	(56)	(55)
Cyst, NOS			1 (2%)	3 (5%)
Hyperplasia, NOS	13 (23%)	9 (16%)	15 (27%)	7 (13%)
Hyperplasia, cystic				1 (2%)
#Pancreas	(56)	(56)	(56)	(54)
Inflammation, chronic	2 (4%)			
Degeneration, NOS	1 (2%)			
Postmortem change				2 (4%)
#Pancreatic acinus	(56)	(56)	(56)	(54)
Atrophy, NOS	2 (4%)	2 (4%)	3 (5%)	2 (4%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
DIGESTIVE SYSTEM (Continued)				
#Stomach	(56)	(55)	(56)	(55)
Congenital malformation, NOS			1 (2%)	
Mineralization				1 (2%)
Inflammation, acute	3 (5%)	1 (2%)	6 (11%)	4 (7%)
Inflammation, acute/chronic				1 (2%)
Inflammation, chronic				1 (2%)
Postmortem change		1 (2%)		
Hyperkeratosis	5 (9%)		5 (9%)	10 (18%)
Acanthosis	3 (5%)		6 (11%)	8 (15%)
#Duodenum	(56)	(56)	(56)	(55)
Postmortem change	1 (2%)	2 (4%)	1 (2%)	8 (15%)
#Colon	(56)	(55)	(56)	(51)
Parasitism	2 (4%)	2 (4%)		
Postmortem change	2 (4%)	1 (2%)		1 (2%)
URINARY SYSTEM				
#Kidney	(56)	(56)	(56)	(55)
Inflammation, acute focal		1 (2%)		
Nephropathy	11 (20%)	6 (11%)	3 (5%)	5 (9%)
Nephrosis, NOS			1 (2%)	
Postmortem change	1 (2%)	1 (2%)		
Hyperplasia, epithelial				1 (2%)
#Kidney/cortex	(56)	(56)	(56)	(55)
Mineralization		1 (2%)		1 (2%)
Cyst, NOS	1 (2%)		1 (2%)	1 (2%)
#Kidney/medulla	(56)	(56)	(56)	(55)
Mineralization			1 (2%)	1 (2%)
#Kidney/pelvis	(56)	(56)	(56)	(55)
Mineralization	30 (54%)	38 (68%)	26 (46%)	23 (42%)
Dilatation, NOS		1 (2%)		1 (2%)
Inflammation, acute			1 (2%)	
Inflammation, chronic		4 (7%)		
#Urinary bladder	(54)	(54)	(53)	(53)
Inflammation, chronic		1 (2%)		
Inflammation, chronic focal		1 (2%)		
Polyp, inflammatory		1 (2%)		
ENDOCRINE SYSTEM				
#Pituitary	(56)	(55)	(56)	(56)
Cyst, NOS	1 (2%)	1 (2%)	3 (5%)	3 (5%)
Hemorrhage	10 (18%)	13 (24%)	8 (14%)	10 (18%)
Hyperplasia, focal	6 (11%)	3 (5%)	6 (11%)	3 (5%)
Hyperplasia, diffuse			1 (2%)	
#Adrenal	(56)	(56)	(55)	(55)
Postmortem change		1 (2%)		
Atrophy, NOS		1 (2%)	1 (2%)	
Angiectasis	32 (57%)	31 (55%)	31 (56%)	25 (45%)
#Adrenal cortex	(56)	(56)	(55)	(55)
Cyst, NOS		1 (2%)		
Necrosis, NOS			1 (2%)	4 (7%)
Infarct, NOS	1 (2%)			
Cytoplasmic vacuolization	11 (20%)	6 (11%)	12 (22%)	8 (15%)
Hyperplasia, focal	7 (13%)	6 (11%)	8 (15%)	8 (15%)
#Adrenal medulla	(56)	(56)	(55)	(55)
Hyperplasia, focal	3 (5%)	2 (4%)	2 (4%)	1 (2%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
ENDOCRINE SYSTEM (Continued)				
#Thyroid	(54)	(54)	(55)	(54)
Ultimobranchial cyst	10 (19%)	8 (15%)	1 (2%)	2 (4%)
Cystic follicles	1 (2%)	1 (2%)		
Postmortem change				1 (2%)
Hyperplasia, C-cell	10 (19%)	13 (24%)	6 (11%)	8 (15%)
Angiectasis			1 (2%)	
REPRODUCTIVE SYSTEM				
*Mammary gland	(56)	(56)	(56)	(56)
Galactocele	1 (2%)	3 (5%)	2 (4%)	6 (11%)
Inflammation, NOS	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Abscess, NOS	1 (2%)			
Hyperplasia, cystic	11 (20%)	27 (48%)	19 (34%)	19 (34%)
*Clitoral gland	(56)	(56)	(56)	(56)
Cystic ducts	1 (2%)	1 (2%)		
*Vagina	(56)	(56)	(56)	(56)
Polyp, inflammatory	1 (2%)			
#Uterus	(55)	(56)	(56)	(54)
Dilatation, NOS	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Hematometra			1 (2%)	
#Uterus/endometrium	(55)	(56)	(56)	(54)
Cyst, NOS	8 (15%)	7 (13%)	14 (25%)	10 (19%)
Inflammation, acute	1 (2%)			2 (4%)
Hyperplasia, cystic	1 (2%)	1 (2%)	1 (2%)	1 (2%)
#Ovary	(55)	(56)	(56)	(55)
Cystic follicles	11 (20%)	11 (20%)	11 (20%)	5 (9%)
Corpus luteum cyst	4 (7%)		1 (2%)	
Parovarian cyst	7 (13%)	7 (13%)	9 (16%)	10 (18%)
Postmortem change				1 (2%)
Amyloidosis		1 (2%)		
NERVOUS SYSTEM				
#Brain/meninges	(56)	(56)	(56)	(56)
Inflammation, chronic				2 (4%)
#Subdural space	(56)	(56)	(56)	(56)
Hemorrhage				1 (2%)
#Brain	(56)	(56)	(56)	(56)
Hemorrhage	1 (2%)		1 (2%)	
SPECIAL SENSE ORGANS				
*Eye	(56)	(56)	(56)	(56)
Inflammation, NOS				1 (2%)
Cataract		1 (2%)		2 (4%)
Postmortem change				1 (2%)
Phthisis bulbi				1 (2%)
*Eye/cornea	(56)	(56)	(56)	(56)
Inflammation, acute				1 (2%)
*Eyeball tunica vasculosa	(56)	(56)	(56)	(56)
Inflammation, NOS				1 (2%)
*Eye/conjunctiva	(56)	(56)	(56)	(56)
Inflammation, acute	1 (2%)			
*Nasolacrimal duct	(56)	(56)	(56)	(56)
Inflammation, acute	4 (7%)		1 (2%)	
Inflammation, acute/chronic	3 (5%)	1 (2%)		
Inflammation, chronic	3 (5%)	3 (5%)	5 (9%)	1 (2%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF 2,6-XYLIDINE (Continued)

	Untreated Control	300 ppm	1,000 ppm	3,000 ppm
SPECIAL SENSE ORGANS (Continued)				
*Harderian gland	(56)	(56)	(56)	(56)
Inflammation, acute	1 (2%)			
*Ear	(56)	(56)	(56)	(56)
Infection, bacterial				1 (2%)
*Auricular cartilage	(56)	(56)	(56)	(56)
Hyperplasia, NOS	2 (4%)			1 (2%)
MUSCULOSKELETAL SYSTEM				
None				
BODY CAVITIES				
*Mesentery	(56)	(56)	(56)	(56)
Abscess, NOS			1 (2%)	
Necrosis, fat	1 (2%)	1 (2%)	1 (2%)	2 (4%)
ALL OTHER SYSTEMS				
*Multiple organs	(56)	(56)	(56)	(56)
Inflammation, acute			1 (2%)	
Inflammation, chronic				1 (2%)
Postmortem change	2 (4%)	8 (14%)	1 (2%)	1 (2%)
SPECIAL MORPHOLOGY SUMMARY				
None				

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX C

MUTAGENICITY OF 2,6-XYLIDINE IN *SALMONELLA TYPHIMURIUM*

MUTAGENICITY OF 2,6-XYLIDINE IN *SALMONELLA TYPHIMURIUM*

Dose (µg/plate)	Trial 1	Trial 2	Trial 3	Mean	Standard Error	Dose (µg/plate)	Trial 1	Trial 2	Trial 3	Mean	Standard Error
TA100											
09/26/80 (−S9)						10/07/80 (−S9)					
0	131	123	105	120	7.7	0	114	107	120	114	3.8
100	95	112	98	102	5.2	100	89	88	97	91	2.8
333	127	156	133	139	8.8	333	96	109	132	112	10.5
1,000	115	134	117	122	6.0	1,000	112	101	116	110	4.5
3,333	123	120	114	119	2.6	3,333	102	105	113	107	3.3
9,900	71	92	77	80	6.2	9,900	43	32	76	50	13.2
09/26/80 (+S9 rat) (a)						10/07/80 (+S9 rat)					
0	148	143	153	148	2.9	0	135	118	148	134	8.7
100	157	133	148	146	7.0	100	131	123	132	129	2.8
333	175	180	181	179	1.9	333	114	124	162	133	14.6
1,000	219	171	198	196	13.9	1,000	122	103	177	134	22.2
3,333	172	137	157	155	10.1	3,333	121	121	140	127	6.3
9,900	119	76	99	98	12.4	9,900	53	89	89	77	12.0
09/26/80 (+S9 hamster) (a)						10/07/80 (+S9 hamster)					
0	163	180	160	168	6.2	0	140	173	168	160	10.3
100	262	230	241	244	9.4	100	156	153	233	181	26.2
333	149	180	161	163	9.0	333	143	134	156	144	6.4
1,000	163	162	159	161	1.2	1,000	140	119	154	138	10.4
3,333	174	226	196	199	15.1	3,333	116	155	190	154	21.4
9,900	120	95	105	107	7.3	9,900	111	62	102	92	15.1
TA1535											
09/26/80 (−S9)						10/07/80 (−S9)					
0	9	5	5	6	1.3	0	5	1	6	4	1.5
100	8	7	6	6	0.3	100	2	6	4	4	1.2
333	7	8	7	7	0.3	333	6	2	5	4	1.2
1,000	8	10	8	9	0.7	1,000	1	1	6	3	1.7
3,333	6	8	6	7	0.7	3,333	(b) 0	(b) 2	(b) 5	--	--
9,900	7	12	9	9	1.5	9,900	(b) 2	(b) 0	(b) 6	--	--
09/26/80 (+S9 rat)						10/07/80 (+S9 rat)					
0	14	10	8	11	1.8	0	10	8	11	10	0.9
100	12	9	9	10	1.0	100	5	5	9	6	1.3
333	5	12	8	8	2.0	333	6	5	7	6	0.6
1,000	9	15	11	12	1.8	1,000	2	10	11	8	2.8
3,333	10	8	8	9	0.7	3,333	(b) 2	(b) 0	(b) 0	--	--
9,900	9	7	7	8	0.7	9,900	(b) 3	(b) 2	(b) 7	--	--
09/26/80 (+S9 hamster)						10/07/80 (+S9 hamster)					
0	9	8	7	8	0.6	0	3	6	8	6	1.5
100	6	16	11	11	2.9	100	5	5	8	6	1.0
333	13	8	10	10	1.5	333	1	6	7	5	1.9
1,000	12	9	10	10	0.9	1,000	2	5	7	5	1.5
3,333	4	3	3	3	0.3	3,333	4	4	2	3	0.7
9,900	0	0	0	0	0.0	9,900	3	5	0	3	1.5
TA1537											
09/26/80 (−S9)						10/07/80 (−S9)					
0	2	3	4	3	0.6	0	2	3	3	3	0.3
100	4	6	6	5	0.7	10	4	5	3	4	0.6
333	1	2	2	2	0.3	33	2	4	3	3	0.6
1,000	4	6	6	5	0.7	100	5	1	4	3	1.2
3,333	(b) 3	(b) 1	(b) 2			333	(b) 3	(b) 1	(b) 2	--	--
9,900	(b) 0	(b) 0	(b) 0			1,000	(b) 0	(b) 1	(b) 4	--	--

MUTAGENICITY OF 2,6-XYLIDINE IN *SALMONELLA TYPHIMURIUM* (Continued)

Dose (µg/plate)	Trial 1	Trial 2	Trial 3	Mean	Standard Error	Dose (µg/plate)	Trial 1	Trial 2	Trial 3	Mean	Standard Error
TA1537 (Continued)											
09/26/80 (+S9 rat)						10/07/80 (+S9 rat)					
0	6	9	8	8	0.9	0	7	8	8	8	0.3
100	13	6	10	10	2.0	10	10	8	10	9	0.7
333	8	14	11	11	1.7	33	1	4	12	6	3.3
1,000	12	(c)	12	12	0.0	100	3	5	10	6	2.1
3,333	(b) 6	(b) 9	(b) 0	--	--	333	3	7	11	7	2.3
9,900	(b) 0	0	0	0	0.0	1,000	(b) 0	(b) 0	(b) 0	--	--
09/26/80 (+S9 hamster)						10/07/80 (+S9 hamster)					
0	13	9	5	9	2.3	0	7	5	9	7	1.2
100	10	11	9	10	0.6	33	3	6	10	6	2.0
333	11	11	9	10	0.7	100	4	5	8	6	1.2
1,000	10	2	5	6	2.3	333	7	1	8	5	2.2
3,333	7	12	8	9	1.5	1,000	(b) 3	(b) 7	(b) 0	--	--
9,900	(b) 3	2	(b) 2	2	--	3,333	(b) 0	(b) 2	(b) 0	--	--
TA98											
09/26/80 (-S9)						10/07/80 (-S9)					
0	18	27	16	20	3.4	0	12	9	18	13	2.6
100	25	28	24	26	1.2	100	7	11	17	12	2.9
333	23	17	18	19	1.9	333	15	13	12	13	0.9
1,000	17	20	17	18	1.0	1,000	12	13	12	12	0.3
3,333	22	17	18	19	1.5	3,333	6	13	12	10	2.2
9,900	23	25	22	23	0.9	9,900	5	7	15	9	3.1
09/26/80 (+S9 rat)						10/07/80 (+S9 rat)					
0	35	27	24	29	3.3	0	23	12	27	21	4.5
100	41	38	37	39	1.2	100	19	19	28	22	3.0
333	34	37	33	35	1.2	333	31	16	25	24	4.4
1,000	53	42	44	46	3.4	1,000	8	14	33	18	7.5
3,333	38	26	30	31	3.5	3,333	24	18	22	21	1.8
9,900	29	31	28	29	0.9	9,900	19	15	21	18	1.8
09/26/80 (+S9 hamster)						10/07/80 (+S9 hamster)					
0	40	37	22	33	5.6	0	10	12	22	15	3.7
100	39	34	31	35	2.3	100	14	17	16	16	0.9
333	33	37	30	33	2.0	333	24	26	15	22	3.4
1,000	44	32	33	38	3.8	1,000	20	25	16	20	2.6
3,333	25	26	22	24	1.2	3,333	17	17	11	15	2.0
9,900	27	20	20	22	2.3	9,900	23	16	10	16	3.8

(a) The S9 fractions were prepared from the liver of Aroclor 1254-induced male Sprague Dawley rats and male Syrian hamsters. Cells and study compound or solvent (dimethyl sulfoxide) were incubated for 20 minutes at 37° C in the presence of either S9 or buffer (Yahagi et al., 1975). After the addition of soft agar, the contents of each tube was poured onto minimal medium, and the plates were incubated at 37° C for 48 hours (Ames et al., 1975). The experiment was performed twice, each in triplicate.
(b) The chemical was toxic.
(c) The plate was contaminated.

APPENDIX D

CHEMICAL CHARACTERIZATION OF 2,6-XYLIDINE

APPENDIX D. CHEMICAL CHARACTERIZATION

I. Identity and Purity Determinations Conducted at the Analytical Chemistry Laboratory (Comparison of Litton Bionetics (Pfaltz and Bauer, Lot No. 37220) and EG&G Mason (Ethyl Corp., Lot No. E121279)

A. Lot no. 37220

Spectral data

Infrared

Instrument:	Beckman IR-12
Cell:	Thin film between silver chloride plates
Results:	Consistent with literature spectrum (Sadtler Standard Spectra) and with Mason sample

Ultraviolet/visible

Instrument:	Cary 219
Solvent:	Methanol

Results:	Determined		Literature Values	
	λ_{max} (nm)	$\varepsilon \times 10^{-3}$	λ_{max} (nm)	$\varepsilon \times 10^{-3}$
	368	0.00634 ± 0.00007 (δ)		
	331	0.00539 ± 0.00058 (δ)		
	285	1.96 ± 0.04 (δ)	283	2.02
	234	7.09 ± 0.06 (δ)	233	7.49
			(Sadtler Standard Spectra)	

Nuclear magnetic resonance

Instrument:	Varian EM-360A
Solvent:	Deuterated chloroform with internal tetramethylsilane
Chemical shift (δ):	a s, 1.94
	b broad s, 3.20
	c m, 6.48-6.81
Integration ratios:	a 6.00
	b 1.92
	c 3.12

Titration: 98.8% ± 0.3 (δ)%; nonaqueous titration of one amine group with 0.1 N perchloric acid in glacial acetic acid medium, monitored potentiometrically using a combination pH/mV electrode.

Water analysis (Karl Fischer): 0.29% ± 0.02(δ)%

Elemental analysis

Element	C	H	N
Theory	79.29	9.15	11.56
Determined	79.26	9.18	11.47
	79.25	9.27	11.66

Chromatographic analysis

Thin-layer chromatography

Plates: Silica Gel 60, F-254, 0.25-mm layer
Reference standard: Diphenylamine, 10 mg
Amount spotted: 1 ml of a 1% (v/v) solution and 1 and 3 ml of a 10% (v/v) solution in diethyl ether
Visualization: Ultraviolet light (254 and 366 nm)

System 1: Diethyl ether:isooctane (70:30)

Spot Intensity	R_f	R_{st}
Minor	0.48	0.86
Major	0.37	0.66
Reference	0.56	--

System 2: Methylene chloride (100%)

Spot Intensity	R_f	R_{st}
Major	0.31	0.49
Minor	0.23	0.36
Reference	0.63	--

Gas chromatography

Instrument: Varian 3700
Detector: Flame ionization
Carrier: Nitrogen
Flow rate: 70 ml/min
Inlet temperature: 200° C
Oven temperature program: 50° C for 5 min, then 50°-170° C at 10° C/min

System 1

Column: 20% SP2100/0.1% Carbowax 1500 on 100/120 Supelcoport: 1.8 m × 4 mm ID, glass

Samples injected: Neat liquid (3 ml) and solutions of 1% and 5% (v/v) 2,6-xylidine in methanol

Results: Major peak with no impurities having areas 0.1% or greater than that of the major peak

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	16.6	1.00	100

System 2

Column: 5% SP2100/1.75% Bentone 34 on 100/120 Supelcoport, 1.8 m × 4 mm ID, glass
Samples injected: Neat liquid (3 ml) and solutions of 1% and 0.5% (v/v) 2,6-xylidine in methanol

Results: Major peak with no impurities having areas 0.1% or greater than that of the major peak

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	19.4	1.00	100

B. Lot no. E121279

	Determined	Literature Values
Boiling point:	216° C (Visual, capillary, Buchi 510 melting point apparatus)	214° C at 739 mm (Aldrich Handbook, 1978)
Spectral data		
Infrared		
Instrument:	Beckman IR-12	
Cell:	Thin film between silver chloride plates	
Results:	See Figure D1	Consistent with literature spectrum (Sadtler Standard Spectra) and with Litton sample
Ultraviolet/visible		
Instrument:	Cary 118	
Solvent:	Methanol	

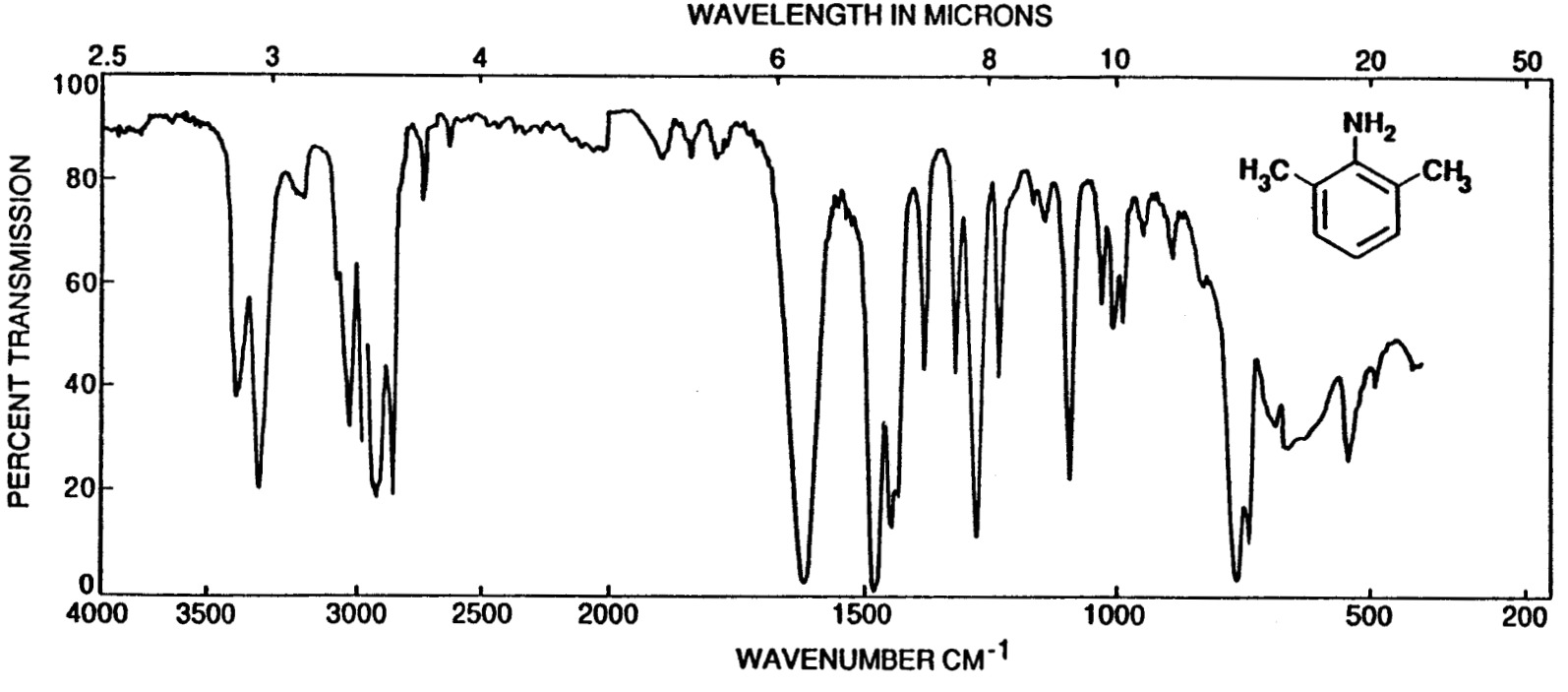


FIGURE D1. INFRARED ABSORPTION SPECTRUM OF 2,6-XYLIDINE (Lot No. E121279)

Results:

λ_{max} (nm)	$\varepsilon \times 10^{-3}$	λ_{max} (nm)	$\varepsilon \times 10^{-3}$
284	2.01 ± 0.04 (δ)	283	2.02
234	7.19 ± 0.06 (δ)	233	7.49
		(Sadtler Standard Spectra)	

Nuclear magnetic resonance

Instrument:	Varian EM-360A
Solvent:	Deuterated chloroform with internal tetramethylsilane
Assignments:	See Figure D2
Chemical shift (δ):	a s, 2.12 ppm
	b broad s, 3.47 ppm
	c m, 6.47-7.10 ppm
Integration ratios:	a 6.00
	b 1.74
	c 3.00

Titration: 98.8% ± 0.2 (δ)%; nonaqueous titration of one amine group with 0.1N perchloric acid in glacial acetic acid, monitored potentiometrically with a combination pH/mV electrode.

Water analysis (Karl Fischer): 0.105% ± 0.002(δ)%

Elemental analysis

Element	C	H	N
Theory	79.29	9.15	11.56
Determined	78.94	8.96	11.29
	79.21	9.26	11.41

Chromatographic analysis

Thin-layer chromatography

Plates: Silica Gel 60, F-254, 0.25-mm layer
Reference standard: Diphenylamine, 10 mg
Amount spotted: 1 ml of a 1% (v/v) solution and 1 and 3 ml of a 10% (v/v) solution in diethyl ether
Visualization: Ultraviolet light (254 nm) and spray of 0.5% aqueous solution of Fast Blue B salt followed by 0.1 N sodium hypochlorite

System 1: Diethyl ether:isooctane (70:30)

Spot Intensity	R_f	R_{st}
Minor	0.51	0.92
Major	0.34	0.62
Trace	0.24	0.44
Slight trace	0.11	0.20
Reference	0.55	--

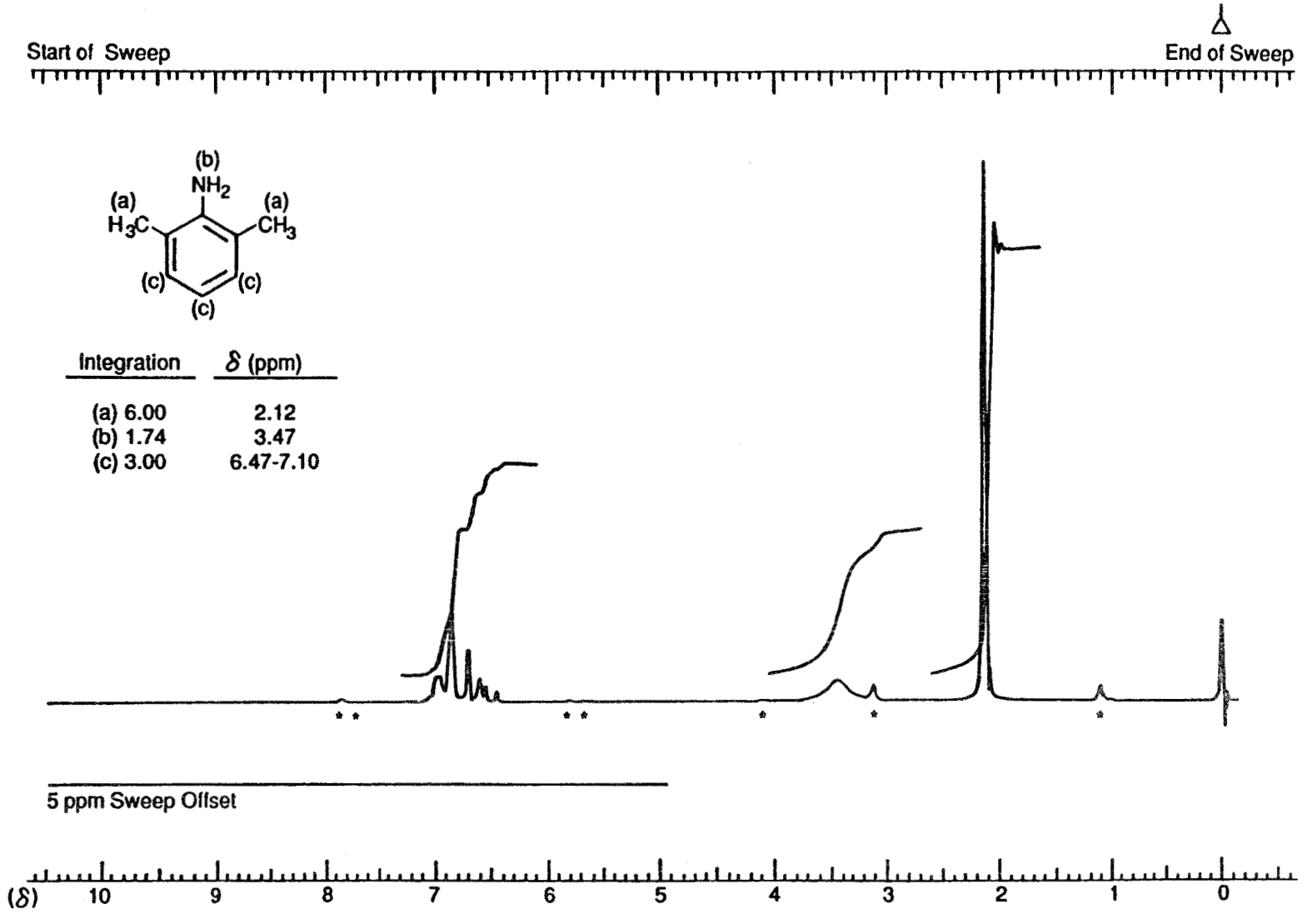


FIGURE D2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF 2,6-XYLIDINE (Lot No. E121279)

System 2: Methylene chloride (100%)

Spot Intensity	R_f	R_{st}
Minor	0.39	0.64
Major	0.22	0.37
Trace	0.12	0.20
Slight trace	Origin	
Reference	0.61	--

Gas chromatography

Instrument: Varian 3700
Detector: Flame ionization
Carrier: Nitrogen
Flow rate: 70 ml/min
Inlet temperature: 200° C
Oven temperature program: 50° C for 5 min, then 50°-170° C at 10° C/min

System 1

Column: 20% SP2100/0.1% Carbowax 1500 on 100/120 Supelcoport: 1.8 m × 4 mm ID, glass
Samples injected: Neat liquid (3 ml) and solutions of 1% and 5% (v/v) 2,6-xylidine in methanol

Results: Major peak and one impurity before the major peak with an area 0.89% that of the major peak

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	15.5	0.94	0.89
2	16.5	1.00	100

System 2

Column: 5% SP2100/1.75% Bentone 34 on 100/120 Supelcoport, 1.8 m × 4 mm ID, glass
Samples injected: Neat liquid (3 ml) and solutions of 1% and 0.5% (v/v) 2,6-xylidine in methanol

Results: Major peak and one impurity before the major peak with an area 0.87% that of the major peak

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	17.7	0.91	0.87
2	19.5	1.00	100

C. Comparison of the purity of the three lots of 2,6-xylidine that were used at EG&G Mason

Purity analysis: During 1978, purity checks were run on three different 2,6-xylidine lots with infrared spectroscopy (IR) and gas chromatography (GC) techniques. The IR spectra of the three lots were identical to one another and to that of the reference 2,6-xylidine sample (Aldrich No. D14,600-5).

The GC results presented in Table D1 indicate that the purity of 2,6-xylidine was greater than 99% in lots I and II and 98.7% in lot III. Lot III showed two impurities, lots I and II, one impurity. The major impurity (relative retention time, 0.71) present in lot III was also present to a similar extent in lot II, but was absent in lot I. The minor impurity in lot III was present in lot I but absent in lot II.

Infrared spectroscopy

Instrument: Perkin Elmer Model 457
Cell: Neat between potassium bromide prisms with 0.015-mm spacer

Results: Spectra of samples were identical to that of Reference No. D14,600-5 (Aldrich) and comparable to each other.

Gas chromatography

Instrument: Varian 2100
Column: 1.8 m × 2 mm ID glass packed with 10% FFAP on 80/100 mesh Chromosorb WAW
Column temperature: 190° C, isothermal
Detector: Flame ionization

Results: The gas chromatographic analysis indicated that the purity of the batches analyzed ranged from 98.7% to greater than 99% based on peak area comparisons.

TABLE D1. GAS CHROMATOGRAPHIC ANALYSIS OF 2,6-XYLIDINE

Peak No.	RRT (a)	Lot I	Lot II	Lot III
1	0.71	--	0.91	1.2
2	1.00	99.91	99.07	98.7
3	2.40	0.09	--	0.13

(a) RRT = Relative retention time

APPENDIX E

CHARACTERIZATION OF FORMULATED DIETS

APPENDIX E. FORMULATED DIETS

Stability Study of Formulated Diets Performed by the Analytical Chemistry Laboratory

I. Sample Preparation and Storage: Three 12-oz screw cap jars were filled with 300 g of the formulated diet and tightly sealed. Individual jars were stored in the dark at −20° C and 5° C and at room temperature and were sampled for analysis after 7 and 14 days. Twelve 200-ml centrifuge bottles, each containing 10 g of the formulated diets, were laid uncapped on the bedding in a rat cage equipped with a filter cap. Three bottles were removed and analyzed after 1, 3, 5, and 7 days.

II. Analytical Method: Formulated diet samples and control feed (10 g, weighed in triplicate into 200-ml centrifuge bottles) were extracted with 100 ml of acetonitrile by shaking for 15 minutes on a wrist-action shaker. The extracts were clarified by centrifuging; then 3-ml aliquots were pipetted into 100-ml volumetric flasks containing 3 ml of internal standard solution (*n*-decane, 0.55 mg/ml in acetonitrile), and diluted to 100 ml with acetonitrile. The 2,6-xylidine content of the solutions was determined by the gas chromatographic system described below:

Instrument: Varian 3700 equipped with autosampler and CDS-11 integrator
Column: 10% SP2100 on 80/100 Supelcoport, 1.8 m × 2 mm ID, glass
Detector: Flame ionization
Temperatures
Injection: 120° C
Oven: 70° C Isothermal for 5 min, then 5° C/min to 130° C
Detector: 180° C
Carrier gas: Nitrogen
Flow rate: 30 ml/min
Injection volume: 4 µl
Retention times: 2,6-Xylidine--14.2 min; *n*-decane (internal standard)--9.5 min

III. Quality Assurance Measures: All stability samples were analyzed in triplicate. The chemical analyses were conducted by making duplicate injections of the solutions prepared from the stability samples, following a randomized order. A standard solution of 2,6-xylidine was injected after every third sample to calibrate detector response. A second, independently prepared standard was used to verify the accuracy of the calibration standard. An internal standard was incorporated into all sample and standard solutions. Recovery of the chemical from feed was determined in triplicate with undosed feed spiked at the same level as the samples.

The chromatographic system was evaluated for linearity of response with standard solutions of 2,6-xylidine in acetonitrile at concentrations of 19.7, 29.5, and 34.3 µg/ml. The correlation coefficient was 1.00000. All sample analysis results were used to calculate the pooled standard deviation for the method.

IV. Results

A. Open storage in a rat cage at ambient temperatures

Days	2,6-Xylidine (a) Found (mg/g)	Percent of Chemical Found (b) Zero-Time Analysis	Total Loss of Chemical (percent)
1	9.04 ± 0.09	91.0 ± 1.0	9.0
3	7.69 ± 0.10	77.3 ± 1.0	22.7
5	7.02 ± 0.09	70.6 ± 0.9	29.4
7	5.57 ± 0.20	56.0 ± 2.0	43.9

(a) Results were corrected for the mean recovery of 2,6-xylidine determined in triplicate with spiked feed on each analysis day. The mean ± standard deviation of the recovery determinations was 99.1% ± 0.3% (n=9).
(b) Recoveries were computed by the mean of the nine zero-time analyses (9.94 mg/g).

B. Storage in sealed bottles in the dark

Days	Temperature	2,6-Xylidine (a) Found (mg/g)	Percent of Chemical Found (b) Zero-Time Analysis	Total Loss of Chemical (percent)
4	Room	8.87 ± 0.03	89.2 ± 0.3	10.8
7	−20° C	9.82 ± 0.02	98.8 ± 0.2	1.2
	5° C	9.57 ± 0.02	96.4 ± 0.2	3.6
	Room	8.78 ± 0.03	88.2 ± 0.3	11.8
14	−20° C	9.81 ± 0.07	98.5 ± 0.4	1.5
	5° C	9.62 ± 0.04	96.8 ± 0.4	3.2
	Room	8.49 ± 0.06	85.4 ± 0.6	14.6

(a) Results were corrected for the mean recovery of 2,6-xylidine determined in triplicate with spiked feed on each analysis day. The mean ± standard deviation of the recovery determinations was 98.9% ± 0.4% (n=9).
(b) Recoveries were computed by the mean of the nine zero-time analyses (9.94 mg/g).

C. Summary of stability loss data

Storage Condition	Mean Daily Loss (percent)
Open/rat cage/room temp	7.2 (n=12)
Sealed/dark/−20° C	0.13 (n=6)
Sealed/dark/5° C	0.38 (n=6)
Sealed/dark/room temp	1.8 (n=9)

V. Discussion: Inspection of the feed stability results showed that 2,6-xylidine was unstable under all conditions of storage. Closer examination of the data revealed two major causes of chemical loss. Samples of formulated diets stored in sealed bottles lost chemical by reacting with feed components. Losses from samples stored open in a rat cage were caused both by reaction with feed and by evaporation.

2,6-Xylidine exhibits considerable volatility at room temperature. For example, 50-ml beakers containing approximately 2 ml of the chemical lost an average of 4.0%, 12.0% and 57.9% of their initial weight after 1, 3, and 18 hours in a hood. Analysis of the samples after 7 days' storage at room temperature in the dark showed no significant difference between the two methods of storage (88.3% vs. 87.2%). These findings confirmed that the losses reported on the sealed bottle samples in the stability study (Section IV. B.) were due to interaction with feed.

It was estimated from a comparison of the average percentage loss per day data (Section IV. C.) that 70%-80% of the loss seen in the rat cage samples was caused by evaporation. With this level of evaporation from formulated diets, it is likely that animals confined in a rat cage would inhale significant levels of 2,6-xylidine during a study of this chemical.

At the conclusion of the stability study, an additional experiment was performed to determine if the low analysis results for sealed samples were due to vaporization of 2,6-xylidine, followed by absorption into the jar cap liner. Formulated diets were stored in the same 12-oz screw-cap jars used for the stability study and in sealed, all-glass containers.

VI. Conclusions: Feed blends with 2,6-xylidine (10 mg/g level) were unstable under all storage conditions. Losses over 14 days' storage in the dark at $-20°$ C and 5° C and at room temperature were 1.5%, 3.2%, and 14.6%, respectively. A minimum of 1.2% loss of chemical was necessary to conclude, at the 95% confidence level, that a sample was unstable.

APPENDIX F

ANALYSIS OF FORMULATED DIETS

APPENDIX F. ANALYSIS OF FORMULATED DIETS

I. Dose Verification Analysis

A. Method: Duplicate aliquots of 1 ml from each sample were diluted to 100 ml with hexane and mixed in 100-ml glass-stoppered graduated cylinders. After further tenfold dilutions with hexane, the absorbances were determined in 1-cm quartz cells at 280 nm with a Perkin-Elmer Lambda 3 spectrophotometer. Spiked and blank corn oil samples were prepared and analyzed in the same manner to provide calibration data. Results are presented in Table F1.

B. Dose verification: In March of 1978, an analytical spectrophotometric procedure was developed to determine 2,6-xylidine levels in formulated diet samples based on "method recovery" (i.e., control feed containing a known amount of 2,6-xylidine was analyzed for percent recovery, and the sample results at each dose level were corrected for the corresponding percent recovery value). In January 1980 a more effective gas chromatographic procedure involving a simple extraction method was developed. To validate the method, a comparative study was made on the same set of samples using the gas chromatographic and spectrophotometric methods (Table F2).

C. Spectrophotometric method for analysis of 2,6-xylidine in feed

Summary: The feed sample is extracted with 0.1 N hydrochloric acid (HCl). The extract is made alkaline with concentrated ammonia and the compound partitioned into benzene. The benzene extract is then partitioned with 0.1 N HCl to isolate the compound. The compound is coupled with diazotized sulfanilic acid and the resulting chromophore determined spectrophotometrically.

Reagents

0.1 N HCl: Dilute 8.3 ml concentrated HCl to 1,000 ml with deionized water
Sulfanilic acid reagent: Dissolve 600 mg sulfanilic acid in 70 ml hot deionized water; cool, add 20 ml concentrated HCl; cool and dilute to 100 ml with water
0.1% Sodium nitrite (prepared fresh at least once per week; sodium nitrite is not stable): Dissolve 100 mg sodium nitrite in 100 ml deionized water
0.5% Ammonium sulfamate: Dissolve 500 mg ammonium sulfamate in 100 ml deionized water
Concentrated ammonia (15 N)
Benzene, reagent grade
Stock standard: Accurately weigh, to the nearest ±0.01 mg, 25 mg of 2,6-xylidine and dilute to 25.0 ml with 0.1 N HCl to obtain 1 mg/ml
Working standard: Dilute stock standard, 5.0 ml to 100.0 ml, with 0.1 N HCl to obtain 50 ppm

TABLE F1. CONCENTRATIONS OF 2,6-XYLIDINE IN CORN OIL MIXTURES IN THE SHORT-TERM STUDIES

Date Mixed	Concentration of 2,6-Xylidine in Corn Oil for Target Concentration (ppm) (w/v%)							
	2	4	8	16	31	62	124	250
11/03/80 (a)	--	--	--	16.2	33.5	59.0	123.0	248.0
11/24/80 (a)	--	--	8.6	16.5	31.6	62.9	157.5	--
02/12/81 (b)	--	4.0	8.4	16.0	31.2	61.5	--	--
04/08/81 (b)	2.0	3.8	7.8	15.91	30.9	59.7	--	--

(a) Two-week study dose mixtures
(b) Thirteen-week study dose mixtures

TABLE F2. COMPARISON OF GAS CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS USED IN THE 2,6-XYLIDINE ANALYSIS

Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Percent of Target
Spectrophotometric Method			
1/9/80	300	275	91.7
		274	91.3
		278	92.7
		213	71.0
		264	88.0
		231	77.0
		260	86.7
		284	94.7
		247	82.3
Mean			86.1
Standard deviation			7.9
	3,000	2,520	84.0
		2,232	74.4
		2,330	77.7
		2,028	67.6
		2,394	79.8
		2,047	68.2
		2,263	75.4
		2,143	71.4
		2,212	73.7
Mean			74.7
Standard deviation			5.3
Gas Chromatographic Method			
1/22/80	300	253	84.2
		259	86.5
		251	83.7
		266	88.8
		248	82.7
		248	82.6
		243	81.1
		252	83.9
Mean			84.2
Standard deviation			2.3
	3,000	2,389	79.6
		2,515	83.8
		2,473	82.4
		2,455	81.8
		2,427	80.9
		2,489	83.0
		2,467	82.2
		2,467	82.2
		2,426	80.9
Mean			81.9
Standard deviation			1.2

Procedure: Weigh 5-g subsamples of control and study diets. For each study diet concentration, weigh a 5-g portion of control diet and add an amount of 2,6-xylidine equivalent to the amount in the study diet. Add 25 ml of 0.1 N HCl, and shake on an automatic shaker for 10 minutes; then centrifuge for 10 minutes at 1,500 rpm. Decant the supernatant through glass wool into a 125-ml Erlenmeyer flask. Repeat the extraction with a second 25-ml portion of dilute acid, and add the extract to the first. Mix well, and transfer 10.0 ml of the extract to a 50-ml centrifuge tube. Add 0.2 ml concentrated ammonia and 10.0 ml benzene. Shake 5 minutes on the shaker, and centrifuge at 9,500 rpm for 30 minutes at 5° C. Transfer 5.0 ml of the upper benzene phase to another 50-ml tube, and add 5.0 ml of 0.1 N HCl. Shake 5 minutes, and centrifuge 5 minutes. Aspirate off the upper benzene layer.

Set up five 15-ml tubes for calibration standards and a similar tube for each study diet and recovery sample. Also set up a background control tube for each diet level. To all tubes add 0.5 ml of sulfanilic acid reagent, and dilute to 10.0 ml with 0.1 N HCl. Add 1.0 ml sodium nitrite solution, mix well, and let stand 3 minutes. Add 1.0 ml ammonium sulfamate solution, mix well, and let stand 3 min. To the tubes for the calibration standards, add 0, 0.1, 0.2, 0.4, and 1.0 ml of the working standard (equivalent to 0, 5, 10, 20, and 50 µg 2,6-xylidine). To the appropriate study diet or recovery sample tube, add an amount of the final 0.1N HCl extract, not to exceed 1.0 ml, containing between 5 and 50 µg. To the appropriate background control tube, add equivalent volumes of the final 0.1 N HCl extract of the control feed sample. Dilute all tubes to 13.0 ml with 0.1N HCl, mix well, and let stand overnight.

Determination of absorbance: Use 1 cm cells to determine the absorbance of each solution. Use the 0-µg calibration standard in the reference cell at 495 nm. Correct the readings for the study diet and recovery samples for their respective control feed values. From the calibration curve obtained with the standards, calculate the micrograms of 2,6-xylidine in each study diet or recovery sample tube. Determine the parts per million of 2,6-xylidine in these samples and correct the results for the study diets for their respective recoveries.

D. Gas chromatographic method for analysis of 2,6-xylidine in feed

Principle: 2,6-xylidine is extracted into toluene containing a fixed amount of biphenyl per milliliter of extractant. Biphenyl serves as an internal standard for gas chromatographic quantitation of 2,6-xylidine.
Equipment: Hewlett-Packard 5880A gas chromatograph with 7672A auto liquid sampler

Procedure

Preparation of standard solutions: Prepare an internal standard solution (ISTD) by dissolving approximately 1.00 g of biphenyl in toluene. Bring to a final volume of 1.00 liter in a volumetric flask to yield a concentration of 1.00 mg biphenyl/ml toluene.

Prepare a stock 2,6-xylidine solution by weighing approximately 0.1000 g 2,6-xylidine into a 50-ml volumetric flask. Bring to volume with ISTD to yield a concentration of 2 mg 2,6-xylidine/ml ISTD.

Preparation of calibration standards: Weigh 5.00 g of control feed into each of six centrifuge tubes. To centrifuge tubes 1 through 6, add 0.50, 1.00, 2.50, 5.00, 7.50, and 10.00 ml, respectively, of stock 2,6-xylidine solution. Bring the final volume of extractant in

each tube to 10.0 ml by adding ISTD. Add 10.00 ml of 1% ammonium hydroxide solution to each calibration standard.

Preparation of dosed feed samples: Weigh 5.00 g of each formulated diet sample into separate centrifuge tubes. Add 10.00 ml of ISTD and 10.00 ml of 1% ammonium hydroxide to each sample. Shake all calibration standards and samples on a shaker box for 15 minutes, centrifuge for 10 minutes at 1,500 rpm, and take an aliquot of the top (organic) layer for gas chromatographic analysis.

Gas chromatographic conditions

Instrument: HP 5880A with 7672A auto liquid sampler
Detector: Flame ionization
Carrier gas: Nitrogen
Flow Rate: 40 ml/min
Temperatures: Injector--250° C; detector--275° C; oven--90° C for 5 minutes and then 90°-200° C at 25° C per minute; post temp--250° C for 5 minutes

System 1

Column: 3% SP2100 on 80/100 mesh Supelcoport, 1.8 m × 2 mm ID, silanized glass
Retention times: 2,6-Xylidine--3.7 min; biphenyl--7.8 min

System 2

Column: 3% SP2550 DB on 100/120 mesh Supelcoport, 1.8 m × 2 mm ID silanized glass
Retention Times: 2,6-Xylidine--3.5 min; biphenyl--7.4 min

II. Homogeneity

Formulated diets were analyzed for homogeneity at the beginning of and midway through the studies at 300, 1,000, and 3,000 ppm (Table F3). The data indicate that homogeneity of 2,6-xylidine throughout the feed was good; however, the 1979 homogeneity study samples assayed less than −15% of the target.

III. Stability

Aliquots of feed from the 3,000-ppm dose were analyzed after storage at ambient laboratory temperatures for 0, 7, 14, 21, and 28 days. The results presented in Table F4 indicate that 2,6-xylidine in feed appears to be stable up to 28 days, since there are no significant variations in concentration at various time intervals. The limitation of the spectrophotometric method to offer consistent results may explain the discrepancies in percent recoveries.

IV. Formulation of Diets

Formulated diets were monitored for 2,6-xylidine concentrations once per week for the first 8 weeks and at various intervals thereafter throughout the duration of the studies (Table F5). Of the 24 mixtures assayed at 300, 1,000, and 3,000 ppm, 22 (92%) were within ±15% of the target at all three concentrations.

V. Conclusions

The 2,6-xylidine used in these studies was stable in the formulated diets for up to 28 days. Formulated diet mixtures were homogeneous at all three concentrations; 92% of the mixtures were within ±15% of the target levels.

TABLE F3. HOMOGENEITY OF 2,6-XYLIDINE DIET MIXTURES IN THE TWO-YEAR STUDIES

Date Mixed	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Percent of Target
Spectrophotometric Method (a)				
5/8/78	5/9/78	300	296	98.5
			278	92.7
			321	107.0
		1,000	976	97.6
			959	95.9
			955	95.5
		3,000	2,683	89.4
			2,724	90.7
			2,483	82.8
Gas Chromatographic Method (b)				
12/7/79	1/22/80	300	253	84.2
			259	86.5
			251	83.7
			266	88.8
			248	82.7
			248	82.6
			243	81.1
			252	83.9
Mean				84.2
Standard deviation				2.3
12/7/79	1/22/80	1,000	722	72.2
			715	71.5
			770	77.0
			755	75.5
			759	75.9
			750	75.0
			741	74.1
			760	76.0
			765	76.5
Mean				74.8
Standard deviation				1.9
12/7/79	1/22/80	3,000	2,389	79.6
			2,515	83.8
			2,473	82.4
			2,455	81.8
			2,427	80.9
			2,489	83.0
			2,467	82.2
			2,467	82.2
			2,426	80.9
Mean				81.9
Standard deviation				1.2

(a) Corrected for method recovery
(b) Built-in recovery method

TABLE F4. STABILITY OF 2,6-XYLIDINE IN THE FORMULATED DIETS (a)

Days from Mixing to Analysis	Target Concentration (ppm)	Determined Concentration (b) (ppm)	Percent of Target
1	3,000	2,703	90.1
7		2,436	81.2
14		2,429	81.0
21		2,550	85.1
28		2,490	83.0

(a) At ambient temperatures
(b) Spectrophotometric method

TABLE F5. CONCENTRATIONS OF 2,6-XYLIDINE IN FEED IN THE TWO-YEAR STUDIES

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	Percent of Target
Spectrophotometric Method			
5/16/78	300	305	102.0
	1,000	882	88.2
	3,000	2,961	98.7
5/23/78	300	297	98.9
	1,000	931	93.1
	3,000	3,054	102.0
5/31/78	300	311	104.0
	1,000	1,130	113.0
	3,000	3,050	102.0
6/6/78	300	322	107.0
	1,000	1,078	108.0
	3,000	3,060	102.0
6/12/78	300	299	99.5
	1,000	1,046	104.6
	3,000	2,820	94.0
6/19/78	300	282	94.0
	1,000	998	99.8
	3,000	2,960	98.8
6/27/78	300	284	94.7
	1,000	992	99.2
	3,000	2,810	93.6
7/3/78	300	299	99.5
	1,000	1,015	101.5
	3,000	2,697	89.9
7/18/78	300	328	109.0
	1,000	1,058	105.8
	3,000	3,027	101.0
8/21/78	300	287	95.6
	1,000	966	96.6
	3,000	2,800	93.5

TABLE F5. CONCENTRATIONS OF 2,6-XYLIDINE IN FEED IN THE TWO-YEAR STUDIES (Continued)

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	Percent of Target
10/16/78	300	290	96.8
	1,000	912	91.1
	3,000	2,900	96.7
11/20/78	300	294	98.1
	1,000	922	92.2
	3,000	3,150	105.0
12/18/78	300	319	106.0
	1,000	1,017	101.7
	3,000	3,028	101.0
1/15/79	300	301	100.0
	1,000	1,079	107.9
	3,000	3,009	100.0
Gas Chromatographic Method			
11/20/79	300	233	77.6
	1,000	713	71.3
	3,000	2,270	75.6
1/18/80	300	319	106.0
	1,000	934	93.4
	3,000	2,744	91.5
2/22/80	300	279	93.0
	1,000	950	95.0
	3,000	2,884	96.1
3/21/80	300	275	91.7
	1,000	944	94.4
	3,000	2,886	96.2
4/18/80	300	282	94.0
	1,000	922	92.2
	3,000	2,750	91.8
5/16/80	300	296	98.7
	1,000	909	90.9
	3,000	2,805	93.5
6/13/80	300	287	95.7
	1,000	922	92.2
	3,000	2,790	93.0
7/22/80	300	273	91.0
	1,000	872	87.2
	3,000	2,700	90.2
8/15/80	300	276	92.2
	1,000	942	94.2
	3,000	2,830	94.4

APPENDIX G

AUDIT SUMMARY

APPENDIX G. AUDIT SUMMARY

The experimental data, documents, and pathology specimens for the 2-year feed studies of 2,6-xylidine in Charles River CD rats were audited for accuracy, consistency, and completeness. The laboratory study was conducted by Litton Bionetics, Inc. (Rockville, MD) for the U.S. Environmental Protection Agency (Cincinnati, OH). The sponsorship of the studies was transferred in September 1980 to the National Toxicology Program (NTP). The gross and microscopic pathologic examinations were conducted by Experimental Pathology Laboratories, Inc. Animal exposure to the chemical in feed began in September 1978. The retrospective audit was conducted for the National Institute of Environmental Health Sciences (NIEHS) at the NTP Archives during April to June 1986 and June 1988 by Dynamac Corporation. The full audit report is on file at the NIEHS. The audit included review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All inlife records including protocol, correspondence, dosing, feed consumption, environmental conditions, animal husbandry, mortality, inlife animal identification, and correlation between clinical and necropsy observations of external masses.
(3) Body weight and clinical observation data for a random sample of five animals per sex per dose group.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning disposition codes, condition codes, and correlations between necropsy observations and microscopic diagnoses.
(6) Individual Animal Data Records from a random 10% sample of animals for data entry errors and the Individual Animal Tumor Pathology Tables for tissue accountability (100%).
(7) All wet tissue bags for inventory and wet tissues from a random 10% sample of all dose groups, plus other relevant cases, to check animal identity and to examine for untrimmed potential lesions.
(8) Blocks and slides of tissues from all control and high dose group animals to examine for proper match and inventory.
(9) Tabulated pathology diagnoses for a random 10% sample of animals to verify computer data entry.

Procedures and events were documented adequately in the archival records, with the exception of the following: (1) quarantine, (2) randomization, (3) cage and rack changes, (4) environmental conditions, (5) clinical observations, (6) records for receipt and shipment of the study chemical, (7) study chemical inventory, (8) dose preparation records before December 22, 1978, (9) bulk chemical reanalysis, (10) corn oil analysis, and (11) referee analysis.

On one occasion, the interval between two dose mixture analyses was 11 months. Of 48 dose mixtures analyzed, 11 were outside the $\pm 10\%$ tolerance limit.

Inspection of wet tissues for individual animal identifiers showed that 54/57 rats were identified correctly by the metallic ear tags. The animals with other than correct identification codes were evaluated against other toxicology and pathology audit findings. The results indicated that one identification error (mid dose male) was related to inconsistent saving of the identifier. Audit of the data trail for two rats (one mid dose male and one high dose male) revealed noncorroborative evidence in the gross and microscopic findings. There were seven untrimmed potential lesions in the wet tissues of 57 rats examined; these potential lesions were in the foot and liver and were distributed among sex and dose groups. Evaluation of 4,560 blocks and 4,781 slides revealed 123 missing slides (replacements were not present for the missing slides).

No changes to the pathology tables were necessitated by the audit findings. Minor revisions of protocol descriptions and in calculation of means for chemical analyses have been incorporated in the Technical Report. In conclusion, the results of the audit support the data presented in this Technical Report.

☆ U. S. GOVERNMENT PRINTING OFFICE : 1990 O - 256-954 QL.3

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF SEPTEMBER 1989

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-p-dioxin (Dermal)
206	Dibromochloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane (Inhalation)
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butylbenzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-*p*-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
229	Guar Gum
230	Agar
231	Stannous Chloride
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	D-Mannitol
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos
250	Benzyl Acetate
251	Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	Propylene Oxide
269	Telone II®
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four strains of rats)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl)phosphonium Sulfate and Tetrakis(hydroxymethyl)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
353	2,4-Dichlorophenol
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
361	Hexachloroethane

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.