NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF *p*-NITROANILINE

(CAS NO. 100-01-6)

IN B6C3F$_1$ MICE

(GAVAGE STUDIES)

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

May 1993

NTP TR 418

NIH Publication No. 93-3149

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

CONTRIBUTORS

National Toxicology Program
C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.D. Irwin, Ph.D.
C.W. Jameson, Ph.D.
G.N. Rao, D.V.M., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Southern Research Institute
Conducted studies, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
D.R. Farnell, D.V.M., Ph.D.
H.D. Giles, D.V.M., Ph.D.
R.B. Thompson, D.V.M., Ph.D.

Integrated Laboratory Systems
Prepared quality assurance audits

J.C. Bhandari, D.V.M., Ph.D., Principal Investigator

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
J.F. Mahler, D.V.M.

NTP Pathology Working Group
Evaluated slides, prepared pathology report on mice (7 August 1990)

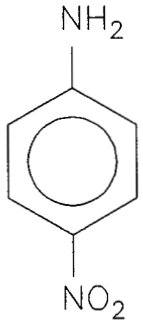
P.K. Hildebrandt, D.V.M., Ph.D., Chair
PATHCO, Inc.
G.A. Boorman, D.V.M., Ph.D
National Toxicology Program
R. Cattley, V.M.D., Ph.D.
North Carolina State University
M.P. Jokinen, D.V.M.
National Toxicology Program
J.F. Mahler, D.V.M.
National Toxicology Program
A. Pinter, M.D., Ph.D.
National Institute of Hygiene, Hungary

Biotechnical Services, Inc.
Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
P. Chaffin, M.S.
G.F. Corley, D.V.M.
J.A. Gregan, M.A.

CONTENTS

ABSTRACT



***p*-NITROANILINE**

CAS No. 100-01-6

Chemical Formula: $C_6H_6N_2O_2$ Molecular Weight: 138.12

p-Nitroaniline is an intermediate in the preparation of several azo dyes used for coloring consumer products. Toxicology and carcinogenicity studies were conducted by administering *p*-nitroaniline (>99% pure) in corn oil by gavage to groups of male and female B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, mouse lymphoma cells, and *Drosophila melanogaster*.

14-Day Studies

Groups of five male and five female B6C3F$_1$ mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 10, 30, 100, 300, or 1,000 mg/kg body weight 5 days per week for 2 weeks. All mice that received 1,000 mg/kg died from chemical-related toxicity by day 4 of the studies. Final mean body weights of mice receiving 300 mg/kg or less were similar to those of the controls. Hematology results were consistent with chemical-related methemoglobinemia and regenerative anemia. Methemoglobin concentrations in all groups of dosed mice were significantly higher than those in controls. Hematocrit values in mice that received 300 mg/kg and total erythrocyte counts in mice that received 100 or 300 mg/kg were significantly lower than those in controls. Reticulocyte counts in 300 mg/kg male mice and in 100 or 300 mg/kg females were significantly higher than controls. Heinz bodies were observed in erythrocytes of all 300 mg/kg mice and in two 100 mg/kg male mice. The absolute and relative spleen weights of 100 and 300 mg/kg mice were significantly greater than those of the controls. Hematopoiesis and pigment (hemosiderin) accumulation were observed in the splenic red pulp of males and females receiving 300 mg/kg; pigment (hemosiderin) accumulation in Kupffer cells of the liver was also seen in male mice at this dose level.

13-Week Studies

Groups of 20 male and 20 female B6C3F$_1$ mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 1, 3, 10, 30, or 100 mg/kg body weight 5 days per week for up to 13 weeks. Eight or nine mice in each group were evaluated at 7 weeks. There were no deaths associated with exposure to *p*-nitroaniline, and final mean body weights of dosed mice were similar to those of the controls. Hematologic and pathologic findings at 7 and 13 weeks were similar to those seen in the 14-day studies and occurred primarily in the 30 and 100 mg/kg groups. Methemoglobin concentrations were increased and hematocrit levels and erythrocyte counts were decreased relative to those of the controls. Heinz bodies were observed in

erythrocytes and nucleated erythrocytes and reticulocytes were increased in number.

Absolute and relative spleen weights of male and female mice receiving 30 and 100 mg/kg were significantly greater than those of controls at 7 and 13 weeks. Absolute and relative liver weights of female mice necropsied at 7 weeks were significantly greater in the 30 and 100 mg/kg groups; by 13 weeks, both absolute and relative liver weights were similar to control values. The incidence or severity of splenic hematopoiesis and pigmentation (hemosiderin) increased with dose at the 7-week interim evaluations and at the end of the studies. Pigment (hemosiderin) was also present in Kupffer cells of the liver in dosed male mice.

2-YEAR STUDIES

Groups of 70 male and 70 female B6C3F$_1$ mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 3, 30, or 100 mg/kg body weight for 5 days per week for up to 103 weeks. The dose selection was based on the hematologic and pathologic findings of the 13-week studies. Nine or ten mice from each group were evaluated at 9 and 15 months for the presence of chemical-related lesions.

Body Weights, Clinical Findings, Survival, and Hematology

Mean body weights of male and female mice that received *p*-nitroaniline were similar to those of control mice throughout the 2-year studies. There were no clinical findings associated with chemical exposure, and survival of dosed mice was similar to that of controls. The hematology findings at the 9- and 15-month interim evaluations were similar to those in the 14-day and 13-week studies. The methemoglobin concentrations were significantly higher in all 30 or 100 mg/kg mice; sulfhemoglobin concentrations were significantly higher at 9 months in all 30 or 100 mg/kg female mice and at 15 months in 100 mg/kg females. Hematocrit and erythrocyte counts in 100 mg/kg mice were significantly lower than those in controls. By 9 months, reticulocyte counts were significantly higher in all 30 or 100 mg/kg mice. At 15 months, only the 100 mg/kg mice exhibited significantly higher reticulocyte counts.

Neoplasms and Nonneoplastic Lesions

Lesions related to the administration of *p*-nitroaniline occurred in the spleen, liver, and bone marrow, primarily in mice receiving 30 or 100 mg/kg; these were observed at the 9- and 15-month interim evaluations and at the end of the studies. There were increases in the incidence or severity of splenic congestion, hematopoiesis, pigment (hemosiderin) accumulation, Kupffer cell pigmentation in the liver, and bone marrow hypercellularity (hyperplasia).

The incidences of hemangiosarcoma of the liver (0 ppm, 0/50; 3 ppm, 1/50; 30 ppm, 2/50; 100 ppm, 4/50) and hemangioma or hemangiosarcoma (combined) at all sites (5/50, 3/50, 4/50, 10/50) were marginally increased in 100 mg/kg male mice. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly decreased (25/50, 26/50, 25/50, 13/50) in 100 mg/kg male mice.

GENETIC TOXICOLOGY

p-Nitroaniline is mutagenic *in vitro*. It was tested in two laboratories for induction of gene mutations in several strains of *Salmonella typhimurium*. Both studies showed positive results in strain TA98, with and without S9 activation; results were negative for all other strains. *p*-Nitroaniline was tested in two laboratories for induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. In the sister chromatid exchange study, one laboratory reported negative results without S9 and positive results with S9; the second laboratory reported equivocal results without S9 and negative results with S9. In the chromosomal aberrations study, both laboratories found positive results with S9. Without S9, one laboratory reported weakly positive results while the other reported negative results. *p*-Nitroaniline induced trifluorothymidine resistance in L5178Y mouse lymphoma cells in the absence of S9; no induction of trifluorothymidine resistance was noted with S9. In contrast to the positive results in the previous tests, *p*-nitroaniline did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered by feeding or injection to adult males or by feeding to larvae.

CONCLUSIONS

Under the conditions of these 2-year gavage studies there was *equivocal evidence of carcinogenic activity** of *p*-nitroaniline in male B6C3F$_1$ mice based on the increased incidences of hemangiosarcoma of the liver and hemangioma or hemangiosarcoma (combined) at all sites. There was *no evidence of carcinogenic activity* of *p*-nitroaniline in female B6C3F$_1$ mice receiving doses of 3, 30, or 100 mg/kg.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of *p*-Nitroaniline

	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses	0, 3, 30, or 100 mg/kg by corn oil gavage	0, 3, 30, or 100 mg/kg by corn oil gavage
Body weights	Similar to controls	Similar to controls
2-Year survival rates	33/50, 32/50, 36/50, 39/50	29/52, 41/50, 32/51, 32/51
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Uncertain findings	Liver: hemangiosarcoma (0/50, 1/50, 2/50, 4/50) All organs: hemangioma or hemangiosarcoma (5/50, 3/50, 4/50, 10/50)	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence
Genetic toxicology		
Salmonella typhimurium gene mutation	Positive with and without S9 in strain TA98; Negative with and without S9 in strains TA100, TA1535, TA1537, and TA97	
Mouse lymphoma gene mutation	Negative with S9; positive without S9	
Sister chromatid exchanges		
Chinese hamster ovary cells *in vitro*:	Positive with S9; equivocal without S9	
Chromosomal aberrations		
Chinese hamster ovary cells *in vitro*:	Positive with S9; weakly positive without S9	
Sex-linked recessive lethal mutations		
Drosophila melanogaster:	Negative when administered by feed or injection	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on *p*-nitroaniline on November 21, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

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

Curtis D. Klaassen, Ph.D., Chair
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Paul T. Bailey, Ph.D., Principal Reviewer
Toxicology Division
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H., Principal Reviewer
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

Gary P. Carlson, Ph.D.
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Kowetha A. Davidson, Ph.D.
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Harold Davis, D.V.M., Ph.D.*
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, D.V.M.
Consultants in Veterinary Pathology
Murrysville, PA

Jay I. Goodman, Ph.D.
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

David W. Hayden, D.V.M., Ph.D.
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Daniel S. Longnecker, M.D.*
Department of Pathology
Dartmouth Medical School
Lebanon, NH

Barbara McKnight, Ph.D.
Department of Biostatistics
University of Washington
Seattle, WA

Ellen K. Silbergeld, Ph.D.*
University of Maryland Medical School
Baltimore, MD

Matthew J. van Zwieten, D.V.M., Ph.D.,
Principal Reviewer
Department of Safety Assessment
Merck, Sharp & Dohme Research Laboratories
West Point, PA

Lauren Zeise, Ph.D.
California Department of Health Services/RCHAS
Berkeley, CA

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 21, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of *p*-nitroaniline received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of *p*-nitroaniline by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on neoplasms in male mice and nonneoplastic lesions in male and female mice. The proposed conclusions were *equivocal evidence of carcinogenic activity* in male B6C3F$_1$ mice and *no evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. M.J. van Zwieten, a principal reviewer, agreed with the conclusions. He thought there was insufficient discussion of the results of the 2-year study in rats recently reported in the literature. Dr. Irwin said the discussion of the rat study would be expanded. Dr. van Zwieten suggested that more discussion would be appropriate regarding selection of gavage administration when previous NTP studies of aniline and substituted anilines used the dietary route. Dr. Irwin said the compound was given by gavage because it was not stable in feed. Dr. van Zwieten said a brief histomorphological description of the vascular neoplasms observed would be useful in indicating the criteria used to distinguish benign from malignant lesions. Dr. Irwin agreed.

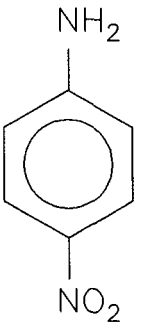
Dr. P.T. Bailey, the second principal reviewer, agreed with the conclusions. He questioned why 1,000 mg/kg was chosen as a dose level for the 14-day studies in view of the oral LD_{50} in mice cited as 750 mg/kg. Dr. Irwin commented that the top dose in the 14-day study is chosen to be sufficiently high enough to elicit a toxic response and, thus, may in some instances exceed the LD_{50}. Dr. Bailey wondered whether dietary administration would have been more akin to actual human exposure to the chemical.

Mr. L.S. Beliczky, the third principal reviewer, did not agree with the conclusions in male mice. He said that hemangioma or hemangiosarcoma (combined) at all sites showed a significant positive trend, and although incidences in the dosed groups were not significantly greater than controls by pairwise comparisons, the incidence of these neoplasms in the high-dose group (20%) exceeded the NTP historical control range (0% to 12%). Therefore, he thought the level of evidence in male mice should be *some evidence of carcinogenic activity*. Dr. Irwin said the level chosen was based on the fact that the neoplasms were only marginally increased in incidence and there was no comparable response in female mice. Mr. Beliczky commented that since these studies may have application to specific industries, the Production and Use section in the Introduction should be expanded to identify which type of industries manufacture and use the end products, among which are antioxidants and antiozonants. He believed that since 1978, NIOSH might have additional use and exposure data. Dr. Irwin asked Mr. Beliczky if he could obtain information about industries that produce these products.

Dr. L. Zeise questioned whether the maximum tolerated dose had been reached in the 2-year studies. Dr. Irwin replied that based on persistent anemia observed in 13-week studies, there was belief that some mortality was likely if 300 mg/kg were the top dose in the 2-year studies. Dr. S.L. Eustis, NIEHS, acknowledged that a higher top dose probably could have been tolerated, and a statement to that effect was added to the Discussion.

Dr. van Zwieten moved that the Technical Report on *p*-nitroaniline be accepted with the revisions discussed and with the conclusions as written for male mice, *equivocal evidence of carcinogenic activity*, and for female mice, *no evidence of carcinogenic activity*. Dr. Bailey seconded the motion, which was accepted by nine yes votes to one no vote (Mr. Beliczky).

INTRODUCTION



p-NITROANILINE

CAS No. 100-01-6

Chemical Formula: $C_6H_6N_2O_2$ Molecular Weight: 138.12

PHYSICAL AND CHEMICAL PROPERTIES

p-Nitroaniline is a bright yellow powder with a melting point of 146° C. It is soluble in methanol and benzene and slightly soluble in water and ether. The water solubility of *p*-nitroaniline can be enhanced by converting it to the salt of a mineral acid such as hydrochloric acid.

PRODUCTION AND USE

The primary use of *p*-nitroaniline is as an intermediate in the production of antioxidants, antiozonants, gasoline additives, and various dyes and pigments. For the latter application, *p*-nitroaniline or a derivative is generally azo coupled through its primary amino group to a more highly substituted dye or pigment nucleus. Eleven million pounds of *p*-nitroaniline were produced or imported in the United States in 1978; however, individual production data for recent years are not available. The NIOSH recommended exposure limit for *p*-nitroaniline is 1 ppm (CFR, 29) while the ACGIH threshold limit value (TLV) is 3 mg/m^3 (ACGIH, 1985). No information concerning occupational or environmental exposure was found.

METABOLISM AND CHEMICAL DISPOSITION

Mate *et al.* (1967) administered 5 mg/kg ^{14}C *p*-nitroaniline orally or intraperitoneally to white rats and collected urine and feces at 24-hour intervals for 72 hours. Approximately 80% of the radioactivity was recovered in the urine during the first 24 hours after dosing by either route. Fecal excretion after 48 hours accounted for approximately 0.6% of the total radioactivity. Analysis of the metabolites by reverse isotope dilution of acid-hydrolyzed urine indicated that 14% was present as the parent compound, 26% as *p*-phenylenediamine, and 43% as 2-amino-5-nitrophenol.

In a more extensive disposition and metabolism study, male F344/N rats received ^{14}C *p*-nitroaniline at doses of 0.276 or 13.8 mg/kg by gavage or 1.38 mg/kg intravenously (Chopade and Matthews, 1984). Absorption and distribution of *p*-nitroaniline-derived radioactivity to all major tissues was rapid and complete for both methods of administration. Within 2 hours 75% to 80% of the radioactivity had cleared from most tissues and within 7 hours the total body burden of *p*-nitroaniline-derived radioactivity (excluding the contents of the large intestine) was

reduced to approximately 5%. There was no indication of any significant bioaccumulation of radioactivity in any tissue. Clearance was best described by a two-component decay curve. The half-life for the first component was approximately 1 hour, a value corresponding to the whole body half-life; approximately 80% of the administered radioactivity exhibited this pattern of clearance kinetics. The second component had a half-life of 16 to 72 hours, depending upon the tissue, and represented the elimination of only a small portion of the total radioactivity. Approximately 64% of the administered radioactivity appeared in the urine within 7 hours after dosing; within 3 days a total of 77% was recovered in urine and 12% to 14% was recovered in the feces.

Following distribution to tissues, *p*-nitroaniline was rapidly metabolized. Within 15 minutes after intravenous administration approximately 50% of *p*-nitroaniline-derived radioactivity was in the form of water-soluble or ether-extractable metabolites in the liver, muscle, and kidney. A total of nine metabolites plus the parent compound were recovered from urine, bile, and feces. Although none of the urinary metabolites were completely characterized, the major ones detected by high-performance liquid chromatography were sulfate conjugates of two *p*-nitroaniline metabolites. These represented approximately 56% of the urinary radioactivity (Chopade and Matthews, 1984).

p-Nitroaniline was incubated with rat liver microsomes *in vitro*, followed by ethyl acetate extraction and high-performance liquid chromatography analysis. Mass spectrometry of the extract revealed a single metabolite, 2-amino-5-nitrophenol. The absence of detectable *N*-hydroxy-4-nitroaniline suggests that, if formed, it is only a minor metabolite (Anderson *et al.*, 1984).

TOXICITY

There are few published studies on the toxicity of *p*-nitroaniline. In one inhalation study, groups of 10 male and 10 female Sprague-Dawley rats were exposed to *p*-nitroaniline at concentrations of 0, 5, 15, or 45 mg/m^3 for 6 hours a day, 5 days per week for 4 weeks (Nair *et al.*, 1986). Body weights and clinical signs were recorded throughout the study, and at the end of the study clinical chemistry, hematology, and gross and histopathologic changes were evaluated. Exposure to *p*-nitroaniline at these levels caused no mortality or body weight reduction. Dose-related increases in methemoglobin concentrations and decreases in erythrocyte counts, hematocrit values, and hemoglobin concentrations were observed in groups exposed to *p*-nitroaniline. Mean spleen weights were increased in all exposed groups. The only reported lesions associated with chemical exposure were hemosiderosis and hematopoiesis in the spleen.

The teratogenic potential of *p*-nitroaniline was evaluated by administering the compound in corn oil by gavage at doses of 25, 85, or 250 mg/kg to mated Sprague-Dawley rats on days 6 through 19 of gestation (Nair *et al.*, 1985). Survivors were sacrificed and evaluated on day 20. Significant maternal toxicity (decreased body weights and increased spleen weights) and embryotoxicity (increased resorptions, decreased fetal body weights, and terata) were observed at the 250 mg/kg dose. Increased maternal spleen weights and fetotoxicity, but no teratogenicity, were noted at the 85 mg/kg dose. The 25 mg/kg dose essentially had no effect.

The reproductive toxicity of *p*-nitroaniline was evaluated by administering 0, 0.25, 1.5, or 9 mg/kg to groups of 15 male and 30 female Sprague-Dawley rats (F_0) for 14 weeks before mating and during mating, gestation, and lactation (Nair *et al.*, 1990). Selected groups of 15 males and 30 females from the F_1 generation were then subjected to the same treatment regimen. Although a slight reduction in the rate of pregnancy was observed in the high-dose F_0 group, no other differences between F_0 and F_1 groups were observed.

CARCINOGENICITY

The carcinogenic potential of *p*-nitroaniline has been evaluated in one study conducted with Sprague-Dawley rats (Nair *et al.*, 1990). Groups of 60 male and 60 female rats received *p*-nitroaniline in corn oil by gavage at doses of 0, 0.25, 1.5, or 9 mg/kg body weight for 2 years. Body weights and feed consumption were recorded weekly for the first 14 weeks of the study and biweekly thereafter. After 6, 10, 12, 18, and 24 months of chemical exposure, blood for hematologic analysis was collected from the orbital sinus of 10 randomly selected rats from each group and complete necropsies were performed on all animals.

Survival and final mean body weights of rats exposed to *p*-nitroaniline were similar to those of the controls. By the 12- and 24-month evaluations erythrocyte counts were significantly decreased in the high-dose rats and methemoglobin concentrations were significantly increased in mid- and high-dose groups. Absolute and relative spleen weights were significantly increased in the high-dose males, and relative spleen weights were increased in the mid-dose males at the end of the study. The only treatment-related lesion observed was pigment accumulation in the liver and spleen. The authors stated that in a previous study of *p*-nitroaniline, a similar brown pigment was shown to be iron positive based on Prussian Blue stain. Based on their studies, Nair *et al.* (1990) concluded that exposure to *p*-nitroaniline was not associated with neoplasia in rats.

Genetic Toxicology

p-Nitroaniline is mutagenic *in vitro*; insufficient data are available to evaluate the *in vivo* genotoxicity of the chemical. *p*-Nitroaniline, in the absence of S9, was positive for growth inhibition due to DNA damage in *Bacillus subtilis* (Shimizu and Yano, 1986). It has been tested extensively for induction of gene mutations in *Salmonella*; in general, responses in base-substitution strains TA100 and TA1535 were negative (Chiu *et al.*, 1978; Malca-Mor and Stark, 1982; Haworth *et al.*, 1983; Thompson *et al.*, 1983; Shahin, 1985), while positive responses were observed, with and without S9, in strains TA98 and TA1538 which mutate by a frameshift mechanism (Garner and Nutman, 1977; Haworth *et al.*, 1983; Thompson *et al.*, 1983; Pai *et al.*, 1985; Shimizu and Yano, 1986). Positive results were reported with *p*-nitroaniline for induction of gene mutations in both TA98 and TA100 using a flavin mononucleotide-modified preincubation technique to promote anaerobic nitroreduction (Dellarco and Prival, 1989). It also induced gene mutations in the bacterium *Photobacterium leiognathi* with S9 (Levi *et al.*, 1986).

In contrast to its demonstrated mutagenicity in bacteria, *p*-nitroaniline did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* treated by feeding or by injection (Valencia *et al.*, 1985; Zimmering *et al.*, 1989). No induction of unscheduled DNA synthesis was observed in rat hepatocytes treated *in vitro* (Mirsalis *et al.*, 1983; Thompson *et al.*, 1983) or *in vivo* (Mirsalis *et al.*, 1983). No induction of sperm head abnormalities occurred in male mice administered 5 mg/kg *p*-nitroaniline by intraperitoneal injection once a day for 5 days (Topham, 1980). However, both sister chromatid exchanges and chromosomal aberrations were induced in Chinese hamster ovary cells *in vitro* by *p*-nitroaniline. Chromosomal aberrations were induced with and without S9; sister chromatid exchanges were induced only in the presence of S9 (Galloway *et al.*, 1987).

Mutagenicity data are available for the structural analogues, *m*- and *o*-nitroaniline. *m*-Nitroaniline was mutagenic in *S. typhimurium* with and without S9 (Garner and Nutman, 1977; Chiu *et al.*, 1978; Melnikow *et al.*, 1981; Shahin *et al.*, 1982; Thompson *et al.*, 1983; Shimizu and Yano, 1986; NTP, unpublished data) while *o*-nitroaniline was negative in most studies (Chiu *et al.*, 1978; Melnikow *et al.*, 1981; Thompson *et al.*, 1983; DeFlora *et al.*, 1984a,b; Shahin, 1985; Shimizu and Yano, 1986; NTP unpublished data). Positive results with *o*-nitroaniline were reported with S9 in strains TA1538 (Garner and Nutman, 1977) and TA98 (Le *et al.*, 1985). Both *m*- and *o*-nitroaniline were mutagenic in *S. typhimurium* strains TA98 and TA100 when tested using a flavin mononucleotide-modified preincubation protocol with hamster S9 (Dellarco and Prival, 1989).

Genotoxicity information is also available for two metabolites of *p*-nitroaniline, 1,4-benzenediamine and 2-amino-5-nitrophenol. Both compounds are mutagenic *in vitro,* but there are insufficient data to allow a conclusion of mutagenicity *in vivo.* 1,4-Benzenediamine was mutagenic in *S. typhimurium* strains TA98 and TA1538 in the presence of S9 (Byeon *et al.*, 1975; Garner and Nutman, 1977; DeGawa *et al.*, 1979; Shahin *et al.*, 1979; Yoshikawa *et al.*, 1979; Watanabe *et al.*, 1980; Crebelli *et al.*, 1981; Burnett *et al.*, 1982; Nohmi *et al.*, 1982; Thompson *et al.*, 1983), but did not induce sex-linked recessive lethal mutations in *D. melanogaster* (Blijleven, 1981) or unscheduled DNA synthesis in rat hepatocytes *in vitro* (Thompson *et al.*, 1983). No induction of sperm head abnormalities was observed in mice after treatment with 1,4-benzenediamine *in vivo* (Topham, 1980), nor was the induction of micronuclei in bone marrow cells (Hossack and Richardson, 1977) or the induction of dominant lethal mutations in germ cells (Burnett *et al.*, 1977) observed in rats.

A second metabolite of *p*-nitroaniline, 2-amino-5-nitrophenol, was mutagenic in *S. typhimurium* with and without S9 (Ames *et al.*, 1975; Chiu *et al.*, 1978; Shahin *et al.*, 1982; Zeiger *et al.*, 1987). Shahin (1985) reported mutagenicity in *S. typhimurium* strains TA1538 and TA98 that were treated with an unpurified sample of 2-amino-5-nitrophenol in the presence of Aroclor-induced rat liver S9. Treatment with a highly purified sample of the compound resulted in no increase in the number of revertant colonies. Shahin (1985) concluded that contaminants in the dye mix were responsible for the earlier reports of mutagenic activity of 2-amino-5-nitrophenol. It should be noted, though, that the test compound used by Zeiger *et al.* (1987), was greater than 99% pure. This same purified sample induced trifluorothymidine resistance in mouse L5178Y cells without S9 (Myhr *et al.*, 1990), and induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, with and without S9 (Anderson *et al.*, 1990). 2-Amino-5-nitrophenol, administered by intraperitoneal injection three times weekly for 8 weeks, did not induce dominant lethal mutations in male rats (Burnett *et al.*, 1977).

STUDY RATIONALE

p-Nitroaniline was nominated for evaluation of carcinogenic potential by the National Cancer Institute because of the possibility for widespread human exposure due to its use as an intermediate in the preparation of dyes and pigments, and because *p*-nitroaniline is a representative of the class of single ring aromatic compounds bearing a nitro and an amino group, several of which are known carcinogens. The NTP did not conduct studies in rats because of the ongoing industry studies in rats (Nair *et al.*, 1990).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *p*-NITROANILINE

p-Nitroaniline was obtained from American Color and Chemical Corporation (Charlotte, NC) in a single lot (lot 990-002) which was used throughout the studies. Identity, purity, and stability analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and confirmed by the study laboratory (Appendix F).

Lot 990-002, a yellow, amorphous powder, was identified as *p*-nitroaniline by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of the lot was found to be greater than 99% by Karl Fischer water analysis, titration of the nitro group, and gas chromatography. Thin-layer chromatography indicated one major spot and two trace impurities, and gas chromatography indicated one major peak and one impurity. Stability studies performed at the analytical chemistry laboratory indicated that *p*-nitroaniline was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when stored protected from light. The stability of the bulk chemical was monitored periodically at the study laboratory with infrared and ultraviolet spectroscopy and gas chromatography methods; no change in purity was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations for gavage administration were prepared by mixing *p*-nitroaniline and corn oil (Table F1). Studies to determine homogeneity and stability of the gavage preparations were conducted by the analytical chemistry laboratory. Dose formulation concentrations greater than 10 mg/mL were suspensions. Homogeneity at the 50 mg/mL level was confirmed using ultraviolet spectroscopy. The stability studies of the dose formulations were performed using gas chromatography. The findings of the studies indicated that the dose formulations were stable for at least 2 weeks at 5° C and room temperature, when stored in the dark, and under simulated dosing conditions (exposed to light and air for 3 hours). No special handling was required during dosing.

Periodic analyses of the dose formulations of *p*-nitroaniline were conducted at the study laboratory and the analytical chemistry laboratory using ultraviolet spectroscopy. During the 14-day studies all dose formulations were analyzed. During the 13-week studies, the dose formulations were analyzed at the initiation, midpoint, and termination of the studies (Tables F2 and F3). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks (Table F4). In the 2-year studies, 98% (45/46) of the dose formulations were within 10% of the target concentrations. Periodic analyses of the corn oil vehicle by the study laboratory indicated that peroxide levels were within the acceptable limit of 10 mEq/kg. Results of the periodic referee analyses performed by the analytical chemistry laboratory were in good agreement with the results obtained by the study laboratory (Table F5).

14-DAY STUDIES

Male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY); at receipt, the mice were 5 to 6 weeks old. The animals were quarantined for 24 to 25 days before dosing began. During this time, two animals of each sex were randomly selected and evaluated for the presence of parasites and other gross indications of disease.

Groups of five male and five female mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 10, 30, 100, 300, or 1,000 mg/kg body weight. All doses were given once daily for 5 days per week, with at least 2 consecutive dosing days at the end of the studies. Animals were housed five per cage; water and feed were available *ad libitum*. Clinical findings were recorded twice daily. The animals were weighed at study initiation, at day 7, and at the end of the

studies. Details of study design and animal maintenance are summarized in Table 1.

At the end of the 14-day studies, blood was collected from the orbital sinus plexus of all animals for clinical pathology analyses; the clinical pathology parameters measured are listed in Table 1. A necropsy was performed on all animals. The brain, heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Histopathologic examinations were conducted on all animals receiving 300 mg/kg. The tissues routinely examined microscopically are listed in Table 1.

13-Week Studies

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to *p*-nitroaniline and to determine the appropriate doses to be used in the 2-year studies.

Male and female B6C3F$_1$ mice, 5 to 6 weeks of age, were obtained from Charles River Breeding Laboratories (Kingston, NY). The animals were quarantined for 12 days before dosing began. At this time, five animals of each sex were randomly selected and evaluated for the presence of parasites and other overt evidence of disease. At the end of the studies, serologic analyses were performed on five control animals of each sex using the protocols of the NTP Sentinel Animal Program (Appendix H).

Groups of 20 male and 20 female mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 1, 3, 10, 30, or 100 mg/kg body weight 5 days per week for 13 weeks. Animals were housed five per cage; water and feed were available *ad libitum*. Clinical findings were recorded twice daily. The animals were weighed at the beginning of the studies and weekly thereafter. Further details of study design and animal maintenance are summarized in Table 1.

Blood was collected for clinical pathology analyses from the orbital sinus plexus of half of the animals at the 7-week interim evaluations and from all remaining animals at the end of the 13-week studies. The clinical pathology parameters measured are listed in Table 1. A necropsy was performed on about half the animals at 7 weeks and on the remaining half at 13 weeks. The brain, heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed at the 7-week interim evaluations. Weights were recorded for these same tissues, in addition to the left epididymis in males, at the end of the studies in the remaining animals. Tissues for microscopic examination were fixed and preserved in phosphate-buffered neutral formalin, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all animals receiving 0 or 100 mg/kg, and on the liver of males and the spleen of males and females receiving 1, 3, 10, or 30 mg/kg. Table 1 lists the tissues routinely examined microscopically.

2-Year Studies

Study Design

Groups of 70 male and 70 female mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 3, 30, or 100 mg/kg body weight 5 days per week for up to 103 weeks. Up to 10 mice per group were designated for interim evaluations after 9 and 15 months of chemical administration.

Source and Specification of Animals

Male and female B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year studies. The animals were quarantined for 11 days before the beginning of the studies. Five mice of each sex were selected for parasite evaluation and gross observation of disease. The animals were approximately 40 days of age at the beginning of the studies. The health of the animals was monitored during the studies according to the NTP Sentinel Animal Program.

Animal Maintenance

Mice were housed individually. Feed and water were available *ad libitum*. Cages were rotated every 2 weeks during the studies. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix G.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded weekly for the first 13 weeks, and monthly thereafter. Animals were weighed at study initiation, weekly for the first 13 weeks, and monthly thereafter. Up to 10 mice from each group were predesignated for interim evaluations after 9 and 15 months. However, several female mice

designated for the interim evaluation died and, thus, were subsequently included with the core study for analysis. Blood was collected by cardiac puncture to determine the following hematology and clinical chemistry parameters: hematocrit, hemoglobin concentration, erythrocyte counts, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, platelets, reticulocyte counts, leukocyte counts, the concentration of segmented neutrophils, lymphocytes, monocytes, and eosinophils, methemoglobin concentration, and sulfhemoglobin concentration. The brain, right kidney, liver, and spleen were weighed at 9 and 15 months; the uterus of females was weighed at 15 months. Further details of the interim evaluations are presented in Table 1.

A complete necropsy was performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Histopathological examinations were performed on all tissues with grossly visible lesions. Tissues examined are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist reviewed the liver, spleen, and all vascular neoplasms for accuracy and consistency of lesion diagnosis.

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

Statistical Methods

Survival Analyses

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

Calculation of Incidence

The incidence of neoplasms or nonneoplastic lesions is given as the number of animals bearing such lesions at a specific anatomic site and 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 neoplasms) before histologic sampling, or when lesions had multiple sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidence

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which

assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

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 neoplasm incidence. Consequently, neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunnett's or Dunn's test). Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff so all had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of *p*-nitroaniline was assessed by testing its ability to induce mutations in *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, trifluorothymidine resistance in mouse L5178Y lymphoma cells, and sex-linked recessive lethal mutations in *Drosophila melanogaster*. The protocols and results of these studies are given in Appendix C.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of *p*-Nitroaniline

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Hazleton Raltech (Madison, WI)	Hazleton Raltech (Madison, WI)	Southern Research Institute (Birmingham, AL)
Strain and Species		
$B6C3F_1$ mice	$B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source		
Charles River Breeding Laboratories (Kingston, NY)	Charles River Breeding Laboratories (Kingston, NY)	Simonsen Laboratories (Gilroy, CA)
Time Held Before Studies		
24 to 25 days	12 days	11 days
Average Age When Placed on Study		
8-9 weeks	7-8 weeks	40 days
Date of First Dose		
25 April 1982	22 November 1982	25 September 1984
Duration of Dosing		
12 days	13 weeks	103 weeks
Date of Last Dose		
10 May 1982	24 February 1983	9-month interim: 21-25 June 1985 15-month interim: 17-19 December 1985 Terminal: 15 September 1986
Average Age When Killed		
12-13 weeks	20-21 weeks	9-month interim: 312 days 15-month interim: 489 days Terminal: 770 days
Size of Study Groups		
5 males and 5 females	20 males and 20 females	70 males and 70 females
Method of Distribution		
Animals were grouped by weight intervals. Animals were assigned to cages, then the cages were assigned to dose groups using an appropriate table of random numbers.	Same as 14-day studies	Same as 14-day studies
Animals per Cage		
5	5	1
Method of Animal Identification		
Metal tags	Metal tags	Toe clip
Diet		
NIH-07 open formula rat and mouse diet (Teklad Test Diets, Winfield, IA), available *ad libitum*	Same as 14-day studies	NIH-07 Open-Formula Pellets, (Zeigler Brothers, Gardners, PA), available *ad libitum*

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of *p*-Nitroaniline (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Water		
Automatic watering system (Systems Engineering, Palo Alto, CA), available *ad libitum*	Same as 14-day studies	Automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*
Cages		
Polycarbonate, changed twice weekly	Same as 14-day studies	Polycarbonate solid-bottom (Lab Products, Inc., Maywood, NJ), changed weekly
Bedding		
BetaChips, hardwood laboratory bedding (Northeastern Products Corp., Warrensburg, NY), changed twice weekly	Same as 14-day studies	Same as 14-day studies
Cage Filters		
Nonwoven polyester, changed at the beginning of the studies	Nonwoven polyester, changed every other week	Reemay spun-bonded polyester (Snow Filtration, Cincinnati, OH, or Andico, Birmingham, AL), changed once every 2 weeks
Racks		
Stainless steel, changed at the beginning of the studies	Stainless steel, changed every other week	Stainless steel (Lab Products, Inc., Maywood, NJ), changed once every 2 weeks
Animal Room Environment		
Temperature: 22° ± 1° C Relative humidity: 50% ± 10% Fluorescent light: 12 hours/day Room air changes: 10-15 changes/hour	Temperature: 22° ± 2° C Relative humidity: 50% ± 20% Fluorescent light: 12 hours/day Room air changes: minimum of 10 changes/hour	Temperature: 22° ± 2° C Relative humidity: 50% ± 5% Fluorescent light: 12 hours/day Room air changes: minimum of 10 changes/hour
Doses		
0, 10, 30, 100, 300, or 1,000 mg/kg *p*-nitroaniline in corn oil by gavage	0, 1, 3, 10, 30, or 100 mg/kg *p*-nitroaniline in corn oil by gavage	0, 3, 30, or 100 mg/kg *p*-nitroaniline in corn oil by gavage
Type and Frequency of Observation		
Observed twice daily; animals weighed initially, on day 7, and at the end of the studies; clinical observations recorded twice daily.	Observed twice daily; animals weighed initially, weekly, and at the end of the studies; clinical findings recorded twice daily.	Observed twice daily; animal weights and clinical findings recorded weekly through week 13, monthly thereafter, and at interim evaluations or at the end of the studies.
Necropsy		
Necropsy performed on all animals. Organ weights were recorded for brain, heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsy performed on all animals. Organ weights were recorded for brain, epididymis (7 weeks only), heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsy performed on all animals. Organ weights were recorded at 9 and 15 months for brain, right kidney, liver, spleen, and uterus (15-month females only).

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of *p*-Nitroaniline (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Clinical Pathology		
Blood was collected from all animals *Hematology:* hematocrit, hemoglobin, erythrocytes, reticulocytes, total leukocyte counts and differentials, and total bone marrow cellularity *Clinical chemistry:* methemoglobin	Blood was collected from half the animals at day 45, and all animals surviving to the end of the studies. *Hematology:* hematocrit, hemoglobin, erythrocytes, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, reticulocytes, total leukocyte counts and differentials, and total bone marrow cellularity *Clinical chemistry:* methemoglobin	Blood was collected from animals designated for 9- and 15-month interim evaluations. *Hematology:* hematocrit, hemoglobin, erythrocytes, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, platelets, reticulocytes, and total leukocyte counts and differentials *Clinical chemistry:* methemoglobin and sulfhemoglobin
Histopathology		
Complete histopathology was performed on all animals receiving 300 mg/kg. In addition to gross lesions, tissue masses, and associated lymph nodes, the tissues examined included: adrenal gland, brain, epididymis, esophagus, femur (including marrow), heart, kidney, large intestine (colon, cecum, rectum), liver, lung and bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis (tunic and scrotal sac), thymus, thyroid gland, tongue, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all animals at the 7-week interim evaluations, and all controls and animals receiving 100 mg/kg at the end of the studies. In addition to gross lesions, tissue masses, and associated lymph nodes, the tissues examined included: adrenal gland, brain, bone marrow, epididymis, esophagus, femur (including marrow), gallbladder, heart, kidney, large intestine (colon, cecum, rectum), liver, lung and bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis (tunic and scrotal sac), thymus, thyroid gland, trachea, urinary bladder, and uterus. Histopathology was also performed on the liver (males) and spleen (males and females) from animals in all dose groups.	Complete histopathology was performed on all early deaths, all control and high-dose animals scheduled for interim evaluations, and all animals surviving to the end of the studies. In addition to gross lesions, tissue masses, and associated lymph nodes, the tissues examined included: adrenal gland, brain, epididymis, esophagus, femur (including marrow), gallbladder, heart, kidney, large intestine (cecum, colon, rectum), liver, lung and mainstem bronchi, lymph node (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Organs examined at the 9-month interim evaluations included liver, lung, spleen, and thyroid gland (all dose groups), uterus (mid-dose females), and the urinary bladder and kidney (mid-dose males). Organs examined at the 15-month interim evaluations included liver and spleen (all dose groups), lung (mid-dose females), and bone marrow, lung, and stomach (mid-dose males).

RESULTS

14-DAY STUDIES

All mice that received 1,000 mg/kg died from compound-related toxicity by day 4 (Table 2). One male and one female receiving 10 mg/kg, one female receiving 30 mg/kg, two males receiving 100 mg/kg, and one female receiving 300 mg/kg died as a result of improper gavage technique. Final mean body weights of dosed mice surviving to the end of the studies were similar to those of controls.

The hematologic and pathologic findings in mice receiving *p*-nitroaniline were characteristic of a process of accelerated erythrocyte destruction caused by methemoglobin and Heinz body formation and a compensatory reaction to maintain erythrocyte mass. The methemoglobin concentrations in all dosed groups of mice were significantly higher than those in controls (Tables 3 and E1). Although hematocrit levels were significantly lower primarily in mice

TABLE 2
Survival and Mean Body Weights of Mice in the 14-Day Gavage Studies of *p*-Nitroaniline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	5/5	24.9 ± 0.6	27.0 ± 0.7	2.1 ± 0.3	
10	4/5[c]	24.1 ± 0.5	27.0 ± 0.5	2.8 ± 0.2	100
30	5/5	25.5 ± 0.7	28.1 ± 0.7	2.6 ± 0.2	104
100	3/5[d]	25.0 ± 0.5	26.5 ± 0.7	1.1 ± 0.1	98
300	5/5	24.3 ± 0.2	26.4 ± 0.5	2.1 ± 0.5	98
1,000	0/5[e]	25.4 ± 0.7	–	–	–
Female					
0	5/5	20.6 ± 0.4	22.1 ± 0.2	1.5 ± 0.5	
10	4/5[f]	19.5 ± 0.3*	21.0 ± 0.3	1.5 ± 0.2	95
30	4/5[g]	20.6 ± 0.2	22.9 ± 0.2	2.1 ± 0.3	104
100	5/5	20.4 ± 0.3	22.0 ± 0.4	1.5 ± 0.2	99
300	4/5[c]	19.4 ± 0.3	23.0 ± 0.5	3.5 ± 0.4**	104
1,000	0/5[h]	20.2 ± 0.2	–	–	–

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. No data were calculated for groups with 100% mortality.
[c] Day of death: 9
[d] Day of death: 11, 13
[e] Day of death: 2, 3, 3, 4, 4
[f] Day of death: 10
[g] Day of death: 8
[h] Day of death: 3, 3, 3, 4, 4

receiving 300 mg/kg, total erythrocyte counts in males and females receiving 100 or 300 mg/kg and in males receiving 30 mg/kg were significantly lower than controls. The reticulocyte counts in 300 mg/kg male mice and in 100 or 300 mg/kg females were significantly higher than controls, indicating the release of immature erythrocytes from the bone marrow or other hematopoietic tissues such as the spleen. Heinz bodies were observed in the erythrocytes of all mice receiving 300 mg/kg and of two male mice receiving 100 mg/kg. Total leukocyte counts were also significantly higher in 100 and 300 mg/kg mice. Although slight increases in total leukocyte counts are often associated with regenerative anemia, the elevated counts in these studies may be due, in part, to artifacts. Heinz bodies and reticulocytes may fail to undergo complete lysis and some will be counted as cells by the electronic cell counter.

At necropsy, the spleens of all 300 mg/kg mice and of two 100 mg/kg males were enlarged and dark purple. Moreover, the absolute and relative spleen weights of 100 and 300 mg/kg mice were significantly greater than those of controls (Tables 3 and D1). On histologic examination, the splenic red pulp of 100 or 300 mg/kg mice was filled with erythrocytes and erythroid precursor cells, indicative of an elevated rate of hematopoiesis, and there were many macrophages filled with granular golden-brown pigment (hemosiderin). In addition, widely scattered Kupffer cells in the liver also contained similar pigment.

There were no gross lesions associated with chemical administration. Increased Kupffer cell pigmentation in the liver of males and increased extramedullary hematopoiesis in males and females were the only lesions associated with exposure to *p*-nitroaniline.

TABLE 3
Selected Organ Weights, Organ-Weight-to-Body-Weight Ratios, and Hematology and Clinical Chemistry Data for Mice in the 14-Day Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Male					
n	5	4	5	3	5
Necropsy body wt	27.0 ± 0.7	27.0 ± 0.5	28.1 ± 0.7	26.5 ± 0.7	26.4 ± 0.5
Organ weights					
Heart					
Absolute	0.146 ± 0.003	0.152 ± 0.006	0.155 ± 0.007	0.152 ± 0.013	0.168 ± 0.004*
Relative	5.40 ± 0.21	5.61 ± 0.21	5.50 ± 0.16	5.71 ± 0.43	6.35 ± 0.16**
Spleen					
Absolute	0.121 ± 0.013	0.118 ± 0.009	0.143 ± 0.012	0.191 ± 0.026**	0.359 ± 0.015**
Relative	4.46 ± 0.38	4.37 ± 0.27	5.06 ± 0.31	7.16 ± 0.81**	13.58 ± 0.41**
Hematology					
Hematocrit (%)	43.0 ± 0.6	41.9 ± 0.7	39.0 ± 1.3*	42.7 ± 0.2	35.9 ± 1.7**
Hemoglobin (g/dL)	15.4 ± 0.2	15.0 ± 0.0	14.6 ± 0.5	19.0 ± 0.6	15.6 ± 0.8
Erythrocytes ($10^6/\mu L$)	9.17 ± 0.15	9.00 ± 0.19	8.21 ± 0.29*	8.44 ± 0.06*	6.75 ± 0.32**
Reticulocytes ($10^6/\mu L$)	2.90 ± 0.27	2.45 ± 0.70	3.32 ± 0.66	4.37 ± 1.78	18.04 ± 1.34**
Leukocytes ($10^3/\mu L$)	4.22 ± 0.35	4.08 ± 0.34	4.22 ± 0.24	12.03 ± 4.63*	16.50 ± 3.38**
Heinz bodies	0	0	0	2	5
Clinical Chemistry					
Methemoglobin (%)	1.70 ± 0.22	3.03 ± 0.56*	5.74 ± 0.55**	13.77 ± 2.10**	11.92 ± 3.15**

(continued)

TABLE 3
Selected Organ Weights, Organ-Weight-to-Body-Weight Ratios, and Hematology and Clinical Chemistry Data for Mice in the 14-Day Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Female					
n	5	4	4	5	4
Necropsy body wt	22.1 ± 0.2	21.0 ± 0.3	22.9 ± 0.2	22.0 ± 0.4	23.0 ± 0.5
Organ weights					
Heart					
Absolute	0.132 ± 0.005	0.134 ± 0.011	0.139 ± 0.009	0.145 ± 0.012	0.133 ± 0.004
Relative	5.98 ± 0.24	6.37 ± 0.48	6.06 ± 0.33	6.62 ± 0.59	5.76 ± 0.07
Spleen					
Absolute	0.109 ± 0.018	0.118 ± 0.011	0.131 ± 0.013	0.184 ± 0.015**	0.300 ± 0.020**
Relative	4.91 ± 0.81	5.61 ± 0.47	5.74 ± 0.56	8.34 ± 0.57**	13.06 ± 0.90**
Hematology					
Hematocrit (%)	43.4 ± 0.5	41.9 ± 0.9	42.6 ± 0.4	42.0 ± 1.2	36.2 ± 1.4**
Hemoglobin (g/dL)	15.4 ± 0.2	15.0 ± 0.4	15.5 ± 0.3	16.0 ± 0.3	17.5 ± 0.3**
Erythrocytes ($10^6/\mu L$)	9.10 ± 0.09	8.78 ± 0.13*	8.80 ± 0.11	8.34 ± 0.24**	7.09 ± 0.25**
Reticulocytes ($10^6/\mu L$)	0.80 ± 0.15	2.03 ± 0.67	2.73 ± 0.69*	4.92 ± 0.88**	5.95 ± 1.49**
Leukocytes ($10^3/\mu L$)	2.90 ± 0.39	2.90 ± 0.35	3.00 ± 0.12	4.58 ± 0.13**	41.90 ± 4.21**
Heinz bodies	0	0	0	0	5
Clinical Chemistry					
Methemoglobin (%)	0.00 ± 0.00	1.35 ± 0.17**	3.20 ± 0.68**	6.16 ± 0.67**	16.73 ± 1.38**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test (organ weights) or by Dunn's or Shirley's test (hematology and clinical chemistry)

** $P \leq 0.01$

[a] Organ and body weights and clinical pathology data (excluding Heinz bodies) are expressed as the mean ± standard error; organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight. All animals that received 1,000 mg/kg died before the end of the studies.

13-WEEK STUDIES

There were no deaths associated with exposure to *p*-nitroaniline during the 13-week studies. Among females, two early deaths in the 10 mg/kg group were considered to be the result of improper gavage technique (Table 4). Among males, one death in each of the 0, 10, and 100 mg/kg groups and two deaths in the 3 mg/kg group were caused by fighting or improper gavage technique. Final mean body weights of dosed mice were similar to those of the controls.

After 7 weeks of chemical exposure the absolute and relative liver weights increased with dose in female mice and were significantly increased in the 30 and 100 mg/kg groups (Tables 5 and D2). However, absolute and relative liver weights of dosed females at 13 weeks were not significantly increased. Absolute and relative spleen weights of male and female mice at 7 and 13 weeks of chemical exposure were significantly increased in groups receiving 30 or 100 mg/kg. Other absolute or relative organ weight differences were considered unrelated to chemical exposure.

TABLE 4
Survival and Mean Body Weights of Mice in the 13-Week Gavage Studies of *p*-Nitroaniline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	9/10[c]	24.0 ± 0.4	32.7 ± 0.8	8.8 ± 0.6	
1	11/11	24.1 ± 0.3	33.7 ± 0.5	9.6 ± 0.4	103
3	8/10[d]	23.5 ± 0.5	31.9 ± 0.7	8.4 ± 0.7	98
10	9/10[e]	24.6 ± 0.3	34.2 ± 0.6	9.5 ± 0.5	105
30	10/10	23.6 ± 0.4	32.4 ± 0.6	8.8 ± 0.5	99
100	9/10[f]	23.5 ± 0.5	33.0 ± 0.7	9.4 ± 0.6	101
Female					
0	10/10	21.1 ± 0.2	26.8 ± 0.4	5.7 ± 0.4	
1	10/10	22.0 ± 0.4	26.7 ± 0.4	4.7 ± 0.5	100
3	10/10	21.1 ± 0.2	26.2 ± 0.3	5.1 ± 0.4	98
10	8/10[g]	21.7 ± 0.2	26.4 ± 0.4	4.6 ± 0.3	99
30	10/10	21.8 ± 0.2	26.2 ± 0.3	4.4 ± 0.2	98
100	10/10	21.6 ± 0.2	27.0 ± 0.4	5.4 ± 0.5	101

[a] Number of animals surviving at 13 weeks/number of animals initially in group
[b] Weights and weight changes given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. Differences from the control group are not significant by Williams' or Dunnett's test.
[c] Week of death: 8
[d] Week of death: 1, 2
[e] Week of death: 1
[f] Week of death: 3
[g] Week of death: 11, 11

TABLE 5
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
7 Weeks						
Male						
n	9	8	8	9	9	8
Necropsy body wt	28.7 ± 0.6	29.9 ± 0.5	29.8 ± 0.6	29.5 ± 0.5	29.2 ± 0.8	28.4 ± 0.5
Liver						
Absolute	1.404 ± 0.043	1.374 ± 0.044	1.564 ± 0.078	1.460 ± 0.028	1.576 ± 0.046	1.488 ± 0.049
Relative	48.92 ± 1.10	45.98 ± 1.00	52.63 ± 2.69	49.58 ± 0.95	53.96 ± 0.84*	52.39 ± 1.28*
Spleen						
Absolute	0.087 ± 0.004	0.084 ± 0.003	0.087 ± 0.004[b]	0.106 ± 0.009	0.142 ± 0.008**	0.200 ± 0.010**
Relative	3.02 ± 0.14	2.82 ± 0.11	2.91 ± 0.17[b]	3.64 ± 0.37	4.88 ± 0.28**	7.04 ± 0.30**
Female						
n	10	10	9	10	10	10
Necropsy body wt	24.7 ± 0.2	25.0 ± 0.2	24.8 ± 0.2	24.5 ± 0.3	25.3 ± 0.2	25.7 ± 0.4
Liver						
Absolute	1.179 ± 0.029	1.227 ± 0.018	1.248 ± 0.033	1.265 ± 0.036	1.306 ± 0.035**	1.384 ± 0.038**
Relative	47.64 ± 1.04	49.18 ± 0.82	50.19 ± 1.09	51.67 ± 1.13**	51.65 ± 1.20**	53.89 ± 0.96**
Spleen						
Absolute	0.105 ± 0.005	0.106 ± 0.002	0.113 ± 0.004	0.117 ± 0.003	0.177 ± 0.012**	0.233 ± 0.011**
Relative	4.24 ± 0.19	4.23 ± 0.07	4.56 ± 0.18	4.78 ± 0.16	7.00 ± 0.47**	9.08 ± 0.45**
13 Weeks						
Male						
n	9	11	8	9	10	9
Necropsy body wt	32.9 ± 0.8	34.0 ± 0.6	31.9 ± 0.7	35.0 ± 0.6	32.4 ± 0.6	33.0 ± 0.7
Liver						
Absolute	1.614 ± 0.058	1.469 ± 0.033	1.508 ± 0.041	1.712 ± 0.046	1.649 ± 0.033	1.483 ± 0.047
Relative	49.01 ± 1.20	43.15 ± 0.53**	47.26 ± 0.79	48.93 ± 0.72	50.92 ± 1.04	44.91 ± 0.73**
Spleen						
Absolute	0.091 ± 0.002[c]	0.075 ± 0.003	0.084 ± 0.004	0.105 ± 0.004	0.147 ± 0.007**	0.239 ± 0.008**
Relative	2.82 ± 0.07[c]	2.21 ± 0.09	2.64 ± 0.13	3.00 ± 0.11	4.53 ± 0.25**	7.27 ± 0.26**
Female						
n	10	10	10	8	10	10
Necropsy body wt	26.5 ± 0.4	26.9 ± 0.5	27.4 ± 0.3	27.7 ± 0.8	28.2 ± 0.4*	28.0 ± 0.5*
Liver						
Absolute	1.354 ± 0.037	1.307 ± 0.030	1.364 ± 0.039	1.411 ± 0.062	1.432 ± 0.054	1.428 ± 0.026
Relative	51.07 ± 1.03	48.74 ± 1.10	49.72 ± 1.33	50.74 ± 0.82	50.64 ± 1.38	51.16 ± 0.96
Spleen						
Absolute	0.097 ± 0.007	0.093 ± 0.004	0.101 ± 0.004	0.114 ± 0.010[b]	0.141 ± 0.006**	0.220 ± 0.009**
Relative	3.65 ± 0.25	3.46 ± 0.14	3.69 ± 0.12	4.07 ± 0.27[b]	5.00 ± 0.17**	7.92 ± 0.39**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)
[b] n=7
[c] n=8

Consistent with the findings in the 14-day studies, the values of several hematologic parameters were significantly affected by exposure to *p*-nitroaniline at the 7-week interim evaluations and at the end of the 13-week studies (Tables 6, E2, and E3); most differences occurred in the 30 and 100 mg/kg groups. Methemoglobin concentrations in all 100 mg/kg mice were significantly higher than controls; this finding was seen both at the 7-week interim evaluations and at the end of the studies. After 7 weeks of chemical exposure, hematocrit values were significantly lower than controls in males receiving 100 mg/kg and in females receiving 30 and 100 mg/kg, yet at the end of the studies, the only significant decrease in hematocrit values occurred in 30 mg/kg females. Erythrocyte counts at 7 weeks were significantly higher than controls in all 30 and 100 mg/kg mice. At the end of the studies the erythrocyte counts of 100 mg/kg males and 30 mg/kg females were significantly lower than controls.

Nucleated erythrocyte counts were significantly higher in 30 mg/kg males and in 30 and 100 mg/kg females at the 7-week interim evaluations. At the end of the studies nucleated erythrocyte counts in all 100 mg/kg mice were significantly greater than the controls. Reticulocyte counts were significantly higher in 30 and 100 mg/kg females at 7 and 13 weeks, 30 and 100 mg/kg males at 13 weeks, and 100 mg/kg males at 7 weeks, reflecting the regenerative response to the accelerated erythrocyte destruction. Further, Heinz bodies were observed in erythrocytes. Although increases in mean erythrocyte hemoglobin and mean erythrocyte hemoglobin concentration are usually associated with a regenerative anemia, the increases in these parameters in these studies may be due in part to artifacts. Heinz bodies, which are composed of aggregates of precipitated hemoglobin, have been shown to produce erroneous hemoglobin values due to abnormal light scattering. Total leukocyte counts were also higher, primarily in 100 mg/kg mice. However, the leukocyte density on Wright's stained blood smears from dosed mice was similar to that of controls, suggesting that the elevated counts were due in part to artifact associated with Heinz bodies, as explained previously.

Lesions associated with the administration of *p*-nitroaniline occurred in the spleen, liver, and bone marrow (Table 7). There was a dose-related increase in the incidence or severity of splenic hematopoiesis and pigmentation (hemosiderin) in mice at the 7-week interim evaluations and at the end of the studies. Golden-brown pigment similar to that in splenic macrophages was also present in a few widely scattered Kupffer cells of the liver in male mice. The incidence of bone marrow hyperplasia in histologic sections appeared to be increased in all dosed groups of male mice by 7 weeks of chemical exposure. There was no increase in the incidence of bone marrow hyperplasia in female mice. However, the histologic appearance did not correlate closely with bone marrow cellularity as determined by direct measurements.

Dose Selection Rationale: The results of the 14-day and 13-week studies indicate that the major effect of repeated exposure to *p*-nitroaniline is a significant increase in the formation of methemoglobin and, ultimately, Heinz bodies, resulting in an increase in the rate of splenic clearance of erythrocytes and a compensatory increase in hematopoiesis. The major risks to the organism include toxicity to the spleen and possibly the liver caused by accumulation of heme and its degradation products, and anemia resulting from the reduced number of mature erythrocytes. During the 14-day studies these differences were most evident at the 100 and 300 mg/kg levels, and during the 13-week studies at the 30 and 100 mg/kg levels. The lesions observed in animals receiving 300 mg/kg were considered severe enough to be potentially life threatening in a 2-year study and, therefore, 100 mg/kg was selected as the high dose for the 2-year studies. Because of the uncertainty about potential cumulative toxicity associated with long-term exposure, 3 mg/kg was selected for the low dose and 30 mg/kg was selected for the mid dose to provide a broad dose range in the 2-year studies.

TABLE 6
Selected Hematology and Clinical Chemistry Data for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
7 Weeks						
Male						
n	9	8	8	9	9	8
Hematology						
Hematocrit (%)	44.0 ± 0.7	45.6 ± 0.7	42.7 ± 1.0	44.0 ± 0.6	42.1 ± 0.9	41.3 ± 0.6*
Erythrocytes ($10^6/\mu L$)	7.84 ± 0.12	8.15 ± 0.12	7.55 ± 0.14	7.89 ± 0.10	7.30 ± 0.14*	7.08 ± 0.10**
Mean cell hemoglobin (pg)	17.7 ± 0.2	17.9 ± 0.1	18.0 ± 0.3	17.8 ± 0.2	19.7 ± 0.2**	24.5 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	31.5 ± 0.3	32.0 ± 0.1	31.8 ± 0.2	32.0 ± 0.2	34.2 ± 0.3**	42.0 ± 0.5**
Reticulocytes (%)	2.64 ± 0.20	2.16 ± 0.25	1.88 ± 0.20	2.60 ± 0.31	4.58 ± 0.76	5.44 ± 0.41**
Nucleated erythrocytes (/100 leukocytes)	0.00 ± 0.00	0.00 ± 0.00[b]	0.50 ± 0.27*	0.44 ± 0.24	0.56 ± 0.24*	0.25 ± 0.16
Clinical Chemistry						
Methemoglobin (g/dL)	0.42 ± 0.11	0.56 ± 0.10	0.53 ± 0.13	0.47 ± 0.09	1.25 ± 0.09**	3.07 ± 0.31**
Female						
n	10	10	9	10	10	10
Hematology						
Hematocrit (%)	49.0 ± 0.6	48.2 ± 0.3	47.6 ± 0.7	47.5 ± 0.4*	42.4 ± 0.8**	44.2 ± 0.7**
Erythrocytes ($10^6/\mu L$)	8.39 ± 0.11	8.25 ± 0.09	8.25 ± 0.09	8.23 ± 0.07	7.42 ± 0.13**	7.62 ± 0.11**
Mean cell hemoglobin (pg)	17.9 ± 0.1	17.8 ± 0.1	17.7 ± 0.1	17.7 ± 0.2	18.5 ± 0.1*	20.2 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	30.7 ± 0.1	30.5 ± 0.1	30.7 ± 0.1	30.6 ± 0.1	32.3 ± 0.2**	34.9 ± 0.3**
Reticulocytes (%)	2.02 ± 0.22	2.28 ± 0.32	1.81 ± 0.18	2.26 ± 0.22	4.64 ± 0.52**	5.93 ± 0.39**
Nucleated erythrocytes (/100 leukocytes)	0.00 ± 0.00	0.20 ± 0.20	0.44 ± 0.18*	0.10 ± 0.10	0.50 ± 0.22*	2.50 ± 0.75**
Clinical Chemistry						
Methemoglobin (g/dL)	0.06 ± 0.03	0.03 ± 0.03	0.04 ± 0.04	0.11 ± 0.03	0.42 ± 0.04**	1.06 ± 0.11**

(continued)

TABLE 6
Selected Hematology and Clinical Chemistry Data for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
13 Weeks						
Male						
n	9	11	8	9	10	9
Hematology						
Hematocrit (%)	40.5 ± 0.7	45.8 ± 0.5	46.8 ± 1.1	41.2 ± 0.7	41.9 ± 0.5	39.7 ± 0.4
Erythrocytes ($10^6/\mu L$)	8.10 ± 0.14	8.89 ± 0.10	9.08 ± 0.18	8.03 ± 0.14	7.79 ± 0.10	7.56 ± 0.08*
Mean cell hemoglobin (pg)	16.5 ± 0.4	16.9 ± 0.2	17.2 ± 0.1	16.6 ± 0.2	19.3 ± 0.2**	24.3 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.4	32.9 ± 0.3	33.4 ± 0.2	32.4 ± 0.3	35.8 ± 0.4**	46.2 ± 0.6**
Reticulocytes (%)	2.56 ± 0.20	1.25 ± 0.19	1.80 ± 0.16	2.46 ± 0.28	5.86 ± 0.62*	9.67 ± 0.86**
Nucleated erythrocytes (/100 leukocytes)	0.10 ± 0.10[c]	0.55 ± 0.21	0.13 ± 0.13	0.67 ± 0.67	0.50 ± 0.17	2.22 ± 0.49**
Clinical Chemistry						
Methemoglobin (g/dL)	0.36 ± 0.02	0.26 ± 0.02[c]	0.29 ± 0.02	0.72 ± 0.03*	0.74 ± 0.04**	1.70 ± 0.20**
Female						
n	10	10	10	8	10	10
Hematology						
Hematocrit (%)	40.8 ± 1.0	42.5 ± 0.4	43.7 ± 0.5	43.7 ± 0.5	44.2 ± 0.7*	39.9 ± 0.9
Erythrocytes ($10^6/\mu L$)	7.76 ± 0.18	8.14 ± 0.07	8.33 ± 0.09*	8.33 ± 0.11	8.41 ± 0.14*	7.70 ± 0.15
Mean cell hemoglobin (pg)	17.0 ± 0.2	16.9 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.0 ± 0.1	20.3 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	32.4 ± 0.3	32.3 ± 0.1	32.9 ± 0.1*	32.5 ± 0.1	32.3 ± 0.2	39.3 ± 0.6**
Reticulocytes (%)	1.64 ± 0.17	1.31 ± 0.19	1.39 ± 0.22	2.11 ± 0.36	4.44 ± 0.49**	6.33 ± 0.41**
Nucleated erythrocytes (/100 leukocytes)	0.60 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.38 ± 0.26	0.70 ± 0.34	1.30 ± 0.26*
Clinical Chemistry						
Methemoglobin (g/dL)	0.37 ± 0.01	0.37 ± 0.04	0.23 ± 0.01	0.34 ± 0.02	1.01 ± 0.03**	1.47 ± 0.03**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error
[b] n=9
[c] n=10

TABLE 7
Incidences of Selected Nonneoplastic Lesions in Mice in the 13-Week Gavage Studies of *p*-Nitroaniline

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
7 Weeks						
Male						
Liver[a]	9	8	7	9	9	8
Kupffer Cell Pigmentation[b]	0	0	0	1 (0.4)[c]	0	8**(3.2)**
Spleen	9	8	7	9	9	8
Extramedullary Hematopoiesis	4 (0.9)	8*(1.8)*	7 (1.8)	9*(2.4)**	9*(2.1)**	8*(3.2)**
Pigmentation	0	3 (0.4)	4*(0.5)*	9**(1.3)**	9**(2.0)**	8**(3.2)**
Bone Marrow	9	8	7	9	9	8
Hyperplasia	3	4	4	5	4	5
Female						
Spleen	10	10	9	10	10	10
Extramedullary Hematopoiesis	10 (2.5)	10 (2.5)	9 (2.8)	10 (2.7)	10 (3.6)**	10 (3.8)**
Pigmentation	9 (1.7)	10 (1.9)	9 (1.9)	10 (2.2)*	10 (3.0)**	10 (3.0)**
Bone Marrow	10	10	9	10	10	10
Hyperplasia	3	0	0	0	0	4
13 Weeks						
Male						
Liver	9	11	8	9	10	9
Kupffer Cell Pigmentation	0	0	0	0	1 (0.2)	9**(2.7)**
Extramedullary Hematopoiesis	1 (0.4)	1 (0.2)	0	7**(2.2)**	10**(3.2)**	9**(3.9)**
Spleen	9	11	8	9	10	9
Pigmentation	0	0	0	3 (0.8)	10**(2.6)**	8**(1.8)**
Bone Marrow	9	11	7	9	10	9
Hyperplasia	3	5	2	5	8	7
Female						
Spleen	10	10	10	8	10	10
Extramedullary Hematopoiesis	0	4*(0.9)*	1 (0.2)	5**(1.8)**	10**(2.9)**	9**(3.6)**
Pigmentation	8 (1.6)	6 (1.2)	6 (1.2)	8 (2.1)	10 (2.9)**	9 (3.5)**
Bone Marrow	9	10	10	8	10	10
Hyperplasia	3	0	0	0	0	5

* Significantly different (P≤0.05) from the control group by the Fisher exact test (incidence) or by the Mann-Whitney U test (average severity grade)
** P≤0.01
[a] Number of mice with organ examined microscopically
[b] Number of mice with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

2-YEAR STUDIES

Survival

Estimates of the probability of survival for control mice and for male and female mice administered *p*-nitroaniline are shown in Table 8 and in the Kaplan-Meier curves in Figure 1. The survival of mice administered *p*-nitroaniline was similar to that of the controls.

TABLE 8
Survival of Mice in the 2-Year Gavage Studies of *p*-Nitroaniline

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
Animals initially in study	70	70	70	70
9-Month interim evaluation[a]	10	10	10	10
15-Month interim evaluation[a]	10	10	10	10
Natural deaths	4	4	3	1
Moribund kills	13	14	10	10
Accidental deaths[a]	0	0	1	0
Animals surviving to study termination	33	32	36	39
Percent probability of survival at end of study[b]	66	64	74	78
Mean survival (days)[c]	597	596	594	607
Survival analysis[d]	P=0.137N	P=0.846	P=0.599N	P=0.270N
Female				
Animals initially in study	70	70	70	70
9-Month interim evaluation[a]	9	10	9	10
15-Month interim evaluation[a]	9	10	10	9
Natural deaths	5	4	5	6
Moribund kills	16	5	11	12
Accidental deaths[a]	2	0	3	1
Animals surviving to study termination	29	41	32	32
Percent probability of survival at end of study	59	82	67	65
Mean survival (days)	568	606	577	592
Survival analysis	P=0.696	P=0.017N	P=0.395N	P=0.538N

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

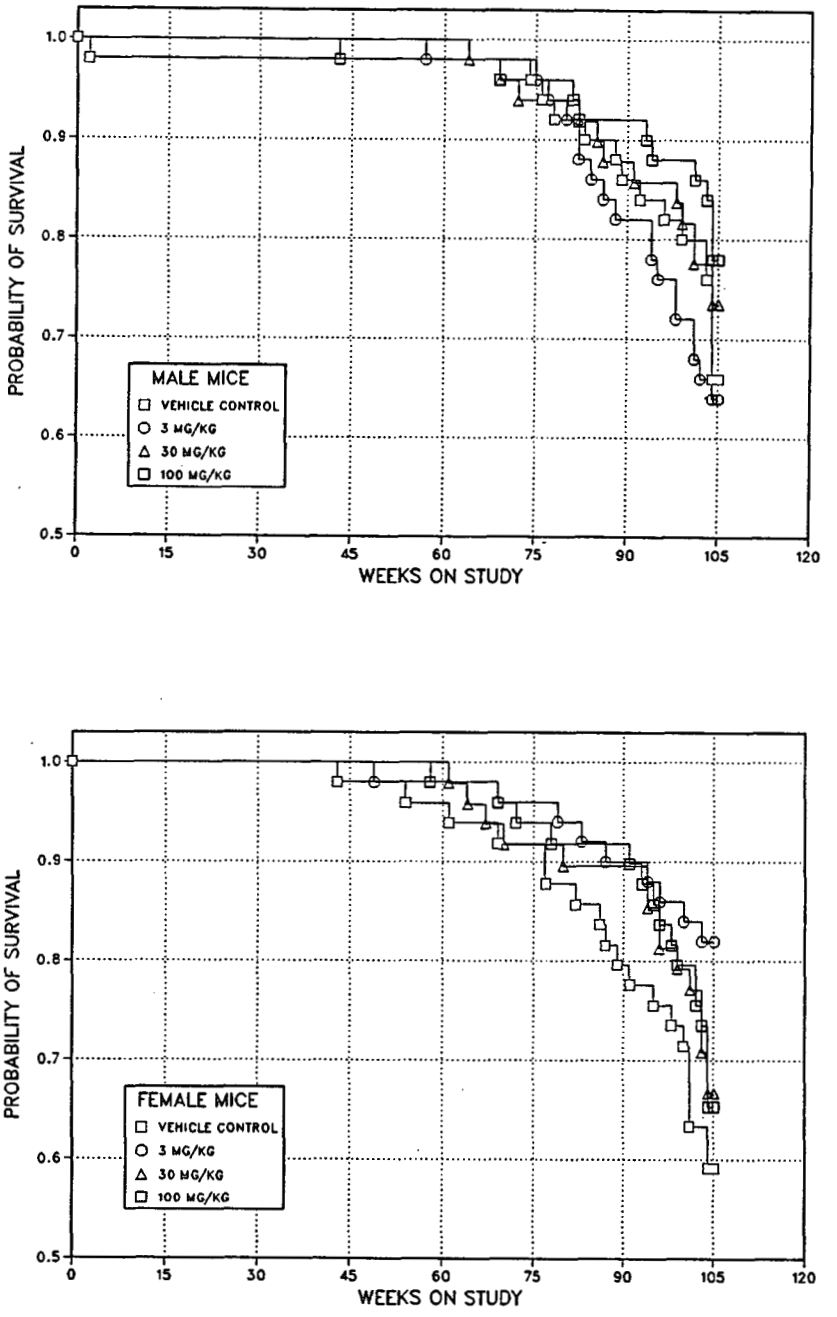


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Mice Administered *p*-Nitroaniline by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of male and female mice that received *p*-nitroaniline were similar to those of controls throughout the 2-year studies (Figure 2 and Tables 9 and 10). There were no clinical findings associated with chemical exposure.

Hematology and Clinical Chemistry

The hematology and clinical chemistry findings at the 9- and 15-month interim evaluations were similar to those in the 14-day and 13-week studies (Tables E4 and E5). The methemoglobin concentrations in male and female mice receiving 30 or 100 mg/kg *p*-nitroaniline for 9 or 15 months were significantly higher than those in controls (Tables E4 and E5). Although at 9 months the sulfhemoglobin concentration was also significantly higher in these groups, at 15 months it was higher only in 100 mg/kg females. The hematocrit values and erythrocyte counts of most 100 mg/kg mice were significantly lower than those of controls at both interim evaluations. Consistent with this evidence of a slight anemia, the number of reticulocytes in 30 or 100 mg/kg mice at 9 months and in 100 mg/kg mice at 15 months was significantly higher than those in controls. Although the increases in mean erythrocyte hemoglobin and mean erythrocyte hemoglobin concentration occurring in the 30 and 100 mg/kg groups are also consistent with a regenerative anemia, these may also be due, in part, to an artifact associated with the presence of Heinz bodies, as explained before. Total leukocyte and lymphocyte counts in 100 mg/kg males at 9 and 15 months and in females receiving the same dose at 15 months were significantly higher than those in controls. Slight increases in total blood leukocytes are observed with regenerative anemias of a variety of causes, apparently as a result of general bone marrow stimulation. However, these increases may also be due, in part, to the presence of Heinz bodies and reticulocytes that are not completely lysed before the blood is placed in the electronic cell counter.

Pathology and Statistical Analyses of Results

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

Vascular System: A hemangioma was seen in the urinary bladder of one male mouse that received 100 mg/kg for 9 months and a hemangiosarcoma was observed in the liver of a male mouse that received 30 mg/kg for 15 months (Table A1). These neoplasms are of interest because of the marginal increase observed in vascular neoplasms at all sites in the 2-year study. Vascular neoplasms occurred in several organs in control mice and in mice receiving *p*-nitroaniline at the end of the 2-year studies (Tables 11, A1, and B1). There was no apparent pattern in the occurrence of hemangioma or hemangiosarcoma except in the liver, where hemangiosarcomas were seen in one 3 mg/kg male, two 30 mg/kg males, and four 100 mg/kg males (Table 11). Although there was a significant positive trend for hemangiosarcoma of the liver and for hemangioma or hemangiosarcoma (combined) at all sites, the incidences in the dosed groups were not significantly greater than those of the controls by pairwise comparisons. The historical incidence of hemangiosarcoma of the liver in NTP control male mice is 15 of 699 (2%) with a range of 0% to 6%, and the incidence of hemangioma or hemangiosarcoma (combined) at all sites is 46 of 700 (7%) with a range of 0% to 12% (Tables A4a and A4b). Thus, the incidence of hemangiosarcoma of the liver and hemangioma or hemangiosarcoma (combined) at all sites in male mice receiving 100 mg/kg *p*-nitroaniline exceeds the range for historical controls.

The incidence of hemangioma or hemangiosarcoma (combined) at all sites was slightly increased in female mice receiving *p*-nitroaniline, but was not significantly different from that in controls by trend or pairwise comparisons (Table 11). Moreover, the incidences in the dosed groups were within the historical control range of 0% to 12% (Table B4).

The benign and malignant vascular neoplasms constituted a morphologic continuum. The hemangiomas were circumscribed masses consisting of irregular, thin-walled vessels with well-differentiated endothelial cells. The nuclei of the endothelial cells were generally evenly spaced along the vascular walls and were normal in appearance. The hemangiosarcomas also consisted of irregular thin-walled vessels, but the nuclei of the endothelial cells were often irregularly spaced and crowded. In some areas the endothelial nuclei were enlarged and pleomorphic.

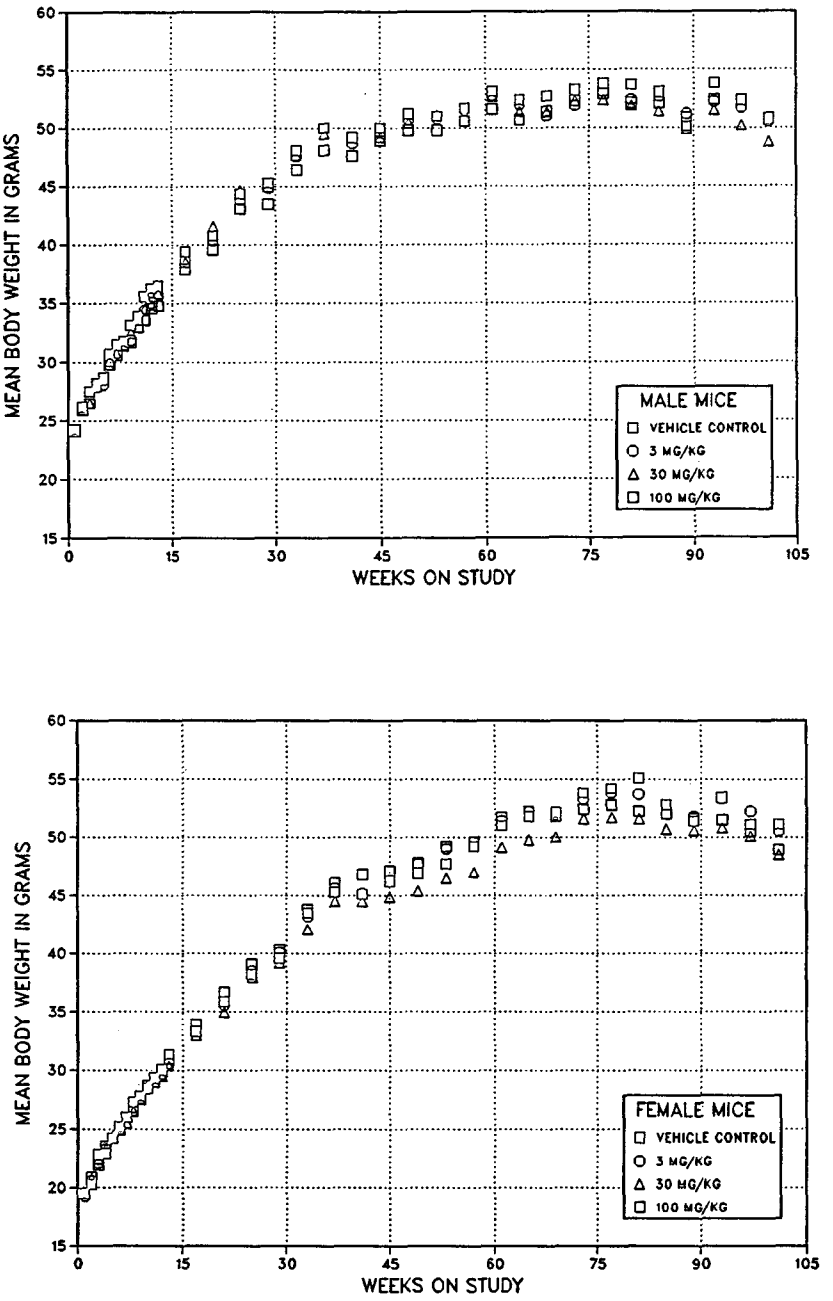


FIGURE 2
Growth Curves for Male and Female Mice Administered *p*-Nitroaniline by Gavage for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline

Weeks on Study	Vehicle Control Av. Wt. (g)	Vehicle Control No. of Survivors	3 mg/kg Av. Wt. (g)	3 mg/kg Wt. (% of controls)	3 mg/kg No. of Survivors	30 mg/kg Av. Wt. (g)	30 mg/kg Wt. (% of controls)	30 mg/kg No. of Survivors	100 mg/kg Av. Wt. (g)	100 mg/kg Wt. (% of controls)	100 mg/kg No. of Survivors
1	23.8	70	24.0	101	70	23.7	100	70	23.7	100	70
2	26.1	69	26.1	100	70	26.0	100	69	25.9	99	70
3	27.3	69	26.6	97	70	26.4	97	69	26.4	97	70
4	27.8	69	28.0	101	70	28.2	101	69	28.2	101	70
5	28.6	69	28.3	99	70	28.5	100	69	28.4	99	70
6	30.6	69	29.9	98	70	29.9	98	69	29.7	97	70
7	31.5	69	31.0	98	70	30.5	97	69	30.5	97	70
8	31.6	69	31.4	99	70	31.2	99	69	31.4	99	70
9	33.1	69	32.1	97	70	32.2	97	69	31.7	96	70
10	33.8	69	33.7	100	70	33.5	99	69	33.0	98	70
11	35.4	69	34.5	98	70	34.3	97	69	33.6	95	70
12	36.1	69	35.1	97	70	34.7	96	69	34.7	96	70
13	36.4	69	36.1	99	70	35.7	98	69	34.8	96	70
17	38.9	69	39.0	100	70	38.3	99	69	37.9	97	70
21	40.4	69	40.8	101	70	41.2	102	69	39.9	99	70
25	44.1	69	44.5	101	70	44.2	100	69	43.1	98	70
29	45.1	69	45.4	101	70	44.5	99	69	43.5	97	70
33	47.8	69	48.1	101	70	47.3	99	69	46.5	97	70
37	49.6	69	50.2	101	70	49.2	99	69	48.4	98	70
41[a]	49.2	59	49.0	100	60	48.9	99	59	47.6	97	60
45	49.7	59	49.9	100	60	49.0	99	59	48.7	98	59
49	51.1	59	51.0	100	60	50.2	98	59	49.7	97	59
53	50.9	59	50.9	100	60	50.9	100	59	49.7	98	59
57	51.6	59	51.8	100	60	51.5	100	59	50.5	98	59
61	53.1	59	53.0	100	59	52.4	99	59	51.3	97	59
65[a]	52.3	59	52.3	100	59	51.1	98	58	50.3	96	59
69	52.7	49	51.0	97	49	51.3	97	48	51.4	98	49
73	53.3	49	51.9	97	49	52.3	98	46	52.3	98	48
77	53.8	47	53.1	99	48	52.4	97	46	52.9	98	48
81	53.7	46	52.4	98	46	51.9	97	46	52.1	97	48
85	53.1	45	52.7	99	43	51.4	97	45	52.1	98	46
89	50.1	44	51.2	102	41	49.9	100	43	50.9	102	46
93	53.8	42	52.2	97	41	51.5	96	42	52.4	97	46
97	52.3	41	51.7	99	38	50.2	96	42	52.4	100	44
101	50.8	40	50.5	99	34	48.8	96	38	50.7	100	43
Terminal sacrifice		33			32			36			39
Mean for weeks											
1-13	30.9		30.5	99		30.4	98		30.2	98	
14-52	46.2		46.4	100		45.9	99		45.0	97	
53-101	52.4		51.9	99		51.2	98		51.5	98	

[a] Interim evaluations occurred during weeks 40 and 65.

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline

Weeks on Study	Vehicle Control		3 mg/kg			30 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.0	70	19.0	100	70	19.2	101	70	19.0	100	70
2	20.4	68	20.6	101	70	20.5	101	69	20.8	102	69
3	22.8	68	22.3	98	70	22.5	99	68	22.1	97	69
4	22.9	68	23.5	103	70	23.5	103	67	23.4	102	69
5	24.1	68	24.1	100	70	24.5	102	67	24.3	101	69
6	25.1	68	25.0	100	70	25.0	100	67	24.8	99	69
7	26.0	68	26.2	101	70	25.6	99	67	26.0	100	69
8	27.3	68	27.0	99	70	26.7	98	67	26.5	97	69
9	27.5	68	27.6	100	70	27.6	100	67	27.5	100	69
10	28.2	68	28.5	101	70	28.8	102	67	28.6	101	69
11	29.0	68	29.1	100	70	29.1	100	67	29.3	101	69
12	29.8	68	29.9	100	70	29.6	99	67	29.8	100	69
13	31.2	68	30.8	99	70	30.5	98	67	30.8	99	69
17	33.2	67	33.3	100	70	33.2	100	67	33.6	101	69
21	35.5	67	35.8	101	70	35.3	99	67	36.5	103	69
25	38.0	67	38.5	101	70	38.1	100	67	39.0	103	69
29	39.5	67	40.1	102	70	39.4	100	67	40.3	102	69
33	43.0	67	43.2	101	70	42.4	99	67	43.8	102	69
37	45.2	67	45.6	101	70	44.7	99	67	46.0	102	69
41[a]	44.8	58	45.0	100	60	44.6	100	58	46.8	105	59
45	45.9	57	45.9	100	60	45.0	98	58	47.2	103	59
49	46.5	57	47.6	102	60	45.5	98	58	47.5	102	59
53	47.4	57	48.7	103	59	46.7	99	58	49.1	104	58
57	49.0	56	49.5	101	59	47.3	97	58	49.4	101	58
61	50.7	56	51.1	101	59	49.1	97	58	51.7	102	57
65[a]	51.2	53	52.1	102	57	50.1	98	54	52.2	102	54
69	52.1	46	51.9	100	49	50.0	96	45	51.8	99	47
73	53.8	45	53.3	99	48	51.6	96	44	52.4	97	46
77	54.2	45	53.8	99	48	51.7	95	44	52.8	97	46
81	55.1	43	53.7	98	47	51.6	94	43	52.2	95	45
85	52.8	42	52.8	100	46	50.7	96	43	52.0	99	45
89	51.4	40	51.8	101	45	50.6	98	43	51.6	100	45
93	53.4	38	53.3	100	45	50.8	95	43	51.5	96	44
97	51.1	37	52.2	102	43	50.1	98	39	50.5	99	41
101	51.1	31	50.5	99	42	48.5	95	38	48.9	96	39
Terminal sacrifice		29			41			32			32
Mean for weeks											
1-13	25.6		25.7	100		25.6	100		25.6	100	
14-52	41.3		41.7	101		40.9	99		42.3	102	
53-101	51.8		51.9	100		49.9	96		51.2	99	

[a] Interim evaluations occurred during weeks 40 and 65.

TABLE 11
Incidences of Selected Vascular Neoplasms in Mice in the 2-Year Gavage Studies of *p*-Nitroaniline

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
Liver				
Hemangiosarcoma[a]				
Overall rate[b]	0/50 (0%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Terminal rate[c]	0/33 (0%)	1/32 (3%)	2/36 (6%)	3/39 (8%)
First incidence (days)	–[e]	729 (T)	729 (T)	563
Logistic regression test[d]	P=0.033	P=0.494	P=0.258	P=0.060
Mesentery				
Hemangioma	0/50	1/50	0/50	0/50
Hemangiosarcoma	1/50	0/50	0/50	0/50
Bone Marrow				
Hemangiosarcoma	1/50	0/50	1/50	2/50
Mesenteric Lymph Node				
Hemangiosarcoma	1/49	0/47	0/49	0/45
Spleen				
Hemangioma	0/50	1/50	1/50	1/50
Hemangiosarcoma	4/50	0/50	2/50	2/50
Skeletal Muscle[f]				
Hemangiosarcoma	0/50	0/50	0/50	1/50
Subcutaneous Tissue				
Hemangioma	1/50	0/50	0/50	1/50
Hemangiosarcoma	1/50	0/50	0/50	0/50
Ear[f]				
Hemangiosarcoma	1/50	0/50	0/50	0/50
All Organs				
Hemangioma or Hemangiosarcoma[g]				
Overall rate	5/50 (10%)	3/50 (6%)	4/50 (8%)	10/50 (20%)
Terminal rate	1/33 (3%)	2/32 (6%)	3/36 (8%)	7/39 (18%)
First incidence (days)	667	681	725	563
Logistic regression test	P=0.026	P=0.379N	P=0.507N	P=0.137
Female				
All Organs				
Hemangioma or Hemangiosarcoma[h]				
Overall rate	1/52 (2%)	3/50 (6%)	3/51 (6%)	4/51 (8%)
Terminal rate	0/29 (0%)	1/41 (2%)	1/32 (3%)	3/32 (9%)
First incidence (days)	701	553	654	716
Logistic regression test	P=0.231	P=0.286	P=0.314	P=0.213

(T)Terminal sacrifice
[a] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation): 15/699 (2.1% ± 2.1%); range 0%-6%
[b] Number of neoplasm-bearing animals/number of animals examined microscopically.
[c] Observed incidence at terminal kill
[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 the controls and that dosed group. The logistic regression test regards these lesions as nonfatal. A lower incidence in a dose group is indicated by N.
[e] Not applicable; no neoplasms in dose group
[f] Diagnosis based on gross observation
[g] Historical incidence: 46/700 (6.6% ± 3.6%); range 0%-12%
[h] Historical incidence: 21/698 (3.0% ± 3.5%); range 0%-12%

Spleen, Liver, and Bone Marrow: The absolute liver weight of 100 mg/kg male mice was significantly greater than controls at 9 months, but not at 15 months. Similarly, that of 100 mg/kg female mice was significantly increased at 9 and 15 months. The absolute and relative spleen weights of 100 mg/kg males and females were also significantly increased at both interim evaluations, with the exception of the relative spleen weight of female mice at 15 months. In most mice receiving 30 or 100 mg/kg at the 9- and 15-month interim evaluations, the splenic red pulp was filled with erythrocytes (congestion) and erythroid precursor cells (hematopoietic cell proliferation or hematopoiesis) with many macrophages containing hemosiderin (Tables 12, A5, and B5). Kupffer cells containing hemosiderin were observed primarily in the liver of 100 mg/kg mice. Increased cellularity of the bone marrow (hyperplasia) was observed primarily in male mice receiving 30 or 100 mg/kg.

At the end of 2 years, the incidence of hematopoietic cell proliferation of the spleen increased with dose and was significantly increased in 30 and 100 mg/kg male mice (Tables 12 and A5), while the incidence was only slightly increased in 100 mg/kg female mice. The incidence of bone marrow hypercellularity (hyperplasia) followed a dose-related increase in male mice and was significantly increased in all dosed male groups and in females that received 100 mg/kg (Tables 12, A5, and B5).

The incidence of pigment deposition in the spleen of male and female mice increased with dose, and the incidence of pigment deposition in Kupffer cells of the liver in male and female mice was increased in the 30 and 100 mg/kg groups (Tables 12, A5, and B5). The pigment was positive for iron using Gomori's iron stain; this finding is consistent with that seen with hemosiderin.

Small Intestine: Adenocarcinomas of the jejunum occurred in two 3 mg/kg males, two 3 mg/kg females, and one 30 mg/kg female (Tables A1 and B1). Neoplasms of the small intestine are uncommon in mice; the current historical database contains only one carcinoma of the jejunum in 700 male mice. In the present study, however, these neoplasms appear to be unrelated to chemical exposure: the incidences are low and are not dose related.

Liver: The incidence of hepatocellular adenoma or carcinoma (combined) was significantly decreased in 100 mg/kg male mice (0 mg/kg, 25/50; 3 mg/kg, 26/50; 30 mg/kg, 25/50; 100 mg/kg, 13/50; Table A3).

Genetic Toxicology

p-Nitroaniline is mutagenic *in vitro*. It was tested (up to 6,666 μg/plate) in two laboratories for the induction of gene mutations in several strains of *Salmonella typhimurium* using a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. Both laboratories showed positive results in strain TA98, with and without S9; negative results were obtained, with and without S9, in strains TA100, TA1535, TA1537, and TA97 (Table C1; Haworth *et al.*, 1983).

p-Nitroaniline was tested in two laboratories for induction of sister chromatid exchanges (Table C2) and chromosomal aberrations (Table C3) in Chinese hamster ovary cells, with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9. In the sister chromatid exchange study, one laboratory (Columbia University) reported negative results in the absence of S9 and positive results with S9 (effective dose range of 1,600 to 3,000 μg/mL) (Galloway *et al.*, 1987). The second laboratory (Environmental Health Research and Testing, Inc.) performed two trials without S9: results of the first trial were weakly positive and the second trial was negative. The results were therefore considered to be equivocal because the initially observed positive response at the high dose did not repeat. In contrast to the results obtained at Columbia in the sister chromatid exchange study, Environmental Health Research and Testing reported negative results with *p*-nitroaniline in the presence of S9; the highest dose tested was 5,000 μg/mL.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Gavage Studies of *p*-Nitroaniline

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
9-Month Interim Evaluation				
Bone Marrow[a]	10	10	10	10
Hyperplasia[b]	0	0	9**	10**
Liver	10	10	10	10
Kupffer Cell Pigmentation	0	0	0	10**
Spleen	10	10	10	10
Congestion	0	0	6**	10**
Hematopoietic Cell Proliferation	0	0	10**	10**
Pigmentation (Hemosiderin)	0	0	10**	10**
15-Month Interim Evaluation				
Bone Marrow	10	10	10	10
Hyperplasia	0	0	4*	9**
Liver	10	10	10	10
Kupffer Cell Pigmentation	1	0	0	0
Spleen	10	10	10	10
Congestion	0	1	10**	10**
Hematopoietic Cell Proliferation	2	0	10**	10**
Pigmentation (Hemosiderin)	0	0	10**	10**
2-Year Study				
Bone Marrow	50	50	50	50
Hypercellularity	1	10**	22**	27**
Liver	50	50	50	50
Kupffer Cell Pigmentation	1	1	8*	50**
Spleen	50	50	50	50
Hematopoietic Cell Proliferation	13	18	37**	48**
Pigmentation	0	1	46**	50**

(continued)

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Female				
9-Month Interim Evaluation				
Liver	9	10	9	10
Kupffer Cell Pigmentation	0	0	0	8**
Spleen	9	10	9	10
Congestion	0	0	9**	10**
Hematopoietic Cell Proliferation	0	0	9**	10**
Pigmentation (Hemosiderin)	0	1	9**	10**
15-Month Interim Evaluation				
Bone Marrow	9	–[c]	–	9
Hyperplasia	1			0
Liver	9	10	10	9
Kupffer Cell Pigmentation	1	0	0	0
Spleen	9	10	10	9
Congestion	0	2	7**	9**
Hematopoietic Cell Proliferation	1	3	10**	9**
Pigmentation (Hemosiderin)	0	0	10**	9**
2-Year Study				
Bone Marrow	52	50	51	51
Hypercellularity	6	4	8	22**
Liver	52	50	51	51
Kupffer Cell Pigmentation	1	1	4	39**
Spleen				
Hematopoietic Cell Proliferation	45	43	47	48
Pigmentation	6	23**	45**	49**

* Significantly different (P≤0.05) from the control group by the Fisher exact test (interims) or the logistic regression test (2-year studies)
** P≤0.01
[a] Number of mice with organ/tissue examined microscopically
[b] Number of mice with lesion
[c] Bone marrow not examined at these dose levels

In the chromosomal aberrations study (Table C3), both testing laboratories found positive results with *p*-nitroaniline in the presence of S9. The first laboratory reported weakly positive results without S9 at an effective dose of 1,600 μg/mL (Galloway *et al.*, 1987) while the second laboratory reported negative results without S9 (highest scorable dose, 800 μg/mL).

p-Nitroaniline induced trifluorothymidine resistance in L5178Y mouse lymphoma cells without S9; results with S9 were negative (Table C4). In this assay, *p*-nitroaniline must remain soluble for the duration of the exposure time. Therefore, the positive responses exhibited for the dose levels at which *p*-nitroaniline precipitation occurred were not included in the evaluation of the experiment (see Trial 1 with S9, for example, Table C4).

p-Nitroaniline did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (Table C5) when administered by feeding (5,000 ppm) or by injection (1,000 ppm) to adult males (Valencia *et al.*, 1985), or by feeding (100 ppm) to larvae (Zimmering *et al.*, 1989).

DISCUSSION AND CONCLUSIONS

The ability to derivatize aromatic amines has made them useful compounds for the preparation of dyes and pigments. *p*-Nitroaniline is an example of a simple primary aromatic amine which, during the manufacture of several different dyes, is first converted to a diazonium salt and then diazo coupled to another aromatic molecule. Because of the potential for widespread human exposure and the absence of data concerning the associated hazard, *p*-nitroaniline was evaluated by the National Toxicology Program in 14-day and 13-week toxicology studies and in 2-year carcinogenicity studies.

The toxic responses observed in both the 14-day and 13-week studies were indicative of that associated with a regenerative hemolytic anemia. These included dose-related decreases in erythrocyte counts and hematocrit values accompanied by dose-related increases in reticulocyte and leukocyte counts. The concentration of methemoglobin was significantly higher in all dosed mice in the 14-day studies and in the 30 and 100 mg/kg groups in the 13-week studies. Heinz bodies were present in the erythrocytes of all 1,000 mg/kg mice and two 100 mg/kg male mice in the 14-day studies and in a number of mice in the 13-week studies. At necropsy the spleens of all mice that received 300 mg/kg for 14 days and all mice that received 30 or 100 mg/kg for 13 weeks were enlarged and red to dark brown in appearance. Microscopic changes associated with chemical exposure included dose-related increased severity of extramedullary hematopoiesis in the spleen, increased severity of bone marrow hyperplasia, and increased pigmentation of Kupffer cells in the liver.

These responses are similar to those observed with aniline and substituted aniline compounds and are caused by the reaction of these compounds or their metabolites with hemoglobin. During the course of these reactions ferrohemoglobin (Fe^{2+}) is oxidized to ferrihemoglobin (methemoglobin; Fe^{3+}) at a faster rate than ferrihemoglobin can be reduced back to ferrohemoglobin by the methemoglobin reducing system of the erythrocyte; this results in the net accumulation of the oxidized form (methemoglobin). The presence of ferric iron (Fe^{3+}) in the heme groups of hemoglobin initiates a series of irreversible changes that lead to denaturation of globin and formation of protein complexes that eventually precipitate within the erythrocyte to form Heinz bodies. The presence of Heinz bodies, precipitated hemoglobin, or both, leads to the premature removal of the affected erythrocytes from the peripheral circulation by the spleen.

Although there are no detailed studies that have evaluated the interaction between *p*-nitroaniline and hemoglobin, the reactions between aniline and hemoglobin have been examined in detail. Eyer and Lierheimer (1980) and Eyer (1983) demonstrated, using rat livers perfused with an aniline-containing perfusate, that N-oxidation of aniline to phenylhydroxylamine occurs in the liver. However, the steady state concentration of the hydroxylamine within the liver is very low because within hepatocytes the rate of reduction of phenylhydroxylamine back to aniline is greater than its rate of formation. Therefore, quantitatively, phenylhydroxylamine is only a very minor metabolite of aniline.

The capacity of erythrocytes to reduce phenylhydroxylamine back to aniline is much less than that of hepatocytes. Thus, any phenylhydroxylamine that escapes from the liver and is taken up by erythrocytes will be rapidly converted to nitrosobenzene in a cooxidation reaction with oxyhemoglobin, resulting in the concomitant formation of methemoglobin. Nitrosobenzene formed in erythrocytes can then be reduced back to phenylhydroxylamine by the methemoglobin reductase system, to be reoxidized to nitrosobenzene along with the conversion of another molecule of oxyhemoglobin to methemoglobin. Thus, in the presence of phenylhydroxylamine, a quasi-catalytic cycle for the oxidation of oxyhemoglobin to methemoglobin is established within erythrocytes, involving the alternate oxidation of phenylhydroxylamine to nitrosobenzene, followed by the reduction of nitrosobenzene back to the hydroxylamine. Nitrosobenzene also reacts with erythrocyte proteins and glutathione; these side reactions eventually deplete the nitrosobenzene and destroy the cycle. However, the overall effect of these reactions is for

small (catalytic) amounts of phenylhydroxylamine (and by analogy hydroxylamines derived from other aniline compounds) to cause the rapid formation of methemoglobin within erythrocytes.

In these 14-day and 13-week studies traditional measures of toxic response, such as mean body weights and survival, were not affected by doses of 300 mg/kg, or approximately one-half the oral LD_{50} of 750 mg/kg, even though the severity of anemia observed at 300 mg/kg was considered potentially life threatening in a 2-year study. Therefore, the major consideration in the selection of doses for the 2-year studies was determining a dose at which the hemolytic anemia would not become life threatening. Although the mice appeared to compensate for accelerated destruction of erythrocytes by increasing hematopoiesis, it was unclear how efficiently this could be sustained throughout the 2-year studies. In previous NTP studies of aniline and substituted anilines the compounds were administered in the diet, and the character of the systemic exposure to the chemical was different from that seen with gavage administration. With dietary administration the chemical is present in the blood at relatively low levels over a 6- to 8-hour period; with gavage administration the chemical is delivered as a bolus and the blood level rises sharply to relatively high levels but decays rapidly thereafter. The half-life of *p*-nitroaniline for clearance from the blood is 0.8 hours. Therefore, it was difficult to evaluate the potential for long-term toxicity associated with chemical-induced anemia in a gavage study based on the previous dietary studies of aniline compounds. The only other aniline compound evaluated by gavage was *p*-chloroaniline hydrochloride, which was administered in deionized water at doses of 3, 10, or 30 mg/kg in the 2-year studies. These doses were selected on the basis of the severity of chemical-induced anemia observed in the 14-day and 13-week studies. In the current studies a similar dose selection rationale was used.

During the 14-day studies, all mice that received 1,000 mg/kg died by study day 4; at necropsy the tissues of these animals were yellow and their urine was dark yellow, observations compatible with the presence of high concentrations of hemoglobin degradation products. There were no compound-related deaths at the 300 mg/kg dose; however, based on decreases in the hematocrit value and erythrocyte count and increases in the reticulocyte count and absolute and relative spleen weight, the severity of anemia at this dose was considered potentially life threatening. Therefore, the dose response for toxicity and lethality increased markedly between 300 and 1,000 mg/kg.

During the 13-week studies there were no treatment-related deaths at the high dose of 100 mg/kg. In addition, hematologic differences including decreased hematocrit values and erythrocyte counts and increased reticulocyte counts and absolute and relative spleen weights, which are indicative of the continuing presence of anemia, were less severe than those observed at 300 mg/kg in the 14-day studies. Therefore, 100 mg/kg was selected as the high dose for the 2-year studies. The remaining doses of 30 mg/kg and 10 mg/kg were selected to provide a wide dose range in the event that life-threatening toxicity developed in the high-dose group. In addition, interim evaluations were scheduled after 9 and 15 months of chemical exposure to monitor any progression in the severity of anemia and to evaluate the development of potential chemical-related lesions.

During the 2-year studies, the survival and mean body weights of mice receiving *p*-nitroaniline were similar to those of controls. Dosed mice evaluated after 9 and 15 months of treatment had enlarged, congested spleens, increased numbers of circulating reticulocytes, increased incidences of extramedullary hematopoiesis, increased incidences of bone marrow hyperplasia, and other evidence of continued anemia associated with the presence of increased turnover of erythrocytes. Mice evaluated at the end of the studies exhibited similar lesions, indicating that increased destruction of erythrocytes continued throughout the 2-year studies. While it is possible that a dose greater than 100 mg/kg but less than 200 mg/kg might have been tolerated, a dose exceeding 200 mg/kg would have increased the severity of anemia and may have resulted in life-shortening toxicity.

Hemangioma or hemangiosarcoma (combined) at all sites occurred with a significant positive trend in male mice. Although the incidences in the dosed groups were not significantly greater than controls by pairwise comparisons, the incidence of these neoplasms in the 100 mg/kg group (20%) exceeded the range for NTP historical control groups of male mice: range 0% to 12%; 46/700 (7%).

For decisions regarding the carcinogenic potential of chemicals, primary emphasis is generally placed on site (organ)-specific statistical analyses. This is justified because chemical carcinogens generally produce neoplasms at one or a few sites. Even for vascular neoplasms, which in theory may occur at multiple sites throughout the vascular system, increased incidences resulting from chemical exposure in experimental animals or humans have occurred at only one or a few specific sites; these sites may differ for each chemical. The incidences of vascular neoplasms were increased in the heart of mice exposed to 1,3-butadiene (NTP, 1984), the spleen of rats exposed to cupferron (NCI, 1978a), and the liver of humans exposed to vinyl chloride (IARC, 1979). In mice receiving *p*-nitroaniline, vascular neoplasms occurred at a low incidence in several organs, and with the exception of the liver, there was no apparent chemical-related pattern. In the liver, hemangiosarcoma occurred with a significant positive trend, and although the incidences in the dosed groups were not greater than concurrent controls by pairwise comparisons, the incidence in the 100 mg/kg group (8%) exceeded the range in NTP historical control groups of male mice: range 0% to 6%; 15/699 (2%). In contrast, the incidence of vascular neoplasms was not increased in female mice.

In studies conducted with other aniline compounds, splenic sarcomas and putative, preneoplastic, fibrotic lesions of the spleen have occurred in rats in NTP 2-year studies of aniline hydrochloride (NCI, 1978b), *p*-chloroaniline (NCI, 1979a), D&C Red No. 9 (NTP, 1982), *N,N*-dimethylaniline (NTP, 1989), 4,4′-sulfonyldianiline (NCI, 1977), *o*-toluidine hydrochloride (NCI, 1979b), and azobenzene (NCI, 1979c), and in a carcinogenicity study of aniline conducted by the Chemical Industry Institute of Toxicology (1982, unpublished data) (Goodman *et al.*, 1984; Weinberger *et al.*, 1985; Bus and Popp, 1987). However, no splenic sarcomas or nonneoplastic splenic lesions were found in mice; the absence of splenic lesions in mice indicates that they are less sensitive to the splenic toxicity of these compounds than are rats. Hemangiosarcomas occurred in mice only in the *p*-chloroaniline and *o*-toluidine hydrochloride studies. *p*-Chloroaniline administered by gavage in deionized water at 3, 10, or 30 mg/kg caused a marginal increased incidence of hemangiosarcoma (all sites) in high-dose male mice (4/50, 4/49, 1/50, 10/50). In all groups including controls, the neoplasms were present in the liver and spleen, or both, and the increase in the high-dose group was the result of a uniform increased incidence in both the liver and spleen rather than a site-specific increase. *o*-Toluidine hydrochloride administered in the feed at doses of 1,000 or 3,000 ppm increased the incidence of hemangiosarcomas in high-dose male mice (1/50, 1/50, 10/50); however, the increase was the result of a site-specific increase. Nine of the ten hemangiosarcomas in the high-dose group were in the abdominal cavity, while none were present in the liver or spleen, a result more indicative of a chemical-specific response than that observed in the *p*-chloroaniline study.

Genetic toxicity was assessed by testing the ability of *p*-nitroaniline to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, mutations in mouse lymphoma cells, and sex-linked recessive lethal mutations in *Drosophila melanogaster*. The genetic toxicology studies of *p*-nitroaniline are part of a larger effort by the NTP to develop a database that would permit the evaluation of the contribution of the four *in vitro* short-term genetic toxicity tests to predicting chemical carcinogenicity in experimental animals. These *in vitro* tests were developed to study mechanisms of chemical-induced DNA damage, but their use has been extended to the prediction of carcinogenicity based on the somatic mutation theory and the electrophilic theory of chemical carcinogenesis (Miller and Miller, 1977; Straus, 1981; Crawford, 1985). A positive response in any of these tests by a chemical that produces increases in neoplasm incidences in experimental animals does not necessarily implicate a specific mechanism of carcinogenicity involving direct DNA damage. Nevertheless, there is a strong correlation between structural alerts to DNA reactivity (electrophilicity), mutagenicity in *S. typhimurium*, and carcinogenicity in two rodent species or at multiple tissue sites (Ashby and Tennant, 1991), providing support for the electrophilic theory of chemical carcinogenesis in a subset of chemical carcinogens. The reader is referred to the article by Ashby and Tennant (1991) for details regarding the correlation of structural alerts (or absence thereof), mutagenicity, and carcinogenicity results of 301 chemicals in the NTP database.

An evaluation of the results of NTP genetic toxicity tests and carcinogenicity studies of 114 chemicals has been reported (Tennant *et al.*, 1987; Zeiger *et al.*,

1990). In this evaluation, the *S. typhimurium* assay was shown to have the lowest sensitivity (0.48 = proportion of carcinogens positive in *S. typhimurium*), the highest specificity (0.91 = proportion of non-carcinogens negative in *S. typhimurium*), and the highest positive predictability for carcinogenicity (89% of the chemicals mutagenic in *S. typhimurium* were carcinogenic in rodents) of the four *in vitro* tests. Positive tests for the induction of mutations in mouse lymphoma cells or for the induction of chromosomal aberrations or sister chromatid exchanges were less predictive of carcinogenicity; 63% of chemicals inducing mutations in mouse lymphoma cells, 73% of chemicals inducing chromosomal aberrations, and 64% of chemicals inducing sister chromatid exchanges were carcinogenic in rodents. The authors also concluded: (1) that there appeared to be little evidence of complementarity among the four assays for prediction of rodent carcinogenicity, and (2) that no battery of tests constructed from the above four substantially improved predictions of carcinogenic potential based on the *Salmonella* assay alone. The reader is referred to the original articles for further details regarding these analyses.

In the specific case of *p*-nitroaniline, both the aromatic nitro and the aromatic amine groups are molecular features which provide an alert to potential DNA reactivity (Tennant and Ashby, 1991). *p*-Nitroaniline gave positive results in all four of the NTP *in vitro* genetic toxicity studies (SAL, MLA, SCE, and ABS), and the metabolites of *p*-nitroaniline are also mutagenic in *Salmonella*. However, these positive results in genotoxicity assays and the structurally alerting nitro and aromatic amine groups were not predictive of the results of the mouse bioassay, where no clear evidence of carcinogenicity was observed.

CONCLUSIONS

Under the conditions of these 2-year gavage studies there was *equivocal evidence of carcinogenic activity** of *p*-nitroaniline in male B6C3F$_1$ mice based on the increased incidences of hemangiosarcoma of the liver and hemangioma or hemangiosarcoma (combined) at all sites. There was *no evidence of carcinogenic activity* of *p*-nitroaniline in female B6C3F$_1$ mice receiving doses of 3, 30, or 100 mg/kg.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH) (1991). Threshold limit values and biological exposure indices for 1991-1992. Cincinnati, OH.

Ames, B.N., Kammen, H.O., and Yamasaki, E. (1975). Hair dyes are mutagenic: Identification of a variety of mutagenic ingredients. *Proc. Natl. Acad. Sci. USA* 72, 2423-2427.

Anderson, B.E., Zeiger, E., Resnick, M.A., Shelby, M.D., Gulati, D.K., Ivett, J.L., and Loveday, K.S. (1990). Chromosome aberration and sister chromatid exchange test results with 42 chemicals. *Environ. Mol. Mutagen.* 16 (Suppl. 18), 55-137.

Anderson, M.M., Mays, J.B., Mitchum, R.K., and Hinson, J.A. (1984). Metabolism of 4-nitroaniline by rat liver microsomes. *Drug Metab. Dispos.* 12, 179-185.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

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

Blijleven, W.G.H. (1981). Re-evaluation of the mutagenic effects of the hair dye *p*-phenylenediamine (BASE) in the sex-linked recessive lethal test in *Drosophila melanogaster*. *Mutat. Res.* 90, 137-141.

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

Burnett, C., Loehr, R., and Corbett, J. (1977). Dominant lethal mutagenicity study on hair dyes. *J. Toxicol. Environ. Health* 2, 657-662.

Burnett, C., Fuchs, C., Corbett, J., and Menkart, J. (1982). The effect of dimethylsulfoxide on the mutagenicity of the hair dye *p*-phenylenediamine. *Mutat. Res.* 103, 1-4.

Bus, J.S., and Popp, J.A. (1987). Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally-related compounds. *Food Chem. Toxicol.* 25, 619-626.

Byeon, W.H., Paik, S.G., and Lee, S.Y. (1975). Mutagenicity of phenylenediamines and their derivatives. (I). *Kor. J. Microbiol.* 13, 51-58.

Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., and Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality control guidelines and response categories. *Environ. Mol. Mutagen.* 12 (Suppl. 13), 19-36.

Chiu, C.W., Lee, L.H., Wang, C.Y., and Bryan, G.T. (1978). Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat. Res.* 58, 11-22.

Chopade, H.M., and Matthews, H.B. (1984). Disposition and metabolism of *p*-nitroaniline in the male F-344 rat. *Fundam. Appl. Toxicol.* 4, 485-493.

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

Code of Federal Regulations (CFR) 29, regulation 1910.100.

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

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology* (W.G. Flamm and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific, Princeton, NJ.

Crebelli, R., Conti, L., Carere, A., and Zito, R. (1981). Mutagenicity of commercial *p*-phenylenediamine and of an oxidation mixture of *p*-phenylenediamine and resorcinol in *Salmonella typhimurium* TA98. *Food Cosmet. Toxicol.* **19**, 79-84.

DeFlora, S., Camoirano, A., Zanacchi, P., and Bennicelli, C. (1984a). Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other *Salmonella* strains. *Mutat. Res.* **134**, 159-165.

DeFlora, S., Zanacchi, P., Camoirano, A., Bennicelli, C., and Badolati, G.S. (1984b). Genotoxic activity and potency of 135 compounds in Ames reversion test and in a bacterial DNA-repair test. *Mutat. Res.* **133**, 161-198.

DeGawa, M., Shoji, Y., Masuko, K., and Hashimoto, Y. (1979). Mutagenicity of metabolites of carcinogenic aminoazo dyes. *Cancer Lett.* **8**, 71-76.

Dellarco, V.L., and Prival, M.J. (1989). Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ. Mol. Mutagen.* **13**, 116-127.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

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

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1095-1121.

Eyer, P., and Lierheimer, E. (1980). Biotransformation of nitrosobenzone in the red cell and the role of glutathione. *Xenobiotica* **10**, 517-526.

Eyer, P. (1983). The red cell as a sensitive target for activated toxic anylamines. *Arch. Toxicol.* Suppl. 6, 3-12.

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

Garner, R.C., and Nutman, C.A. (1977). Testing of some azo dyes and their reduction products for mutagenicity using *Salmonella typhimurium* TA1538. *Mutat. Res.* **44**, 9-19.

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

Goodman, D.G., Ward, J.M., and Reichardt, W.D. (1984). Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, *p*-chloroaniline, azobenzene, *o*-toluidine hydrochloride, 4,4′-sulfonyldianiline, or D&C Red 9. *JNCI* **73**, 265.

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

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

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* **75**, 975-984.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutagen.* 5 (Suppl. 1), 3-142.

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

Hossack, J.N., and Richardson, J.C. (1977). Examination of the potential mutagenicity of hair dye constituents using the micronucleus test. *Experientia* 33, 377-378.

International Agency for Research on Cancer (IARC) (1979). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Some monomers, plastic and synthetic elastomers, and acrolein. Vol. 19, pp. 377-438. IARC, Lyon, France.

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

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

Le, J., Jung, R., and Kramer, M. (1985). Effects of using liver fractions from different mammals, including man, on results of mutagenicity assays in *Salmonella typhimurium*. *Food Chem. Toxicol.* 23, 695-700.

Levi, B.Z., Kuhn, J.C., and Ulitzur, S. (1986). Determination of the activity of 16 hydrazine derivatives in the bioluminescence test for genotoxic agents. *Mutat. Res.* 173, 233-237.

Malca-Mor, L., and Stark, A.A. (1982). Mutagenicity and toxicity of carcinogenic and other hydrazine derivatives: Correlation between toxic potency in animals and toxic potency in *Salmonella typhimurium* TA1538. *Appl. Environ. Microbiol.* 44, 801-808.

Margolin, B.H., Collings, B.J., and Mason, J.M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* 5, 705-716.

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

Mate, C., Ryan, A.J., and Wright, S.E. (1967). Metabolism of some 4-nitroaniline derivatives in the rat. *Food Cosmet. Toxicol.* 5, 657-663.

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

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* 79, 639-648.

Melnikow, J., Keeffe, J.R., and Bernstein, R.L. (1981). Carcinogens and mutagens in the undergraduate laboratory. *J. Chem. Educ.* 58, A11-A14.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-628. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mirsalis, J., Tyson, K., Beck, J., Loh, E., Steinmetz, K., Contreras, C., Austere, L., Martin, S., and Spalding, J. (1983). Induction of unscheduled DNA synthesis (UDS) in hepatocytes following *in vitro* and *in vivo* treatment. *Environ. Mutagen.* 5, 482.

Myhr, B., Bowers, L., and Caspary, W.J. (1985). Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. *Prog. Mutat. Res.* 5, 555-568.

Myhr, B., McGregor, D., Bowers, L., Riach, C., Brown, A.G., Edwards, I., McBride, D., Martin, R., and Caspary, W.J. (1990). L5178Y mouse lymphoma cell mutation assay results with 41 compounds. *Environ. Mol. Mutagen.* 16 (Suppl. 18), 138-167.

Nair, R.S., Johannsen, F.R., and Schroeder, R.E. (1985). Evaluation of teratogenic potential of para-nitroaniline and para-nitrochlorobenzene in rats and rabbits. In *Toxicity of Nitroaromatic Compounds* (D.E. Rickert, Ed.), pp. 61-85. Hemisphere Publish ing Corporation, New York.

Nair, R.S., Johannsen, F.R., Levinskas, G.J., and Terrill, J.B. (1986). Subchronic inhalation toxicity of *p*-nitroaniline and *p*-nitrochlorobenzene in rats. *Fundam. Appl. Toxicol.* **6**, 618-627.

Nair, R.S., Auletta, C.S., Schroeder, R.E., and Johannsen, F.R. (1990). Chronic toxicity, oncogenic potential, and reproductive toxicity of *p*-nitroaniline in rats. *Fundam. Appl. Toxicol.* **15**, 607-621.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1977). Bioassay of Dapsone for Possible Carcinogenicity (CAS No. 80-08-0). Technical Report Series No. 20. NIH Publication No. 77-820. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978a). Bioassay of Cupferron for Possible Carcinogenicity (CAS No. 135-20-6). Technical Report Series No. 100. NIH Publication No. 78-1350. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978b). Bioassay of Aniline Hydrochloride for Possible Carcinogenicity (CAS No. 142-04-1). Technical Report Series No. 130. NIH Publication No. 78-1385. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1979a). Bioassay of *p*-Chloroaniline for Possible Carcinogenicity (Feed Studies) (CAS No. 106-47-8). Technical Report Series No. 189. NIH Publication No. 79-1745. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1979b). Bioassay of *o*-Toluidine Hydrochloride for Possible Carcinogenicity (CAS No. 636-21-5). Technical Report Series No. 153. NIH Publication No. 79-1709. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1979c). Bioassay of Azobenzene for Possible Carcinogenicity (CAS No. 103-33-3). Technical Report Series No. 154. NIH Publication No. 79-1710. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). NIH Publication No. NIH-11-1335. National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1982). Carcinogenesis Bioassay of D & C Red No. 9 (CAS No. 5160-02-1) in F344 Rats and B6C3F$_1$ Mice (Feed Study). Technical Report Series No. 225. NIH Publication No. 82-1781. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1984). Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 288. NIH Publication No. 84-2544. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1989). Toxicology and Carcinogenesis Studies of *N,N*-Dimethylaniline (CAS No. 121-69-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 360. NIH Publication No. 90-2815. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Nohmi, T., Miyata, R., Yoshikawa, K., and Ishidate, M., Jr. (1982). Effects of 7,8-benzo-flavone and SKF 525-A on the enzyme-mediated mutagenicity of phenylenediamines. *J. Toxicol. Sci.* 7, 61-69.

Pai, V., Bloomfield, S.F., and Gorrod, J.W. (1985). Mutagenicity of N-hydroxylamines and N-hydroxy-carbamates towards strains of *Escherichia coli* and *Salmonella typhimurium*. *Mutat. Res.* 151, 201-207.

Sadtler Standard Spectra. IR No. 8451, NMR No. 9378M. Sadtler Research Laboratories, Philadelphia, PA.

Shahin, M.M. (1985). Mutagenicity of nitroanilines and nitroaminophenols in *Salmonella typhimurium*. *Intl. J. Cosmet. Sci.* 7, 277-289.

Shahin, M.M., Bugaut, A., and Kalopissis, G. (1982). Mutagenicity of aminonitrophenol compounds in *Salmonella typhimurium*: A study of structure-activity relationships. *Intl. J. Cosmet. Sci.* 4, 25-35.

Shahin, M.M., Andrillon, P., Goetz, N., Bore, P., Bugaut, A., and Kalopissis, G. (1979). Studies on the mutagenicity of *p*-phenylenediamine in *Salmonella typhimurium*. Presence of PCB's in rat-liver microsomal fraction induced by Aroclor. *Mutat. Res.* 68, 327-336.

Shimizu, M., and Yano, E. (1986). Mutagenicity of mononitrobenzene derivatives in the Ames test and rec assay. *Mutat. Res.* 170, 11-22.

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

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

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

Tennant, R.W., and Ashby, J. (1991). Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 39 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. *Mutat. Res.* 257, 209-227.

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

Thompson, C.Z., Hill, L.E., Epp, J.K., and Probst, G.S. (1983). The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. *Environ. Mutagen.* 5, 803-811.

Topham, J.C. (1980). The detection of carcinogen-induced sperm head abnormalities in mice. *Mutat. Res.* 69, 149-155.

Valencia, R., Mason, J.M., Woodruff, R.C., and Zimmering, S. (1985). Chemical mutagenesis testing in *Drosophila*. III. Results of 48 coded compounds tested for the National Toxicology Program. *Environ. Mutagen.* 7, 325-348.

Valencia, R., Mason, J.M., and Zimmering, S. (1989). Chemical mutagenesis testing in *Drosophila*. VI. Interlaboratory comparison of mutagenicity tests after treatment of larvae. *Environ. Mol. Mutagen.* 14, 238-244.

Watanabe, T., Kawata, A., and Koyanagi, T. (1980). Mutagenicity of hair dyes. Influence of couplers on mutagenicity of primary intermediates and couplers. *J. Soc. Cosmet. Chem. Japan* 14, 20-25.

Weinberger, M.A., Albert, R.H., and Montgomery, S.B. (1985). Splenotoxicity associated with splenic sarcomas in rats fed high doses of D&C Red No. 9 or aniline hydrochloride. *JNCI* 75, 681-690.

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

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

Yoshikawa, K., Nohmi, T., Harada, R., Inokawa, Y., and Ishidate, M., Jr. (1979). Differential mutagenicities of tramino benzenes against *Salmonella typhimurium* TA98 in the presence of S9 fractions from polychlorinated biphenyls-, phenobarbital- or 3-methyl-cholanthrene-pretreated rats, hamsters and mice. *J. Toxicol. Sci.* **4**, 317-326.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987). *Salmonella* mutagenicity tests. III. Results from the testing of 255 chemicals. *Environ. Mutagen.* **9** (Suppl. 9), 1-110.

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

Zimmering, S., Mason, J.M., and Valencia, R. (1989). Chemical mutagenesis testing in *Drosophila*. VII. Results of 22 coded compounds tested in larval feeding experiments. *Environ. Mol. Mutagen.* **14**, 245-251.

APPENDIX A
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF *p*-NITROANILINE

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	70	70	70	70
9-Month interim evaluation	10	10	10	10
15-Month interim evaluation	10	10	10	10
Early deaths				
Accidental deaths			1	
Moribund	13	14	10	10
Natural deaths	4	4	3	1
Survivors				
Terminal sacrifice	33	32	36	39
Animals examined microscopically	70	70	70	70
9-Month Interim Evaluation[b]				
Urinary System				
Urinary bladder	(10)		(10)	(10)
Hemangioma				1 (10%)
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hemangiosarcoma			1 (10%)	
Hepatocellular carcinoma		1 (10%)	1 (10%)	1 (10%)
Hepatocellular adenoma		1 (10%)	1 (10%)	
Hepatocellular adenoma, two, multiple	1 (10%)	1 (10%)		1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	1 (10%)			
Cardiovascular System				
None				
Endocrine System				
Thyroid gland	(10)	(1)		(10)
Follicular cell, carcinoma		1 (100%)		
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Spleen	(10)	(10)	(10)	(10)

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
15-Month Interim Evaluation (continued)				
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma				1 (10%)
Special Senses System				
None				
Urinary System				
None				
Systemic Lesions				
Multiple organs[c]	(10)	(10)	(10)	(10)
Lymphoma malignant lymphocytic	1 (10%)			
2-Year Study				
Alimentary System				
Intestine large, rectum	(49)	(49)	(50)	(49)
Fibrous histiocytoma	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(48)
Polyp adenomatous		1 (2%)		
Intestine small, jejunum	(50)	(49)	(50)	(50)
Adenocarcinoma		2 (4%)		
Liver	(50)	(50)	(50)	(50)
Cholangiocarcinoma			1 (2%)	
Cholangiocarcinoma, two, multiple		1 (2%)		
Hemangiosarcoma		1 (2%)	2 (4%)	4 (8%)
Hepatocellular carcinoma	8 (16%)	10 (20%)	11 (22%)	5 (10%)
Hepatocellular carcinoma, two, multiple	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Hepatocellular carcinoma, three, multiple			1 (2%)	
Hepatocellular adenoma	11 (22%)	12 (24%)	11 (22%)	8 (16%)
Hepatocellular adenoma, two, multiple	8 (16%)	6 (12%)	3 (6%)	1 (2%)
Hepatocellular adenoma, three, multiple			1 (2%)	
Hepatocellular adenoma, four, multiple			1 (2%)	
Ito cell tumor benign, two, multiple		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(2)	(4)	(6)	(1)
Cholangiocarcinoma, metastatic, liver		1 (25%)	1 (17%)	
Fibrosarcoma			1 (17%)	
Hemangioma		1 (25%)		
Hemangiosarcoma	1 (50%)			
Pancreas	(50)	(50)	(50)	(49)
Cholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Fibrosarcoma, metastatic, mesentery			1 (2%)	
Sarcoma	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal gland, cortex	(50)	(50)	(50)	(50)
Spindle cell, adenoma		1 (2%)	1 (2%)	
Adrenal gland, medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant			1 (2%)	
Pheochromocytoma benign	1 (2%)		1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma		1 (2%)	1 (2%)	
Pituitary gland	(48)	(47)	(48)	(45)
Pars distalis, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)	1 (2%)		
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Prostate	(49)	(49)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)	2 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node	(50)	(50)	(50)	(49)
Mediastinal, cholangiocarcinoma, metastatic, liver		1 (2%)		
Lymph node, mandibular	(50)	(48)	(49)	(48)
Lymph node, mesenteric	(49)	(47)	(49)	(45)
Hemangiosarcoma	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)	1 (2%)	1 (2%)
Hemangiosarcoma	4 (8%)		2 (4%)	2 (4%)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Thymus	(46)	(47)	(49)	(47)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Mediastinum, hemangiosarcoma				1 (2%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Sebaceous gland, adenoma			1 (2%)	
Subcutaneous tissue, fibrosarcoma		2 (4%)		
Subcutaneous tissue, hemangioma	1 (2%)			1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(2)	(1)
Cholangiocarcinoma, metastatic, liver		1 (100%)		
Fibrosarcoma	1 (100%)			
Fibrosarcoma, metastatic, mesentery			1 (50%)	
Hemangiosarcoma				1 (100%)
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	6 (12%)	7 (14%)	6 (12%)	3 (6%)
Alveolar/bronchiolar adenoma, two, multiple	1 (2%)		2 (4%)	
Alveolar/bronchiolar carcinoma	5 (10%)	2 (4%)	1 (2%)	6 (12%)
Alveolar/bronchiolar carcinoma, two, multiple		1 (2%)		
Carcinoma, metastatic, harderian gland	1 (2%)		2 (4%)	
Cholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Hepatocellular carcinoma, metastatic, liver		2 (4%)	2 (4%)	2 (4%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Polyp			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Special Senses System				
Ear	(2)	(1)	(2)	
Fibrosarcoma	1 (50%)		2 (100%)	
Hemangiosarcoma	1 (50%)			
Harderian gland	(8)	(6)	(4)	(9)
Adenoma	4 (50%)	5 (83%)	3 (75%)	7 (78%)
Carcinoma	1 (13%)	2 (33%)	2 (50%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Lymphoma malignant histiocytic		1 (2%)	1 (2%)	
Lymphoma malignant mixed	4 (8%)	1 (2%)	3 (6%)	
Neoplasm Summary				
Total animals with primary neoplasms[d]				
9-Month interim evaluation				1
15-Month interim evaluation	3	3	3	2
2-Year study	33	38	36	28
Total primary neoplasms				
9-Month interim evaluation				1
15-Month interim evaluation	3	4	3	3
2-Year study	70	67	66	46
Total animals with benign neoplasms				
9-Month interim evaluation				1
15-Month interim evaluation	2	2	1	2
2-Year study	27	32	26	19
Total benign neoplasms				
9-Month interim evaluation				1
15-Month interim evaluation	2	2	1	2
2-Year study	36	41	35	24
Total animals with malignant neoplasms				
15-Month interim evaluation	1	2	2	1
2-Year study	23	22	20	15
Total malignant neoplasms				
15-Month interim evaluation	1	2	2	1
2-Year study	34	26	31	22
Total animals with metastatic neoplasms				
2-Year study	2	3	5	2
Total metastatic neoplasms				
2-Year study	3	8	9	2

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] All organ systems listed in Table 1 (Materials and Methods) were evaluated, but neoplasms were found only in the urinary system.
[c] Number of animals with any tissue examined microscopically
[d] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control

Number of Days on Study	0	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	1	2	4	7	1	1	4	6	9	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	8	8	7	0	5	1	9	0	7	2	1	1	4	5	5	5	5	9	9	9	9	9	9	9
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	3	0	2	3	0	3	1	0	2	1	1	1	0	2	3	3	0	0	0	0	1	1	1
	9	3	6	5	0	8	1	5	2	4	0	1	3	3	2	6	7	1	4	5	7	2	4	6
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																								
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma					X	X	X				X		X							X				
Hepatocellular carcinoma, two, multiple				X											X									
Hepatocellular adenoma					X						X					X								
Hepatocellular adenoma, two, multiple		X				X		X															X	
Mesentery										+		+												
Hemangiosarcoma												X												
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma						X																		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma														X										
Squamous cell papilloma																								
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth		+					+	+		+	+	+	+	+	+	+	+	+	+	+	+			
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign											X													
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 1 8 1	0 1 9 1	0 2 0 1	0 2 1 1	0 2 3 1	0 2 6 1	0 2 7 1	0 2 8 1	0 2 9 1	0 3 2 1	0 3 4 1	0 3 5 1	0 3 8 1	0 3 9 1	0 4 0 1	0 4 1 1	0 4 2 1	0 4 3 1	0 4 4 1	0 4 5 1	0 4 6 1	0 4 7 1	0 4 8 1	0 4 9 1	0 5 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Fibrous histiocytoma						X																				1
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma					X										X											8
Hepatocellular carcinoma, two, multiple																										2
Hepatocellular adenoma	X			X	X			X					X				X					X	X			11
Hepatocellular adenoma, two, multiple						X										X				X					X	8
Mesentery																										2
Hemangiosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Squamous cell papilloma																		X			X	X				3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+			+		+	+	+	+	+	+		+	+		+	+	+	+	+			+	+	+	35
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	49

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control
(continued)

Number of Days on Study	0	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	1	2	4	7	1	1	4	6	9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	8	8	7	0	5	1	9	0	7	2	1	1	4	5	5	5	5	9	9	9	9	9	9	9	9
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	3	0	2	3	0	3	1	0	2	1	1	1	0	2	3	3	0	0	0	0	1	1	1	1
	9	3	6	5	0	8	1	5	2	4	0	1	3	3	2	6	7	1	4	5	7	2	4	6	7
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Endocrine System (continued)																									
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Coagulating gland				+			+	+								+		+	+						
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland		+	+		+	+	+				+	+	+		+	+	+			+	+		+		+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma									X																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																	X								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma									X			X		X			X								
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangioma																			X						
Subcutaneous tissue, hemangiosarcoma																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle						+																			
Fibrosarcoma						X																			

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 1 8 1	0 1 9 1	0 2 0 1	0 2 1 1	0 2 3 1	0 2 6 1	0 2 7 1	0 2 8 1	0 2 9 1	0 3 2 1	0 3 4 1	0 3 5 1	0 3 8 1	0 3 9 1	0 4 0 1	0 4 1 1	0 4 2 1	0 4 3 1	0 4 4 1	0 4 5 1	0 4 6 1	0 4 7 1	0 4 8 1	0 4 9 1	0 5 0 1	Total Tissues/ Tumors
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																		X								1
General Body System																										
None																										
Genital System																										
Coagulating gland			+	+		+	+		+					+					+			+	+	+	+	17
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+		+					+				+			+		+		+		M	+		+	+	25
Prostate	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										4
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangioma																										1
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Fibrosarcoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control
(continued)

Number of Days on Study	0	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	1	2	4	7	1	1	4	6	9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	8	8	7	0	5	1	9	0	7	2	1	1	4	5	5	5	5	9	9	9	9	9	9	9	9
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	3	0	2	3	0	3	1	0	2	1	1	1	0	2	3	3	0	0	0	0	1	1	1	1
	9	3	6	5	0	8	1	5	2	4	0	1	3	3	2	6	7	1	4	5	7	2	4	6	7
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X	X		X		X			X										
Alveolar/bronchiolar adenoma, two, multiple																								X	
Alveolar/bronchiolar carcinoma								X		X															
Carcinoma, metastatic, harderian gland																									
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear												+													
Fibrosarcoma																									
Hemangiosarcoma												X													
Eye													+												
Harderian gland										+	+		+												
Adenoma													X												
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed					X					X															

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 1 8 1	0 1 9 1	0 2 0 1	0 2 1 1	0 2 3 1	0 2 6 1	0 2 7 1	0 2 8 1	0 2 9 1	0 3 2 1	0 3 4 1	0 3 5 1	0 3 8 1	0 3 9 1	0 4 0 1	0 4 1 1	0 4 2 1	0 4 3 1	0 4 4 1	0 4 5 1	0 4 6 1	0 4 7 1	0 4 8 1	0 4 9 1	0 5 0 1	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																				X						6
Alveolar/bronchiolar adenoma, two, multiple																										1
Alveolar/bronchiolar carcinoma																		X		X	X					5
Carcinoma, metastatic, harderian gland				X																						1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																+										2
Fibrosarcoma																X										1
Hemangiosarcoma																										1
Eye																										1
Harderian gland				+					+	+				+											+	8
Adenoma				X					X																X	4
Carcinoma				X																						1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed					X																				X	4

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg

Number of Days on Study	3 5 5 5 5 5 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 9 2 3 5 7 7 8 0 1 5 5 5 8 8 0 0 1 2 2 2 2 2 2 2 2 9 5 6 5 0 1 4 2 1 4 4 9 1 1 1 1 4 5 9 9 9 9 9 9 9
Carcass ID Number	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 4 6 3 4 4 4 3 5 1 5 1 1 5 2 3 5 3 1 1 1 1 4 4 4 4 8 0 2 1 0 3 1 4 1 8 3 7 6 2 3 5 5 2 4 5 6 6 7 9 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Alimentary System	
Esophagus	+ +
Gallbladder	M + + + + + + + + + + + + + + + M + + + + + + + +
Intestine large	+ +
Intestine large, cecum	+ +
Intestine large, colon	+ +
Intestine large, rectum	+ + + + M +
Intestine small	+ +
Intestine small, duodenum	+ + + + A +
Intestine small, ileum	+ +
Polyp adenomatous	X
Intestine small, jejunum	+ + + + A +
Adenocarcinoma	
Liver	+ +
Cholangiocarcinoma, two, multiple	X
Hemangiosarcoma	
Hepatocellular carcinoma	X X X X X X X X
Hepatocellular carcinoma, two, multiple	X X
Hepatocellular adenoma	X X X X X X X X X
Hepatocellular adenoma, two, multiple	X X X
Ito cell tumor benign, two, multiple	
Mesentery	+ + + +
Cholangiocarcinoma, metastatic, liver	X
Hemangioma	X
Pancreas	+ +
Cholangiocarcinoma, metastatic, liver	X
Salivary glands	+ +
Stomach	+ +
Stomach, forestomach	+ +
Squamous cell papilloma	
Stomach, glandular	+ +
Tooth	+ +
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Spindle cell, adenoma	
Adrenal gland, medulla	+ +

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 1 8 1	2 1 9 1	2 2 0 1	2 2 1 1	2 2 3 1	2 2 4 1	2 2 5 1	2 2 6 1	2 2 7 1	2 2 8 1	2 2 9 1	2 3 0 1	2 5 0 1	2 5 1 1	2 5 2 1	2 5 3 1	2 5 7 1	2 5 9 1	2 6 6 1	2 7 7 1	2 3 8 1	2 3 9 1	2 4 2 1	2 4 4 1	2 4 5 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma													X								X					2
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma, two, multiple																										1
Hemangiosarcoma																									X	1
Hepatocellular carcinoma													X	X			X	X								10
Hepatocellular carcinoma, two, multiple																										2
Hepatocellular adenoma										X	X			X							X					12
Hepatocellular adenoma, two, multiple		X					X																X			6
Ito cell tumor benign, two, multiple																									X	1
Mesentery																										4
Cholangiocarcinoma, metastatic, liver																										1
Hemangioma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma, metastatic, liver																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma												X									X					2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+		+	35
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spindle cell, adenoma							X																			1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	3	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7
	9	2	3	5	7	7	8	0	1	5	5	5	8	8	0	0	1	2	2	2	2	2	2	2
	9	5	6	5	0	1	4	2	1	4	4	9	1	1	1	1	4	5	9	9	9	9	9	9
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	3	4	6	3	4	4	4	3	5	1	5	1	1	5	2	3	5	3	1	1	1	1	4	4
	4	8	0	2	1	0	3	1	4	1	8	3	7	6	2	3	5	5	2	4	5	6	6	7
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Endocrine System (continued)																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pars distalis, adenoma												X												
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								
General Body System																								
None																								
Genital System																								
Coagulating gland													+	+								+		+
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis											+													
Preputial gland					+			+		+		+				+	+	+	+	+		+		+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma, metastatic, liver									X															
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																								
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, cholangiocarcinoma, metastatic, liver									X															
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X			
Histiocytic sarcoma																X								
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	M	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma							X																	

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 1 8 1	2 1 9 1	2 2 0 1	2 2 1 1	2 2 3 1	2 2 4 1	2 2 5 1	2 2 6 1	2 2 7 1	2 2 8 1	2 2 9 1	2 3 0 1	2 5 0 1	2 5 1 1	2 5 2 1	2 5 3 1	2 5 7 1	2 5 9 1	2 6 1 1	2 7 1 1	2 8 1 1	2 9 1 1	2 4 2 1	2 4 4 1	2 4 5 1	Total Tissues/ Tumors
Endocrine System (continued)																										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X													1
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Pituitary gland	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma							X																			1
General Body System																										
None																										
Genital System																										
Coagulating gland							+	+																		6
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland	+	+	+			+		+	+	+	+				+	+	+		+		+	+				25
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma, metastatic, liver																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																							X			1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, cholangiocarcinoma, metastatic, liver																										1
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																X										2

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	3	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7
	9	2	3	5	7	7	8	0	1	5	5	5	8	8	0	0	1	2	2	2	2	2	2	2
	9	5	6	5	0	1	4	2	1	4	4	9	1	1	1	1	4	5	9	9	9	9	9	9
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	3	4	6	3	4	4	4	3	5	1	5	1	1	5	2	3	5	3	1	1	1	1	4	4
	4	8	0	2	1	0	3	1	4	1	8	3	7	6	2	3	5	5	2	4	5	6	6	7
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle									+															
Cholangiocarcinoma, metastatic, liver									X															
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X								X	X	X					
Alveolar/bronchiolar carcinoma																								
Alveolar/bronchiolar carcinoma, two, multiple											X													
Cholangiocarcinoma, metastatic, liver									X															
Hepatocellular carcinoma, metastatic, liver		X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Ear								+																
Eye		+																						
Harderian gland		+										+	+					+						
Adenoma		X										X	X											
Carcinoma													X					X						
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																X								
Lymphoma malignant histiocytic																X								
Lymphoma malignant mixed														X										

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 1 8 1	2 1 9 1	2 2 0 1	2 2 1 1	2 2 3 1	2 2 4 1	2 2 5 1	2 2 6 1	2 2 7 1	2 2 8 1	2 2 9 1	2 3 0 1	2 5 0 1	2 5 1 1	2 5 2 1	2 5 3 1	2 5 7 1	2 5 9 1	2 3 6 1	2 3 7 1	2 3 8 1	2 3 9 1	2 4 2 1	2 4 4 1	2 4 5 1	Total Tissues/ Tumors
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Cholangiocarcinoma, metastatic, liver																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X														X				X				7
Alveolar/bronchiolar carcinoma								X												X						2
Alveolar/bronchiolar carcinoma, two, multiple																										1
Cholangiocarcinoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, liver													X													2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye																										1
Harderian gland					+																		+			6
Adenoma					X																		X			5
Carcinoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										1

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg

Number of Days on Study	0 0 7	4 5	4 7 8	5 0 1	5 7 0	5 9 1	5 9 9	6 3 1	6 8 0	6 9 0	7 0 1	7 0 1	7 2 5	7 2 5	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	1 6 3 1	1 4 7 1	1 8 2 1	1 8 9 1	1 8 0 1	1 8 7 1	1 4 9 1	1 6 1 1	1 7 1 1	1 4 3 1	1 5 5 1	1 5 8 1	1 4 2 1	1 8 4 1	1 9 0 1	1 4 8 1	1 5 0 1	1 5 1 1	1 5 2 1	1 5 3 1	1 5 4 1	1 5 6 1	1 5 7 1	1 5 9 1	1 6 0 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma			X																						
Hemangiosarcoma																									
Hepatocellular carcinoma		X	X	X			X		X			X						X							
Hepatocellular carcinoma, two, multiple																			X						
Hepatocellular carcinoma, three, multiple														X											
Hepatocellular adenoma				X							X			X	X							X			X
Hepatocellular adenoma, two, multiple																X	X	X							
Hepatocellular adenoma, three, multiple										X															
Hepatocellular adenoma, four, multiple																									
Mesentery			+				+		+									+					+		
Cholangiocarcinoma, metastatic, liver			X																						
Fibrosarcoma			X																						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma, metastatic, liver			X																						
Fibrosarcoma, metastatic, mesentery			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma			X								X														
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+	+			+	+	+		+		+	+			+	+		+	+	+	+
Cardiovascular System																									
Blood vessel																		+							
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	1 6 2 1	1 6 4 1	1 6 5 1	1 6 6 1	1 6 7 1	1 6 8 1	1 6 9 1	1 7 0 1	1 7 2 1	1 7 3 1	1 7 4 1	1 7 5 1	1 7 6 1	1 7 7 1	1 7 8 1	1 7 9 1	1 8 1 1	1 8 3 1	1 8 5 1	1 8 6 1	1 8 8 1	1 4 1 1	1 4 4 1	1 4 5 1	1 4 6 1	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M		47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+		49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Cholangiocarcinoma																											1
Hemangiosarcoma							X																	X			2
Hepatocellular carcinoma	X								X		X														X		11
Hepatocellular carcinoma, two, multiple																											1
Hepatocellular carcinoma, three, multiple																											1
Hepatocellular adenoma				X						X		X						X				X					11
Hepatocellular adenoma, two, multiple																											3
Hepatocellular adenoma, three, multiple																											1
Hepatocellular adenoma, four, multiple	X																										1
Mesentery												+															6
Cholangiocarcinoma, metastatic, liver																											1
Fibrosarcoma																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Cholangiocarcinoma, metastatic, liver																											1
Fibrosarcoma, metastatic, mesentery																											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Squamous cell papilloma																											2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Tooth		+	+	+			+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+				33
Cardiovascular System																											
Blood vessel																											1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	0	4	4	5	5	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	4	7	0	7	9	9	3	8	9	0	0	2	2	2	3	3	3	3	3	3	3	3	3
	7	5	8	1	0	1	9	1	0	0	1	1	5	5	9	1	1	1	1	1	1	1	1	1
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6	4	8	8	8	8	4	6	7	4	5	5	4	8	9	4	5	5	5	5	5	5	5	6
	3	7	2	9	0	7	9	1	1	3	5	8	2	4	0	8	0	1	2	3	4	6	7	9	0
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spindle cell, adenoma		X																						
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant								X																
Pheochromocytoma benign																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																			
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								
Genital System																								
Coagulating gland																						+		
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland					+		+		+		+			+	+		+	+			+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																								
Hemangiosarcoma														X										
Histiocytic sarcoma																								
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, adenoma													X											

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	1 6 2 1	1 6 4 1	1 6 5 1	1 6 6 1	1 6 7 1	1 6 8 1	1 6 9 1	1 7 0 1	1 7 2 1	1 7 3 1	1 7 4 1	1 7 5 1	1 7 6 1	1 7 7 1	1 7 8 1	1 7 9 1	1 8 1 1	1 8 3 1	1 8 5 1	1 8 6 1	1 8 8 1	1 4 1 1	1 4 4 1	1 4 5 1	1 4 6 1	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spindle cell, adenoma																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign																	X									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Coagulating gland	+																									2
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+		+	+		+		+				+	+	+	+	+	+	+		+			+			27
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma							X																			1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																				X						1
Hemangiosarcoma							X																			2
Histiocytic sarcoma											X															1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sebaceous gland, adenoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg (continued)

Number of Days on Study	007	445	478	501	570	591	599	631	680	690	701	701	725	725	729	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	1631	1471	1821	1891	1801	1871	1491	1611	1711	1431	1451	1581	1421	1841	1901	1481	1501	1511	1521	1531	1541	1561	1571	1591	1601
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+															+							
Fibrosarcoma, metastatic, mesentery			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X			X						X		
Alveolar/bronchiolar adenoma, two, multiple																		X							
Alveolar/bronchiolar carcinoma											X														
Carcinoma, metastatic, harderian gland								X																	
Cholangiocarcinoma, metastatic, liver			X																						
Hepatocellular carcinoma, metastatic, liver														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp						X																			
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Fibrosarcoma																									
Eye													+												
Harderian gland								+																	+
Adenoma								X																	X
Carcinoma								X																	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymphoma malignant histiocytic									X																
Lymphoma malignant mixed																									

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	1 6 2 1	1 6 4 1	1 6 5 1	1 6 6 1	1 6 7 1	1 6 8 1	1 6 9 1	1 7 0 1	1 7 2 1	1 7 3 1	1 7 4 1	1 7 5 1	1 7 6 1	1 7 7 1	1 7 8 1	1 7 9 1	1 8 1 1	1 8 3 1	1 8 5 1	1 8 6 1	1 8 8 1	1 4 1 1	1 4 4 1	1 4 5 1	1 4 6 1	Total Tissues/ Tumors
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																											2
Fibrosarcoma, metastatic, mesentery																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X		X					X																			6
Alveolar/bronchiolar adenoma, two, multiple																	X										2
Alveolar/bronchiolar carcinoma																											1
Carcinoma, metastatic, harderian gland												X															2
Cholangiocarcinoma, metastatic, liver																											1
Hepatocellular carcinoma, metastatic, liver									X																		2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp																											1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Ear																		+						+			2
Fibrosarcoma																		X						X			2
Eye																											1
Harderian gland												+			+												4
Adenoma															X												3
Carcinoma												X															2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma											X																1
Lymphoma malignant histiocytic																											1
Lymphoma malignant mixed								X			X	X															3

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg

Number of Days on Study	2 9 8	4 7 7	5 6 3	5 7 1	6 4 9	6 5 6	7 0 1	7 1 6	7 2 4	7 2 5	7 2 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	0 9 3 1	1 1 6 1	0 8 2 1	0 9 4 1	0 7 6 1	1 1 9 1	0 7 2 1	1 0 3 1	0 8 3 1	0 8 7 1	1 0 4 1	0 7 1 1	0 7 3 1	0 7 4 1	0 7 5 1	0 7 7 1	0 7 0 1	1 2 8 1	0 7 9 1	0 7 0 1	0 8 1 1	0 8 4 1	0 9 2 1	0 9 5 1	0 9 6 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X															X							
Hepatocellular carcinoma						X	X	X		X															
Hepatocellular carcinoma, two, multiple		X																							
Hepatocellular adenoma												X			X				X	X	X				
Hepatocellular adenoma, two, multiple							X																		
Mesentery												+													
Pancreas	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma									X																
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth				+	+		+		+	+	+	+		+	+	+		+	+		+	+		+	+
Cardiovascular System																									
Blood vessel	+																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 9 7 1	0 9 8 1	1 1 3 1	1 1 4 1	1 1 5 1	0 8 5 1	0 8 6 1	0 8 8 1	0 8 9 1	0 9 0 1	0 9 1 1	0 9 9 1	0 9 0 1	1 0 0 1	1 0 1 1	1 0 2 1	1 0 5 1	1 0 6 1	1 0 7 1	1 0 8 1	1 0 9 1	1 1 0 1	1 1 1 1	1 1 2 1	1 1 7 1	1 1 8 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma						X													X							4
Hepatocellular carcinoma		X																								5
Hepatocellular carcinoma, two, multiple																										1
Hepatocellular adenoma	X	X													X											8
Hepatocellular adenoma, two, multiple																										1
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma				X																						2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth			+	+	+	+	+	+			+	+	+		+	+	+		+	+	+	+	+	+	+	35
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign					X																					1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	M	+	+	+	M	+	+	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	2	4	5	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	9	7	6	7	4	5	0	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3
	8	7	3	1	9	6	1	6	4	5	5	9	9	9	9	9	9	1	1	1	1	1	1	1	1	1
Carcass ID Number	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	9	1	8	9	7	1	7	0	8	8	0	7	7	7	7	7	2	7	7	8	8	8	9	9	9	9
	3	6	2	4	6	9	2	3	3	7	4	1	3	4	5	7	0	8	9	0	1	4	2	5	6	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
General Body System																										
None																										
Genital System																										
Coagulating gland												+	+	+			+									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland									+		+			+			+	+	+	+			+			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X																				X
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X					
Hemangiosarcoma						X		X																		
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	
Mediastinum, hemangiosarcoma																										
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangioma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																										+
Hemangiosarcoma																										X
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+																									

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 9 7 1	0 9 8 1	1 1 3 1	1 1 4 1	1 1 5 1	0 1 8 5 1	0 8 8 6 1	0 8 8 8 1	0 8 8 9 1	0 9 9 0 1	0 9 9 1 1	0 9 9 9 1	0 9 9 0 1	1 0 0 1 1	1 0 0 2 1	1 0 0 5 1	1 0 0 6 1	1 0 0 7 1	1 0 0 8 1	1 0 0 9 1	1 1 0 0 1	1 1 1 1 1	1 1 1 2 1	1 1 1 7 1	1 1 1 8 1	Total Tissues/ Tumors	
General Body System																										
None																										
Genital System																										
Coagulating gland																			+	+					6	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Preputial gland	+			+		+			+	+			+	+				+			+		+	+	19	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hemangiosarcoma																									2	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	M	45	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Hemangioma																									1	
Hemangiosarcoma																									2	
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Mediastinum, hemangiosarcoma								X																	1	
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Subcutaneous tissue, hemangioma													X												1	
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Skeletal muscle																									1	
Hemangiosarcoma																									1	
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Spinal cord																									1	

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	2 9 8	4 7 7	5 6 3	5 7 1	6 4 9	6 5 6	7 0 1	7 1 6	7 2 4	7 2 5	7 2 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	0 9 3 1	1 1 6 1	0 8 2 1	0 9 4 1	0 7 6 1	1 1 9 1	0 7 2 1	1 0 3 1	0 8 3 1	0 8 7 1	1 0 4 1	0 7 1 1	0 7 3 1	0 7 4 1	0 7 5 1	0 7 7 1	0 7 0 1	1 2 8 1	0 7 9 1	0 7 0 1	0 8 1 1	0 8 1 1	0 8 4 1	0 9 2 1	0 9 5 1	0 9 6 1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Alveolar/bronchiolar carcinoma						X			X									X		X					X
Hepatocellular carcinoma, metastatic, liver		X					X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland									+		+		+					+				+			
Adenoma									X		X							X				X			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 9 7 1	0 9 8 1	1 1 3 1	1 1 4 1	1 1 5 1	0 8 5 1	0 8 6 1	0 8 8 1	0 8 9 1	0 9 0 1	0 9 1 1	0 9 9 1	0 9 0 1	1 0 1 1	1 0 1 1	1 0 2 1	1 0 5 1	1 0 6 1	1 0 7 1	1 0 8 1	1 0 9 1	1 1 0 1	1 1 1 1	1 1 2 1	1 1 7 1	1 1 8 1	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X	X																							3
Alveolar/bronchiolar carcinoma														X												6
Hepatocellular carcinoma, metastatic, liver																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland		+	+															+						+		9
Adenoma			X															X						X		7
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	4/50 (8%)	5/50 (10%)	3/50 (6%)	7/50 (14%)
Adjusted rate[b]	11.5%	12.9%	7.8%	17.0%
Terminal rate[c]	3/33 (9%)	2/32 (6%)	2/36 (6%)	5/39 (13%)
First incidence (days)	724	525	631	724
Life table test[d]	P=0.305	P=0.471	P=0.467N	P=0.355
Logistic regression test[d]	P=0.219	P=0.499	P=0.506N	P=0.308
Cochran-Armitage test[d]	P=0.204			
Fisher exact test[d]		P=0.500	P=0.500N	P=0.266
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	6/50 (12%)	4/50 (8%)	7/50 (14%)
Adjusted rate	11.5%	15.5%	10.5%	17.0%
Terminal rate	3/33 (9%)	2/32 (6%)	3/36 (8%)	5/39 (13%)
First incidence (days)	724	525	631	724
Life table test	P=0.392	P=0.342	P=0.606N	P=0.355
Logistic regression test	P=0.297	P=0.368	P=0.638	P=0.308
Cochran-Armitage test	P=0.274			
Fisher exact test		P=0.370	P=0.643N	P=0.262
Liver: Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	3.1%	5.6%	9.6%
Terminal rate	0/33 (0%)	1/32 (3%)	2/36 (6%)	3/39 (8%)
First incidence (days)	-[e]	729 (T)	729 (T)	563
Life table test	P=0.050	P=0.494	P=0.258	P=0.083
Logistic regression test	P=0.033	P=0.494	P=0.258	P=0.060
Cochran-Armitage test	P=0.031			
Fisher exact test		P=0.500	P=0.247	P=0.059
Liver: Hepatocellular Adenoma				
Overall rate	19/50 (38%)	18/50 (36%)	16/50 (32%)	9/50 (18%)
Adjusted rate	47.4%	42.6%	39.6%	22.3%
Terminal rate	13/33 (39%)	9/32 (28%)	12/36 (33%)	8/39 (21%)
First incidence (days)	518	399	501	701
Life table test	P=0.005N	P=0.557N	P=0.279N	P=0.010N
Logistic regression test	P=0.011N	P=0.499N	P=0.345N	P=0.020N
Cochran-Armitage test	P=0.012N			
Fisher exact test		P=0.500N	P=0.338N	P=0.022N
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	12/50 (24%)	13/50 (26%)	6/50 (12%)
Adjusted rate	23.2%	28.2%	29.1%	13.1%
Terminal rate	3/33 (9%)	4/32 (13%)	6/36 (17%)	1/39 (3%)
First incidence (days)	540	525	445	477
Life table test	P=0.070N	P=0.362	P=0.359	P=0.178N
Logistic regression test	P=0.114N	P=0.400	P=0.325	P=0.232N
Cochran-Armitage test	P=0.094N			
Fisher exact test		P=0.405	P=0.318	P=0.207N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/50 (50%)	26/50 (52%)	25/50 (50%)	13/50 (26%)
Adjusted rate	57.1%	56.0%	55.1%	29.1%
Terminal rate	15/33 (45%)	12/32 (38%)	16/36 (44%)	8/39 (21%)
First incidence (days)	518	399	445	477
Life table test	P=0.002N	P=0.426	P=0.485N	P=0.008N
Logistic regression test	P=0.003N	P=0.507	P=0.578	P=0.012N
Cochran-Armitage test	P=0.002N			
Fisher exact test		P=0.500	P=0.579N	P=0.011N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	7/50 (14%)	7/50 (14%)	8/50 (16%)	3/50 (6%)
Adjusted rate	17.0%	19.0%	21.6%	7.7%
Terminal rate	2/33 (6%)	3/32 (9%)	7/36 (19%)	3/39 (8%)
First incidence (days)	619	602	725	729 (T)
Life table test	P=0.066N	P=0.547	P=0.538	P=0.125N
Logistic regression test	P=0.090N	P=0.609	P=0.492	P=0.154N
Cochran-Armitage test	P=0.106N			
Fisher exact test		P=0.613N	P=0.500	P=0.159N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Adjusted rate	13.4%	8.5%	2.5%	14.3%
Terminal rate	3/33 (9%)	2/32 (6%)	0/36 (0%)	4/39 (10%)
First incidence (days)	640	654	701	656
Life table test	P=0.337	P=0.383N	P=0.101N	P=0.591
Logistic regression test	P=0.260	P=0.364N	P=0.105N	P=0.514
Cochran-Armitage test	P=0.242			
Fisher exact test		P=0.357N	P=0.102N	P=0.500
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	9/50 (18%)	10/50 (20%)	9/50 (18%)	9/50 (18%)
Adjusted rate	22.4%	26.4%	23.5%	21.7%
Terminal rate	4/33 (12%)	5/32 (16%)	7/36 (19%)	7/39 (18%)
First incidence (days)	619	602	701	656
Life table test	P=0.347N	P=0.440	P=0.555N	P=0.483N
Logistic regression test	P=0.458N	P=0.489	P=0.594	P=0.584N
Cochran-Armitage test	P=0.508N			
Fisher exact test		P=0.500	P=0.602N	P=0.602N
Spleen: Hemangiosarcoma				
Overall rate	4/50 (8%)	0/50 (0%)	2/50 (4%)	2/50 (4%)
Adjusted rate	10.0%	0.0%	5.3%	4.5%
Terminal rate	0/33 (0%)	0/32 (0%)	1/36 (3%)	0/39 (0%)
First incidence (days)	667	–	725	656
Life table test	P=0.534N	P=0.084N	P=0.332N	P=0.309N
Logistic regression test	P=0.587N	P=0.066N	P=0.342N	P=0.334N
Cochran-Armitage test	P=0.602N			
Fisher exact test		P=0.059N	P=0.339N	P=0.339N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	9.1%	6.3%	4.5%	4.9%
Terminal rate	3/33 (9%)	2/32 (6%)	0/36 (0%)	1/39 (3%)
First incidence (days)	729 (T)	729 (T)	478	724
Life table test	P=0.431N	P=0.514N	P=0.478N	P=0.431N
Logistic regression test	P=0.501N	P=0.514N	P=0.498N	P=0.461N
Cochran-Armitage test	P=0.506N			
Fisher exact test		P=0.500N	P=0.500N	P=0.500N
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	11.5%	6.3%	4.5%	4.9%
Terminal rate	3/33 (9%)	2/32 (6%)	0/36 (0%)	1/39 (3%)
First incidence (days)	725	729 (T)	478	724
Life table test	P=0.329N	P=0.358N	P=0.320N	P=0.276N
Logistic regression test	P=0.393N	P=0.396N	P=0.336N	P=0.299N
Cochran-Armitage test	P=0.400N			
Fisher exact test		P=0.339N	P=0.339N	P=0.339N
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	8/50 (16%)
Adjusted rate	10.0%	3.1%	8.0%	18.5%
Terminal rate	0/33 (0%)	1/32 (3%)	2/36 (6%)	5/39 (13%)
First incidence (days)	667	729 (T)	725	563
Life table test	P=0.040	P=0.219N	P=0.485N	P=0.246
Logistic regression test	P=0.020	P=0.191N	P=0.506N	P=0.180
Cochran-Armitage test	P=0.017			
Fisher exact test		P=0.181N	P=0.500N	P=0.178
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/50 (10%)	3/50 (6%)	4/50 (8%)	10/50 (20%)
Adjusted rate	12.7%	8.7%	10.7%	23.3%
Terminal rate	1/33 (3%)	2/32 (6%)	3/36 (8%)	7/39 (18%)
First incidence (days)	667	681	725	563
Life table test	P=0.053	P=0.408N	P=0.475N	P=0.205
Logistic regression test	P=0.026	P=0.379N	P=0.507N	P=0.137
Cochran-Armitage test	P=0.021			
Fisher exact test		P=0.357N	P=0.500N	P=0.131
All Organs: Malignant Lymphoma and Histiocytic Sarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	10.3%	5.3%	10.5%	0.0%
Terminal rate	2/33 (6%)	0/32 (0%)	3/36 (8%)	0/39 (0%)
First incidence (days)	575	681	680	–
Life table test	P=0.066N	P=0.373N	P=0.613N	P=0.054N
Logistic regression test	P=0.082N	P=0.337N	P=0.642	P=0.065N
Cochran-Armitage test	P=0.084N			
Fisher exact test		P=0.339N	P=0.643N	P=0.059N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
All Organs: Malignant Lymphoma (Histiocytic or Mixed)				
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	10.3%	5.3%	10.5%	0.0%
Terminal rate	2/33 (6%)	0/32 (0%)	3/36 (8%)	0/39 (0%)
First incidence (days)	575	681	680	–
Life table test	P=0.066N	P=0.373N	P=0.613N	P=0.054N
Logistic regression test	P=0.082N	P=0.337N	P=0.642	P=0.065N
Cochran-Armitage test	P=0.084N			
Fisher exact test		P=0.339N	P=0.643N	P=0.059N
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	32/50 (64%)	26/50 (52%)	19/50 (38%)
Adjusted rate	60.7%	69.2%	57.4%	45.1%
Terminal rate	16/33 (48%)	18/32 (56%)	17/36 (47%)	16/39 (41%)
First incidence (days)	518	399	445	701
Life table test	P=0.005N	P=0.198	P=0.415N	P=0.036N
Logistic regression test	P=0.010N	P=0.208	P=0.505N	P=0.064N
Cochran-Armitage test	P=0.011N			
Fisher exact test		P=0.208	P=0.500N	P=0.080N
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	22/50 (44%)	20/50 (40%)	15/50 (30%)
Adjusted rate	49.6%	48.4%	43.8%	32.2%
Terminal rate	10/33 (30%)	9/32 (28%)	11/36 (31%)	8/39 (21%)
First incidence (days)	540	525	445	477
Life table test	P=0.031N	P=0.541	P=0.321N	P=0.057N
Logistic regression test	P=0.056N	P=0.499N	P=0.341N	P=0.077N
Cochran-Armitage test	P=0.050N			
Fisher exact test		P=0.500N	P=0.343N	P=0.074N
All Organs: Benign and Malignant Neoplasms				
Overall rate	33/50 (66%)	38/50 (76%)	36/50 (72%)	28/50 (56%)
Adjusted rate	68.7%	79.0%	75.0%	59.5%
Terminal rate	18/33 (55%)	22/32 (69%)	24/36 (67%)	20/39 (51%)
First incidence (days)	518	399	445	477
Life table test	P=0.018N	P=0.199	P=0.483	P=0.108N
Logistic regression test	P=0.039N	P=0.189	P=0.325	P=0.189N
Cochran-Armitage test	P=0.042N			
Fisher exact test		P=0.189	P=0.333	P=0.206N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[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 the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no neoplasms in dose group

TABLE A4a
Historical Incidence of Liver Neoplasms in Male B6C3F$_1$ Mice Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls		
	Hemangioma	Hemangiosarcoma	Hemangioma or Hemangiosarcoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	1/50	0/50	1/50
Dichlorvos	0/50	1/50	1/50
Furan	0/50	2/50	2/50
Furfural	1/50	2/50	3/50
γ-Butyrolactone	0/50	2/50	2/50
p-Nitroaniline	0/50	0/50	0/50
Pentachloroanisole	0/50	2/50	2/50
Overall Historical Incidence			
Total	3/699 (0.4%)	15/699 (2.1%)	18/699 (2.6%)
Standard deviation	0.9%	2.1%	2.3%
Range	0%-2%	0%-6%	0%-6%

[a] Data as of 3 April 1991

TABLE A4b
Historical Incidence of Hemangiomas or Hemangiosarcomas in Male B6C3F$_1$ Mice Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls		
	Hemangioma	Hemangiosarcoma	Hemangioma or Hemangiosarcoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	1/50	1/50	2/50
Dichlorvos	1/50	2/50	3/50
Furan	0/50	5/50	5/50
Furfural	1/50	2/50	3/50
γ-Butyrolactone	0/50	3/50	3/50
p-Nitroaniline	1/50	4/50	5/50
Pentachloroanisole	1/50	4/50	5/50
Overall Historical Incidence			
Total	10/700 (1.4%)	36/700 (5.1%)	46/700 (6.6%)
Standard deviation	1.8%	3.7%	3.6%
Range	0%-6%	0%-12%	0%-12%

[a] Data as of 3 April 1991

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	70	70	70	70
9-Month interim evaluation	10	10	10	10
15-Month interim evaluation	10	10	10	10
Early deaths				
Accidental deaths			1	
Moribund	13	14	10	10
Natural deaths	4	4	3	1
Survivors				
Terminal sacrifice	33	32	36	39
Animals examined microscopically	70	70	70	70
9-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Kupffer cell, pigmentation, hemosiderin				10 (100%)
Cardiovascular System				
None				
Endocrine System				
Adrenal gland, cortex	(10)			(10)
Vacuolization cytoplasmic, focal	1 (10%)			
Thyroid gland	(10)	(10)	(10)	(10)
Ultimobranchial cyst	1 (10%)			1 (10%)
Follicle, degeneration, cystic	2 (20%)	3 (30%)	3 (30%)	4 (40%)
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Bone marrow	(10)	(10)	(10)	(10)
Hyperplasia			9 (90%)	10 (100%)
Pigmentation, hemosiderin			8 (80%)	10 (100%)
Spleen	(10)	(10)	(10)	(10)
Congestion			6 (60%)	10 (100%)
Hematopoietic cell proliferation			10 (100%)	10 (100%)
Pigmentation, hemosiderin			10 (100%)	10 (100%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
9-Month Interim Evaluation (continued)				
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Erythrophagocytosis, multifocal		1 (10%)		
Hemorrhage, multifocal		1 (10%)		
Infiltration cellular, histiocyte, multifocal		2 (20%)	3 (30%)	4 (40%)
Pigmentation, hemosiderin, multifocal		1 (10%)	3 (30%)	4 (40%)
Alveolar epithelium, hyperplasia, focal		1 (10%)	1 (10%)	
Special Senses System				
None				
Urinary System				
Kidney	(10)		(10)	(10)
Bowman's capsule parietal layer, hyperplasia, focal				1 (10%)
Renal tubule, hyperplasia, multifocal	1 (10%)		1 (10%)	9 (90%)
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Developmental malformation			1 (10%)	
Hematopoietic cell proliferation	1 (10%)			
Kupffer cell, pigmentation, hemosiderin	1 (10%)		5 (50%)	10 (100%)
Mesentery		(1)		
Fat, necrosis, focal		1 (100%)		
Stomach, forestomach	(10)	(10)	(10)	(10)
Erosion				1 (10%)
Hyperplasia	1 (10%)	1 (10%)		1 (10%)
Inflammation, subacute	1 (10%)	1 (10%)		1 (10%)
Stomach, glandular	(10)	(10)	(10)	(10)
Inflammation, subacute				1 (10%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
15-Month Interim Evaluation (continued)				
Cardiovascular System				
None				
Endocrine System				
Islets, pancreatic	(10)			(10)
Hyperplasia	1 (10%)			
Parathyroid gland	(10)			(10)
Cyst	1 (10%)			1 (10%)
Thyroid gland	(10)	(1)		(10)
Follicle, degeneration, cystic	2 (20%)	1 (100%)		4 (40%)
General Body System				
None				
Genital System				
Preputial gland	(1)	(4)	(2)	(2)
Duct, cyst	1 (100%)	4 (100%)	2 (100%)	2 (100%)
Hematopoietic System				
Bone marrow	(10)	(10)	(10)	(10)
Hyperplasia			4 (40%)	9 (90%)
Pigmentation, hemosiderin			1 (10%)	6 (60%)
Lymph node, mandibular	(10)			(9)
Congestion				1 (11%)
Spleen	(10)	(10)	(10)	(10)
Congestion		1 (10%)	10 (100%)	10 (100%)
Hematopoietic cell proliferation	2 (20%)		10 (100%)	10 (100%)
Pigmentation, hemosiderin			10 (100%)	10 (100%)
Thymus	(10)			(10)
Cyst	4 (40%)			3 (30%)
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
15-Month Interim Evaluation (continued)				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte, multifocal		3 (30%)	4 (40%)	1 (10%)
Pigmentation, hemosiderin, multifocal		3 (30%)	4 (40%)	1 (10%)
Alveolar epithelium, hyperplasia, focal		1 (10%)		
Nose	(10)			(10)
Exudate				2 (20%)
Foreign body	2 (20%)			1 (10%)
Inflammation, suppurative, acute				1 (10%)
Special Senses System				
None				
Urinary System				
Kidney	(10)			(10)
Casts protein	1 (10%)			
Cortex, cyst				2 (20%)
Renal tubule, hyperplasia, multifocal	3 (30%)			1 (10%)
2-Year Study				
Alimentary System				
Intestine small, jejunum	(50)	(49)	(50)	(50)
Hyperplasia				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Basophilic focus	3 (6%)		2 (4%)	
Clear cell focus	6 (12%)	2 (4%)	4 (8%)	3 (6%)
Clear cell focus, multiple	1 (2%)			1 (2%)
Eosinophilic focus	8 (16%)	4 (8%)	3 (6%)	2 (4%)
Hematopoietic cell proliferation			2 (4%)	
Hemorrhage		1 (2%)		
Inclusion body intracytoplasmic				1 (2%)
Mixed cell focus	5 (10%)	9 (18%)	5 (10%)	2 (4%)
Necrosis, focal		4 (8%)	2 (4%)	3 (6%)
Necrosis, multifocal	1 (2%)			
Biliary tract, cyst			1 (2%)	
Kupffer cell, pigmentation	1 (2%)	1 (2%)	8 (16%)	50 (100%)
Mesentery	(2)	(4)	(6)	(1)
Fat, necrosis		2 (50%)	3 (50%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(49)
Basophilic focus	2 (4%)		2 (4%)	1 (2%)
Eosinophilic focus			2 (4%)	
Inflammation, subacute				1 (2%)
Acinus, atrophy	6 (12%)	2 (4%)	4 (8%)	1 (2%)
Artery, inflammation, subacute			1 (2%)	
Duct, dilatation		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia	21 (42%)	20 (40%)	24 (48%)	18 (36%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion				1 (2%)
Hyperplasia	2 (4%)			
Tooth	(35)	(35)	(33)	(35)
Dysplasia	35 (100%)	35 (100%)	33 (100%)	35 (100%)
Inflammation, subacute		1 (3%)	1 (33%)	
Cardiovascular System				
Blood vessel			(1)	(1)
Abdominal, aneurysm				1 (100%)
Abdominal, hemorrhage				1 (100%)
Abdominal, inflammation, subacute			1 (100%)	
Abdominal, thrombosis				1 (100%)
Aorta, inflammation, subacute			1 (100%)	
Heart	(50)	(50)	(50)	(50)
Inflammation, subacute	1 (2%)			
Mineralization			1 (2%)	
Endocrine System				
Adrenal gland, cortex	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Hyperplasia	1 (2%)			
Hypertrophy, focal	8 (16%)	8 (16%)	10 (20%)	12 (24%)
Necrosis				1 (2%)
Capsule, accessory adrenal cortical nodule	2 (4%)		1 (2%)	1 (2%)
Spindle cell, hyperplasia	10 (20%)	13 (26%)	10 (20%)	11 (22%)
Adrenal gland, medulla	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	1 (2%)		
Necrosis				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia	7 (14%)	6 (12%)	6 (12%)	3 (6%)
Pituitary gland	(48)	(47)	(48)	(45)
Pars distalis, cyst	1 (2%)			
Pars distalis, hyperplasia	1 (2%)		1 (2%)	1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst	9 (18%)	3 (6%)	6 (12%)	6 (12%)
Follicle, degeneration, cystic	8 (16%)	17 (34%)	15 (30%)	10 (20%)
Follicle, foreign body				1 (2%)
Follicular cell, hyperplasia	2 (4%)	1 (2%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	2 (4%)		1 (2%)	2 (4%)
Necrosis				1 (2%)
Penis		(1)		
Developmental malformation		1 (100%)		
Preputial gland	(25)	(25)	(27)	(19)
Inflammation, subacute	5 (20%)	1 (4%)	1 (4%)	1 (5%)
Duct, cyst	23 (92%)	22 (88%)	24 (89%)	18 (95%)
Prostate	(49)	(49)	(50)	(50)
Inflammation, subacute			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Atrophy		1 (2%)		
Mineralization		1 (2%)		
Necrosis				1 (2%)
Seminiferous tubule, dilatation			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hypercellularity	1 (2%)	10 (20%)	22 (44%)	27 (54%)
Necrosis				1 (2%)
Lymph node	(50)	(50)	(50)	(49)
Inguinal, hyperplasia			1 (2%)	
Lymph node, mesenteric	(49)	(47)	(49)	(45)
Angiectasis	1 (2%)	3 (6%)	3 (6%)	
Hyperplasia			1 (2%)	
Inflammation, subacute			1 (2%)	
Thrombosis		1 (2%)		1 (2%)
Spleen	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Hematopoietic cell proliferation	13 (26%)	18 (36%)	37 (74%)	48 (96%)
Pigmentation		1 (2%)	46 (92%)	50 (100%)
Thrombosis				1 (2%)
Thymus	(46)	(47)	(49)	(47)
Cyst	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		1 (2%)
Inflammation, subacute, focal			1 (2%)	
Epithelium, hyperplasia, focal				1 (2%)
Subcutaneous tissue, edema, focal				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, hypertrophy, focal			1 (2%)	
Skeletal muscle	(1)	(1)	(2)	(1)
Artery, inflammation, subacute			1 (50%)	
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Embolus, multiple		1 (2%)		
Alveolar epithelium, hyperplasia	5 (10%)	3 (6%)	5 (10%)	8 (16%)
Mediastinum, thrombosis				1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	8 (16%)	7 (14%)	8 (16%)	4 (8%)
Fungus	1 (2%)	3 (6%)		
Inflammation, suppurative, acute	8 (16%)	6 (12%)	8 (16%)	4 (8%)
Nasolacrimal duct, hyperplasia	1 (2%)			
Special Senses System				
Eye	(1)	(1)	(1)	
Cornea, inflammation, subacute	1 (100%)	1 (100%)		
Harderian gland	(8)	(6)	(4)	(9)
Hyperplasia	3 (38%)			1 (11%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis	1 (2%)	1 (2%)		
Inflammation, suppurative, acute		1 (2%)		
Metaplasia, osseous	1 (2%)	1 (2%)		2 (4%)
Nephropathy, chronic	38 (76%)	48 (96%)	46 (92%)	39 (78%)
Cortex, cyst	1 (2%)	2 (4%)	12 (24%)	3 (6%)
Renal tubule, dilatation			1 (2%)	
Renal tubule, hyperplasia	1 (2%)			1 (2%)
Renal tubule, necrosis				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Urinary System (continued)				
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Artery, inflammation, subacute			1 (2%)	

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

APPENDIX B
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF *p*-NITROANILINE

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	70	70	70	70
9-Month interim evaluation	9	10	9	10
15-Month interim evaluation	9	10	10	9
Early deaths				
Accidental deaths	2		3	1
Moribund	16	5	11	12
Natural deaths	5	4	5	6
Survivors				
Terminal sacrifice	29	41	32	32
Animals examined microscopically	70	70	70	70
9-Month Interim Evaluation[b]				
15-Month Interim Evaluation				
Alimentary System				
Liver	(9)	(10)	(10)	(9)
Hepatocellular carcinoma	1 (11%)			
Hepatocellular adenoma	1 (11%)	1 (10%)		1 (11%)
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
Ovary	(9)		(1)	(9)
Teratoma benign			1 (100%)	
Hematopoietic System				
None				
Integumentary System				
None				
Musculoskeletal System				
None				

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
15-Month Interim Evaluation (continued)				
Nervous System				
None				
Respiratory System				
Lung	(9)		(10)	(9)
Hepatocellular carcinoma, metastatic, liver	1 (11%)			
Special Senses System				
None				
Urinary System				
None				
2-Year Study				
Alimentary System				
Gallbladder	(51)	(50)	(51)	(44)
Intestine small, duodenum	(52)	(50)	(50)	(51)
Polyp adenomatous		1 (2%)		
Intestine small, ileum	(52)	(50)	(50)	(50)
Intestine small, jejunum	(52)	(50)	(51)	(50)
Adenocarcinoma		2 (4%)	1 (2%)	
Liver	(52)	(50)	(51)	(51)
Cholangiocarcinoma			1 (2%)	
Hemangioma		1 (2%)		
Hemangiosarcoma	1 (2%)	1 (2%)		
Hepatocellular carcinoma	7 (13%)	6 (12%)	10 (20%)	7 (14%)
Hepatocellular carcinoma, two, multiple				2 (4%)
Hepatocellular adenoma	9 (17%)	9 (18%)	14 (27%)	8 (16%)
Hepatocellular adenoma, two, multiple	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Hepatocellular adenoma, three, multiple	1 (2%)	2 (4%)		1 (2%)
Hepatocholangiocarcinoma		1 (2%)		
Mesentery	(8)	(2)	(9)	(6)
Cholangiocarcinoma, greater than five, metastatic, multiple, liver			1 (11%)	
Hemangiosarcoma			1 (11%)	
Sarcoma	1 (13%)			
Pancreas	(52)	(50)	(51)	(51)
Salivary glands	(52)	(50)	(51)	(50)
Stomach, forestomach	(52)	(50)	(51)	(51)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Stomach, glandular	(52)	(50)	(51)	(51)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Cardiovascular System				
Heart	(52)	(50)	(51)	(51)
Endocrine System				
Adrenal gland, cortex	(52)	(50)	(51)	(51)
Spindle cell, adenoma		1 (2%)		
Adrenal gland, medulla	(52)	(50)	(51)	(51)
Pheochromocytoma malignant	1 (2%)			
Pheochromocytoma benign		1 (2%)	2 (4%)	
Islets, pancreatic	(52)	(50)	(51)	(51)
Adenoma	1 (2%)		1 (2%)	1 (2%)
Pituitary gland	(50)	(50)	(49)	(48)
Pars distalis, adenoma	4 (8%)	3 (6%)	5 (10%)	2 (4%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(52)	(50)	(51)	(51)
Follicular cell, adenoma		3 (6%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(1)		(1)	
Ovary	(49)	(50)	(50)	(49)
Cystadenoma			1 (2%)	
Hemangioma			1 (2%)	1 (2%)
Mixed neoplasm benign				1 (2%)
Teratoma benign	1 (2%)			
Granulosa cell, adenoma	1 (2%)		1 (2%)	
Uterus	(52)	(50)	(51)	(51)
Adenocarcinoma	1 (2%)			
Polyp stromal		3 (6%)		1 (2%)
Sarcoma stromal	1 (2%)		1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(52)	(50)	(51)	(51)
Hemangiosarcoma			1 (2%)	
Lymph node	(52)	(50)	(51)	(51)
Mediastinal, osteosarcoma, metastatic, bone	1 (2%)			
Lymph node, mandibular	(50)	(49)	(51)	(48)
Lymph node, mesenteric	(52)	(47)	(50)	(46)
Spleen	(52)	(49)	(51)	(51)
Hemangioma				1 (2%)
Hemangiosarcoma		1 (2%)	1 (2%)	2 (4%)
Histiocytic sarcoma				1 (2%)
Thymus	(51)	(48)	(49)	(49)
Schwannoma NOS			1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Integumentary System				
Skin	(52)	(50)	(51)	(51)
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	
Musculoskeletal System				
Bone	(52)	(50)	(51)	(51)
Hemangiosarcoma			1 (2%)	
Osteosarcoma	1 (2%)	1 (2%)		
Skeletal muscle	(2)		(1)	
Carcinoma, metastatic, harderian gland	1 (50%)			
Osteosarcoma, metastatic, bone	1 (50%)			
Nervous System				
Brain	(52)	(49)	(51)	(51)
Glioma malignant		1 (2%)		
Respiratory System				
Lung	(52)	(50)	(51)	(51)
Alveolar/bronchiolar adenoma	2 (4%)	5 (10%)	4 (8%)	3 (6%)
Alveolar/bronchiolar carcinoma			2 (4%)	1 (2%)
Cholangiocarcinoma, greater than five, metastatic, multiple, liver			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Osteosarcoma, metastatic, bone	1 (2%)			
Schwannoma NOS			1 (2%)	
Mediastinum, schwannoma NOS			1 (2%)	
Nose	(52)	(50)	(51)	(51)
Carcinoma, metastatic, harderian gland	1 (2%)			
Special Senses System				
Harderian gland	(4)	(3)	(10)	(7)
Adenoma	3 (75%)	3 (100%)	4 (40%)	5 (71%)
Carcinoma	1 (25%)		2 (20%)	
Urinary System				
Kidney	(52)	(50)	(51)	(51)
Urinary bladder	(52)	(50)	(50)	(51)
Systemic Lesions				
Multiple organs[c]	(52)	(50)	(51)	(51)
Histiocytic sarcoma				1 (2%)
Lymphoma malignant histiocytic		1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)		1 (2%)	
Lymphoma malignant mixed	9 (17%)	3 (6%)	4 (8%)	5 (10%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[d]				
15-Month interim evaluation	2	1	1	1
2-Year study	35	33	34	36
Total primary neoplasms				
15-Month interim evaluation	2	1	1	1
2-Year study	54	54	68	52
Total animals with benign neoplasms				
15-Month interim evaluation	1	1	1	1
2-Year study	23	25	23	23
Total benign neoplasms				
15-Month interim evaluation	1	1	1	1
2-Year study	29	36	36	28
Total animals with malignant neoplasms				
15-Month interim evaluation	1			
2-Year study	21	14	20	22
Total malignant neoplasms				
15-Month interim evaluation	1			
2-Year study	25	18	29	24
Total animals with metastatic neoplasms				
15-Month interim evaluation	1			
2-Year study	3	2	2	3
Total metastatic neoplasms				
15-Month interim evaluation	1			
2-Year study	6	2	4	3
Total animals with neoplasms uncertain-benign or malignant				
2-Year study			1	
Total uncertain neoplasms				
2-Year study			3	

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] All organ systems listed in Table 1 (Materials and Methods) were evaluated, but no neoplasms were found.
[c] Number of animals with any tissue examined microscopically
[d] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control

Number of Days on Study	0 0 1 2 3 4 4 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 0 0 0 9 7 2 8 3 3 7 9 0 2 3 6 8 0 0 0 0 0 2 2 2 2 2 2 2 7 5 8 3 3 3 4 6 0 9 6 0 1 2 0 0 1 1 1 1 5 5 9 9 9 9
Carcass ID Number	2 3 3 2 2 3 3 3 3 3 2 3 2 3 3 2 3 2 2 3 3 3 3 3 3 3 3 8 4 3 9 8 1 1 2 0 1 9 2 9 1 2 8 0 8 9 0 2 0 3 2 2 2 2 1 3 3 7 2 2 3 2 2 7 0 9 2 4 0 4 4 6 4 3 3 7 0 4 5 6 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Alimentary System	
Esophagus	+ +
Gallbladder	M +
Intestine large	+ +
Intestine large, cecum	+ +
Intestine large, colon	+ M + + + +
Intestine large, rectum	+ +
Intestine small	+ +
Intestine small, duodenum	+ +
Intestine small, ileum	+ +
Intestine small, jejunum	+ +
Liver	+ +
Hemangiosarcoma	X
Hepatocellular carcinoma	X X X X
Hepatocellular adenoma	X X X X
Hepatocellular adenoma, two, multiple	X
Hepatocellular adenoma, three, multiple	
Mesentery	+ + + + +
Sarcoma	X
Pancreas	+ +
Salivary glands	+ +
Stomach	+ +
Stomach, forestomach	+ +
Squamous cell papilloma	X X
Stomach, glandular	+ +
Tongue	+
Cardiovascular System	
Blood vessel	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adrenal gland, medulla	+ +
Pheochromocytoma malignant	
Islets, pancreatic	+ +
Adenoma	X
Parathyroid gland	+ + M + + + + + + M + + M + + + + + + + + + + + + +

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 2 8 1	2 9 5 1	2 9 6 1	2 9 8 1	2 9 9 1	3 0 0 1	3 0 1 1	3 0 9 1	3 1 0 1	3 1 1 1	3 1 5 1	3 0 5 1	3 0 6 1	3 0 8 1	3 2 8 1	3 2 8 1	3 2 8 1	3 2 8 1	3 2 8 1	3 2 9 1	3 2 9 1	3 3 1 1	3 3 6 1	3 3 8 1	3 3 9 1	3 3 1 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Hemangiosarcoma																										1
Hepatocellular carcinoma							X							X			X									7
Hepatocellular adenoma				X						X				X					X	X		X				9
Hepatocellular adenoma, two, multiple																					X			X		3
Hepatocellular adenoma, three, multiple																	X									1
Mesentery					+												+					+				8
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Squamous cell papilloma						X																				3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Tongue																										1
Cardiovascular System																										
Blood vessel								+																		1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pheochromocytoma malignant																								X		1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma																										1
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control
(continued)

Number of Days on Study	0 0 1 2 3 4 4 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 0 0 0 9 7 2 8 3 3 7 9 0 2 3 6 8 0 0 0 0 0 2 2 2 2 2 2 2 7 5 8 3 3 3 4 6 0 9 6 0 1 2 0 0 1 1 1 1 5 5 9 9 9 9
Carcass ID Number	2 3 3 2 2 3 3 3 3 3 2 3 2 3 3 2 3 2 2 3 3 3 3 3 3 3 3 8 4 3 9 8 1 1 2 0 1 9 2 9 1 2 8 0 8 9 0 2 0 3 2 2 2 2 1 3 3 7 2 2 3 2 2 7 0 9 2 4 0 4 4 6 4 3 3 7 0 4 5 6 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Endocrine System (continued)	
Pituitary gland	+ + + + M + + + + + + + M + + + + + + + + + + + + +
Pars distalis, adenoma	X X
Pars intermedia, adenoma	
Thyroid gland	+ +
General Body System	
None	
Genital System	
Clitoral gland	+
Ovary	+ +
Teratoma benign	X
Granulosa cell, adenoma	X
Uterus	+ +
Adenocarcinoma	
Sarcoma stromal	X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ +
Mediastinal, osteosarcoma, metastatic, bone	X
Lymph node, mandibular	+ M M +
Lymph node, mesenteric	+ +
Spleen	+ +
Thymus	+ +
Integumentary System	
Mammary gland	+ + + + + + + M + + + + + + + + + + + + + + + + + +
Skin	+ +
Subcutaneous tissue, fibrosarcoma	
Musculoskeletal System	
Bone	+ +
Osteosarcoma	X
Skeletal muscle	+ +
Carcinoma, metastatic, harderian gland	X
Osteosarcoma, metastatic, bone	X

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 2 8 1	2 9 5 1	2 9 6 1	2 9 8 1	2 9 9 1	3 0 0 1	3 0 1 1	3 0 9 1	3 1 0 1	3 1 1 1	3 1 5 1	3 0 5 1	3 0 6 1	3 0 8 1	2 8 3 1	2 8 5 1	2 8 7 1	2 8 8 1	2 8 9 1	2 9 1 1	2 9 3 1	3 1 6 1	3 1 8 1	3 1 9 1	3 2 1 1	Total Tissues/ Tumors
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma													X						X							4
Pars intermedia, adenoma																	X									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
General Body System																										
None																										
Genital System																										
Clitoral gland																										1
Ovary	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Teratoma benign																										1
Granulosa cell, adenoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenocarcinoma						X																				1
Sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Mediastinal, osteosarcoma, metastatic, bone																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Subcutaneous tissue, fibrosarcoma							X																			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Osteosarcoma																										1
Skeletal muscle																										2
Carcinoma, metastatic, harderian gland																										1
Osteosarcoma, metastatic, bone																										1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control
(continued)

Number of Days on Study	0 0 1 2 3 4 4 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 0 0 0 9 7 2 8 3 3 7 9 0 2 3 6 8 0 0 0 0 0 0 2 2 2 2 2 2 2 7 5 8 3 3 3 4 6 0 9 6 0 1 2 0 0 1 1 1 1 5 5 9 9 9 9
Carcass ID Number	2 3 3 2 2 3 3 3 3 3 2 3 2 3 3 2 3 2 2 3 3 3 3 3 3 3 3 8 4 3 9 8 1 1 2 0 1 9 2 9 1 2 8 0 8 9 0 2 0 3 2 2 2 2 1 3 3 7 2 2 3 2 2 7 0 9 2 4 0 4 4 6 4 3 3 7 0 4 5 6 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	
Hepatocellular carcinoma, metastatic, liver	X
Osteosarcoma, metastatic, bone	X
Nose	+ +
Carcinoma, metastatic, harderian gland	X
Trachea	+ +
Special Senses System	
Ear	+
Eye	+
Harderian gland	+ +
Adenoma	X
Carcinoma	X
Urinary System	
Kidney	+ +
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant lymphocytic	X
Lymphoma malignant mixed	X X X X X X X

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: Vehicle Control (continued)

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	9	0	0	0	0	0	0	0	0	0	0	2	2	2	5	5	5	5	5	5	5	5	5	5	5	
Carcass ID Number	3	2	2	2	2	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	3	3	3	3	
	2	9	9	9	9	0	0	0	1	1	1	0	0	0	8	8	8	8	8	9	9	1	1	1	2	**Total**
	8	5	6	8	9	0	1	9	0	1	5	5	6	8	3	5	7	8	9	1	3	6	8	9	1	**Tissues/**
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**Tumors**
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Alveolar/bronchiolar adenoma									X										X							2
Hepatocellular carcinoma, metastatic, liver																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, harderian gland																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Special Senses System																										
Ear																										1
Eye																										1
Harderian gland										+	+															4
Adenoma										X	X															3
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed	X																	X								9

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg

	3	4	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Number of Days on Study	4	7	5	8	0	5	6	0	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	1	8	3	1	6	3	7	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5
Carcass ID Number	3	3	0	1	2	2	1	0	1	9	9	9	9	9	9	9	0	0	0	1	1	1	1	1	1
	8	9	7	5	9	4	8	8	0	1	2	3	4	5	6	7	5	6	9	1	2	3	4	6	7
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous					X																				
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																			X					X	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma											X														
Hemangiosarcoma			X																						
Hepatocellular carcinoma			X				X	X									X							X	
Hepatocellular adenoma								X		X								X							
Hepatocellular adenoma, two, multiple																									
Hepatocellular adenoma, three, multiple											X														
Hepatocholangiocarcinoma																									
Mesentery						+						+													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma				X								X													
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spindle cell, adenoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	5 2 6 1	5 2 7 1	5 2 8 1	5 3 0 1	5 3 1 1	5 3 2 1	5 3 3 1	5 3 4 1	5 3 5 1	5 3 6 1	5 3 7 1	5 4 8 1	4 9 9 1	5 0 0 1	5 0 1 1	5 0 2 1	5 0 3 1	5 0 4 1	5 1 9 1	5 2 0 1	5 2 1 1	5 2 2 1	5 2 3 1	5 2 5 1	5 4 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										2
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Hemangiosarcoma																										1
Hepatocellular carcinoma																									X	6
Hepatocellular adenoma		X			X				X						X				X					X		9
Hepatocellular adenoma, two, multiple							X																			1
Hepatocellular adenoma, three, multiple						X																				2
Hepatocholangiocarcinoma																					X					1
Mesentery																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																								X		3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel					+																					1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spindle cell, adenoma						X																				1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign								X																		1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	3 4 1	4 7 8	5 5 3	5 8 1	6 0 6	6 5 3	6 6 7	7 0 0	7 1 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	5 3 8 1	5 3 9 1	5 0 7 1	5 1 5 1	5 2 9 1	5 2 4 1	5 1 8 1	5 0 8 1	5 1 0 1	4 9 1 1	4 9 2 1	4 9 3 1	4 9 4 1	4 9 5 1	4 9 6 1	4 9 7 1	5 0 5 1	5 0 6 1	5 0 9 1	5 1 1 1	5 1 2 1	5 1 3 1	5 1 4 1	5 1 6 1	5 1 7 1
Endocrine System (continued)																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma									X																
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																					X				
General Body System																									
None																									
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal		X															X			X					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Hemangiosarcoma								X																	
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant	X																								
Spinal cord		+																							

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	5 2 6 1	5 2 7 1	5 2 8 1	5 3 0 1	5 3 1 1	5 3 2 1	5 3 3 1	5 3 4 1	5 3 5 1	5 3 6 1	5 3 7 1	5 4 8 1	4 9 9 1	5 0 0 1	5 0 1 1	5 0 2 1	5 0 3 1	5 0 4 1	5 1 9 1	5 2 0 1	5 2 1 1	5 2 2 1	5 2 3 1	5 2 5 1	5 4 0 1	Total Tissues/ Tumors
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma									X												X					3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																			X					X		3
General Body System																										
None																										
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal																										3
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																						X				1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Glioma malignant																										1
Spinal cord																										1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	3 4 1	4 7 8	5 5 3	5 8 1	6 0 6	6 5 3	6 6 7	7 0 0	7 1 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	5 3 8 1	5 3 9 1	5 0 7 1	5 1 5 1	5 2 9 1	5 2 4 1	5 1 8 1	5 0 8 1	5 1 0 1	4 9 1 1	4 9 2 1	4 9 3 1	4 9 4 1	4 9 5 1	4 9 6 1	4 9 7 1	5 0 5 1	5 0 6 1	5 0 9 1	5 1 1 1	5 1 2 1	5 1 3 1	5 1 4 1	5 1 6 1	5 1 7 1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X		X	X													
Hepatocellular carcinoma, metastatic, liver								X																X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Harderian gland								+																+	
Adenoma								X																X	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic						X																			
Lymphoma malignant mixed						X																			

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 3 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	5 2 6 1	5 2 7 1	5 2 8 1	5 3 0 1	5 3 1 1	5 3 2 1	5 3 3 1	5 3 4 1	5 3 5 1	5 3 6 1	5 3 7 1	5 4 8 1	4 9 9 1	5 0 0 1	5 0 1 1	5 0 2 1	5 0 3 1	5 0 4 1	5 1 9 1	5 2 0 1	5 2 1 1	5 2 2 1	5 2 3 1	5 2 5 1	5 4 0 1	Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X												X						5
Hepatocellular carcinoma, metastatic, liver																									2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																									
Eye			+	+																					2
Harderian gland			+																						3
Adenoma			X																						3
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																									1
Lymphoma malignant mixed	X				X																				3

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg

Number of Days on Study	0 0 0 4 4 4 4 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	0 1 1 2 4 6 8 6 5 5 6 7 9 0 1 1 2 2 2 3 3 3 3 3 3 3 3 3
	3 1 7 4 8 5 4 0 4 5 7 2 2 3 6 6 0 3 5 0 0 0 0 0 0 0 0 0
Carcass ID Number	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
	7 4 2 5 5 3 5 2 3 4 2 4 5 6 3 3 6 6 2 2 2 2 2 4 5 5 5
	9 6 7 4 5 8 9 4 9 8 1 4 3 0 2 6 7 4 9 2 3 5 6 9 0 1 2
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Alimentary System	
Esophagus	+ +
Gallbladder	+ +
Intestine large	+ +
Intestine large, cecum	+ +
Intestine large, colon	+ + A +
Intestine large, rectum	+ + A +
Intestine small	+ +
Intestine small, duodenum	+ + A +
Intestine small, ileum	+ + A +
Intestine small, jejunum	+ +
Adenocarcinoma	X
Liver	+ +
Cholangiocarcinoma	X
Hepatocellular carcinoma	X X X X X X X
Hepatocellular adenoma	X X X X X X
Hepatocellular adenoma, two, multiple	
Mesentery	+ + + + +
Cholangiocarcinoma, greater than five, metastatic, multiple, liver	X
Hemangiosarcoma	
Pancreas	+ +
Salivary glands	+ +
Stomach	+ +
Stomach, forestomach	+ +
Cholangiocarcinoma, metastatic, liver	X
Squamous cell papilloma	
Stomach, glandular	+ +
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adrenal gland, medulla	+ +
Pheochromocytoma benign	
Islets, pancreatic	+ +
Adenoma	
Parathyroid gland	+ + + + + + + + + + + + + + + + + M + + + M + + + M + +

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 2 8 1	4 3 0 1	4 3 1 1	4 3 3 1	4 3 4 1	4 4 2 1	4 4 3 1	4 4 5 1	4 4 7 1	4 3 5 1	4 3 7 1	4 4 0 1	4 4 1 1	4 5 6 1	4 5 7 1	4 5 8 1	4 6 1 1	4 6 2 1	4 6 3 1	4 6 5 1	4 6 6 1	4 6 8 1	4 6 9 1	4 7 0 1	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenocarcinoma																									1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cholangiocarcinoma																									1
Hepatocellular carcinoma	X	X	X											X											10
Hepatocellular adenoma						X	X	X	X				X	X								X			14
Hepatocellular adenoma, two, multiple											X														1
Mesentery								+			+								+				+		9
Cholangiocarcinoma, greater than five, metastatic, multiple, liver																									1
Hemangiosarcoma																			X						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cholangiocarcinoma, metastatic, liver																									1
Squamous cell papilloma						X																			1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Pheochromocytoma benign									X														X		2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenoma						X																			1
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	0 3	0 1	0 1	4 2	4 4	4 6	4 8	5 6	6 5	6 5	6 6	6 7	6 9	7 0	7 1	7 1	7 2	7 2	7 3	7 3	7 3	7 3	7 3	7 3	7 3	7 3
Carcass ID Number	4 7 9 1	4 4 6 1	4 2 7 1	4 5 4 1	4 5 5 1	4 3 8 1	4 5 9 1	4 2 4 1	4 3 9 1	4 4 8 1	4 2 1 1	4 4 4 1	4 5 3 1	4 6 0 1	4 3 2 1	4 3 6 1	4 6 7 1	4 6 4 1	4 2 9 1	4 2 2 1	4 2 3 1	4 2 5 1	4 2 6 1	4 4 9 1	4 5 0 1	4 5 1 1	4 5 2 1
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pars distalis, adenoma																						X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																	X									
General Body System																										
None																										
Genital System																										
Clitoral gland															+											
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Cystadenoma																										
Hemangioma																	X									
Granulosa cell, adenoma																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal								X																		
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma									X																	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma									X																	
Thymus	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Schwannoma NOS						X																				
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma				X																						
Subcutaneous tissue, hemangiosarcoma									X																	
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma									X																	
Skeletal muscle															+											

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 2 8 1	4 3 0 1	4 3 1 1	4 3 3 1	4 3 4 1	4 4 2 1	4 4 3 1	4 4 5 1	4 4 7 1	4 4 3 1	4 3 5 1	4 3 7 1	4 4 0 1	4 4 1 1	4 5 6 1	4 5 7 1	4 5 8 1	4 6 1 1	4 6 2 1	4 6 3 1	4 6 5 1	4 6 6 1	4 6 8 1	4 6 9 1	4 7 0 1	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma								X	X		X		X													5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma											X															1
Hemangioma																										1
Granulosa cell, adenoma																			X							1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Hemangiosarcoma																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Schwannoma NOS																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Hemangiosarcoma																										1
Skeletal muscle																										1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	0	0	0	4	4	4	4	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	1	1	2	4	6	8	6	5	5	6	7	9	0	1	1	2	2	2	3	3	3	3	3	3	3
	3	1	7	4	8	5	4	0	4	5	7	2	2	3	6	6	0	3	5	0	0	0	0	0	0	0
Carcass ID Number	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	7	4	2	5	5	3	5	2	3	4	2	4	5	6	3	3	6	6	2	2	2	2	2	4	5	5
	9	6	7	4	5	8	9	4	9	8	1	4	3	0	2	6	7	4	9	2	3	5	6	9	0	1
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord							+																			
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X																	X			
Alveolar/bronchiolar carcinoma						X					X															
Cholangiocarcinoma, greater than five, metastatic, multiple, liver					X																					
Hepatocellular carcinoma, metastatic, liver																X										
Schwannoma NOS						X																				
Mediastinum, schwannoma NOS						X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye																	+									
Harderian gland															+		+									
Adenoma																										
Carcinoma																	X									
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																			X							
Lymphoma malignant lymphocytic															X											
Lymphoma malignant mixed																					X					

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 30 mg/kg
(continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 2 8 1	4 3 0 1	4 3 1 1	4 3 3 1	4 3 4 1	4 4 2 1	4 4 3 1	4 4 5 1	4 4 7 1	4 4 5 1	4 3 5 1	4 3 7 1	4 4 0 1	4 4 1 1	4 5 6 1	4 5 7 1	4 5 8 1	4 6 1 1	4 6 2 1	4 6 3 1	4 6 5 1	4 6 6 1	4 6 8 1	4 6 9 1	4 7 0 1	**Total Tissues/ Tumors**
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Spinal cord																									1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Alveolar/bronchiolar adenoma			X										X												4
Alveolar/bronchiolar carcinoma																									2
Cholangiocarcinoma, greater than five, metastatic, multiple, liver																									1
Hepatocellular carcinoma, metastatic, liver																									1
Schwannoma NOS																									1
Mediastinum, schwannoma NOS																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Special Senses System																									
Eye																									1
Harderian gland	+	+				+				+				+				+			+				10
Adenoma										X				X						X			X		4
Carcinoma		X																							2
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Urinary bladder	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymphoma malignant histiocytic																									1
Lymphoma malignant lymphocytic																									1
Lymphoma malignant mixed	X						X				X														4

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg

Number of Days on Study	0 0 8	3 4 7	4 0 2	4 7 7	5 0 1	5 4 2	6 3 4	6 5 1	6 6 5	6 6 7	6 8 1	6 9 2	7 0 8	7 1 1	7 1 6	7 2 3	7 2 4	7 2 5	7 2 5	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	4 2 0 1	3 9 7 1	3 7 4 1	3 6 4 1	3 9 4 1	3 9 9 1	3 8 3 1	3 5 1 1	3 6 9 1	3 9 8 1	3 9 2 1	3 7 3 1	3 5 4 1	3 5 9 1	3 8 1 1	3 9 0 1	3 7 0 1	3 5 7 1	3 5 7 1	3 5 8 1	3 6 0 1	3 6 1 1	3 6 2 1	3 6 3 1	3 6 6 1	3 8 8 1
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	M	+	+	+	M	+	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma						X						X	X	X	X						X					
Hepatocellular carcinoma, two, multiple																	X									
Hepatocellular adenoma						X											X							X		
Hepatocellular adenoma, two, multiple																				X						
Hepatocellular adenoma, three, multiple																X										
Mesentery							+																		+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																										
Squamous cell papilloma																										
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X																	
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									X	

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 8 9 1	3 9 1 1	3 9 3 1	3 9 5 1	3 9 6 1	4 0 0 1	3 6 5 1	3 6 6 1	3 6 7 1	3 6 8 1	3 7 1 1	3 7 2 1	3 7 5 1	3 7 6 1	3 7 9 1	3 8 0 1	3 8 2 1	3 8 4 1	3 8 5 1	3 5 2 1	3 5 3 1	3 5 5 1	3 5 6 1	3 7 7 1	3 7 8 1	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Hepatocellular carcinoma							X																			7
Hepatocellular carcinoma, two, multiple			X																							2
Hepatocellular adenoma			X					X											X		X			X		8
Hepatocellular adenoma, two, multiple																										1
Hepatocellular adenoma, three, multiple																										1
Mesentery					+									+								+		+		6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Squamous cell carcinoma		X																								1
Squamous cell papilloma								X			X															2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenoma																										1
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma					X																	X				2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Follicular cell, adenoma																										1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	0	3	4	4	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	4	0	7	0	4	3	5	6	6	8	9	0	1	1	2	2	2	2	3	3	3	3	3	3	3	3
	8	7	2	7	1	2	4	1	5	7	1	2	8	1	6	3	4	5	5	0	0	0	0	0	0	0	0
Carcass ID Number	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	2	9	7	6	9	9	8	5	6	9	9	7	5	5	8	9	7	5	8	5	6	6	6	6	8	8	
	0	7	4	4	4	9	3	1	9	8	2	3	4	9	1	0	0	7	7	8	0	1	2	3	6	8	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
General Body System																											
None																											
Genital System																											
Clitoral gland																								M			
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																											
Mixed tumor benign																X											
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																											
Sarcoma stromal																											
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																											
Hemangiosarcoma															X												
Histiocytic sarcoma										X																	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma				X	X											X											
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 8 9 1 1	3 9 1 1	3 9 3 1	3 9 5 1	3 9 6 1	4 0 0 1	3 6 5 1	3 6 6 1	3 6 7 1	3 6 8 1	3 7 1 1	3 7 2 1	3 7 5 1	3 7 6 1	3 7 9 1	3 8 0 1	3 8 2 1	3 8 4 1	3 8 5 1	3 5 2 1	3 5 3 1	3 5 5 1	3 5 6 1	3 7 7 1	3 7 8 1	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Clitoral gland																										
Ovary	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																			X							1
Mixed tumor benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Polyp stromal													X													1
Sarcoma stromal				X																						1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Hemangioma																									X	1
Hemangiosarcoma														X												2
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Subcutaneous tissue, fibrosarcoma																										3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	0	3	4	4	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	4	0	7	0	4	3	5	6	6	8	9	0	1	1	2	2	2	2	3	3	3	3	3	3	3	3
	8	7	2	7	1	2	4	1	5	7	1	2	8	1	6	3	4	5	5	0	0	0	0	0	0	0	0
Carcass ID Number	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	2	9	7	6	9	9	8	5	6	9	9	7	5	5	8	9	7	5	8	5	6	6	6	6	8	8	
	0	7	4	4	4	9	3	1	9	8	2	3	4	9	1	0	0	7	7	8	0	1	2	3	6	8	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X														X		
Alveolar/bronchiolar carcinoma																											
Hepatocellular carcinoma, metastatic, liver						X						X		X													
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																											
Harderian gland									+	+			+	+					+								
Adenoma													X	X					X								
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X																	
Lymphoma malignant histiocytic										X																	
Lymphoma malignant mixed							X	X											X								

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline: 100 mg/kg
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 8 9 1 1	3 9 1 1	3 9 3 1	3 9 5 1	3 9 6 1	4 0 0 1	3 6 5 1	3 6 6 1	3 6 7 1	3 6 8 1	3 7 1 1	3 7 2 1	3 7 5 1	3 7 6 1	3 7 9 1	3 8 0 1	3 8 2 1	3 8 4 1	3 8 5 1	3 5 2 1	3 5 3 1	3 5 5 1	3 5 6 1	3 7 7 1	3 7 8 1	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Alveolar/bronchiolar adenoma																			X							3
Alveolar/bronchiolar carcinoma								X																		1
Hepatocellular carcinoma, metastatic, liver																										3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Special Senses System																										
Harderian gland												+				+										7
Adenoma												X				X										5
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Histiocytic sarcoma																										1
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed	X																					X				5

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	3/52 (6%)	3/50 (6%)	4/51 (8%)	5/51 (10%)
Adjusted rate[b]	9.9%	7.1%	12.5%	13.7%
Terminal rate[c]	2/29 (7%)	2/41 (5%)	4/32 (13%)	2/32 (6%)
First incidence (days)	725	700	729 (T)	708
Life table test[d]	P=0.238	P=0.515N	P=0.551	P=0.429
Logistic regression test[d]	P=0.276	P=0.575N	P=0.563	P=0.425
Cochran-Armitage test[d]	P=0.262			
Fisher exact test[d]		P=0.642	P=0.489	P=0.347
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/52 (8%)	3/50 (6%)	6/51 (12%)	5/51 (10%)
Adjusted rate	11.9%	7.1%	18.1%	13.7%
Terminal rate	2/29 (7%)	2/41 (5%)	5/32 (16%)	2/32 (6%)
First incidence (days)	534	700	723	708
Life table test	P=0.338	P=0.357N	P=0.423	P=0.569
Logistic regression test	P=0.382	P=0.473N	P=0.394	P=0.537
Cochran-Armitage test	P=0.366			
Fisher exact test		P=0.522N	P=0.358	P=0.488
Liver: Hepatocellular Adenoma				
Overall rate	13/52 (25%)	12/50 (24%)	15/51 (29%)	10/51 (20%)
Adjusted rate	39.8%	28.5%	39.8%	27.8%
Terminal rate	10/29 (34%)	11/41 (27%)	10/32 (31%)	7/32 (22%)
First incidence (days)	606	700	655	542
Life table test	P=0.351N	P=0.178N	P=0.536	P=0.231N
Logistic regression test	P=0.254N	P=0.321N	P=0.482	P=0.238N
Cochran-Armitage test	P=0.285N			
Fisher exact test		P=0.545N	P=0.389	P=0.338N
Liver: Hepatocellular Carcinoma				
Overall rate	7/52 (13%)	6/50 (12%)	10/51 (20%)	9/51 (18%)
Adjusted rate	20.1%	13.4%	24.8%	22.5%
Terminal rate	4/29 (14%)	3/41 (7%)	4/32 (13%)	3/32 (9%)
First incidence (days)	536	553	448	542
Life table test	P=0.278	P=0.321N	P=0.372	P=0.494
Logistic regression test	P=0.288	P=0.501N	P=0.292	P=0.422
Cochran-Armitage test	P=0.284			
Fisher exact test		P=0.531N	P=0.283	P=0.377
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	17/52 (33%)	17/50 (34%)	21/51 (41%)	16/51 (31%)
Adjusted rate	47.1%	38.4%	51.7%	40.2%
Terminal rate	11/29 (38%)	14/41 (34%)	13/32 (41%)	9/32 (28%)
First incidence (days)	536	553	448	542
Life table test	P=0.510N	P=0.212N	P=0.406	P=0.363N
Logistic regression test	P=0.407N	P=0.467N	P=0.286	P=0.412N
Cochran-Armitage test	P=0.437N			
Fisher exact test		P=0.528	P=0.246	P=0.527N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/52 (4%)	5/50 (10%)	4/51 (8%)	3/51 (6%)
Adjusted rate	6.9%	11.9%	11.3%	8.5%
Terminal rate	2/29 (7%)	4/41 (10%)	3/32 (9%)	2/32 (6%)
First incidence (days)	729 (T)	719	465	681
Life table test	P=0.549N	P=0.373	P=0.376	P=0.548
Logistic regression test	P=0.501N	P=0.326	P=0.340	P=0.549
Cochran-Armitage test	P=0.513N			
Fisher exact test		P=0.202	P=0.330	P=0.491
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/52 (4%)	5/50 (10%)	5/51 (10%)	4/51 (8%)
Adjusted rate	6.9%	11.9%	13.5%	11.6%
Terminal rate	2/29 (7%)	4/41 (10%)	3/32 (9%)	3/32 (9%)
First incidence (days)	729 (T)	719	465	681
Life table test	P=0.469	P=0.373	P=0.255	P=0.386
Logistic regression test	P=0.517	P=0.326	P=0.218	P=0.390
Cochran-Armitage test	P=0.504			
Fisher exact test		P=0.202	P=0.210	P=0.330
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	5/49 (10%)	2/48 (4%)
Adjusted rate	12.5%	7.1%	16.1%	6.5%
Terminal rate	3/29 (10%)	2/41 (5%)	5/31 (16%)	2/31 (6%)
First incidence (days)	599	719	729 (T)	729 (T)
Life table test	P=0.363N	P=0.340N	P=0.539	P=0.307N
Logistic regression test	P=0.318N	P=0.437N	P=0.524	P=0.316N
Cochran-Armitage test	P=0.333N			
Fisher exact test		P=0.500N	P=0.487	P=0.359N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/52 (2%)	1/50 (2%)	1/51 (2%)	3/51 (6%)
Adjusted rate	3.4%	2.1%	2.1%	6.8%
Terminal rate	1/29 (3%)	0/41 (0%)	0/32 (0%)	0/32 (0%)
First incidence (days)	729 (T)	581	424	477
Life table test	P=0.159	P=0.709N	P=0.745N	P=0.338
Logistic regression test	P=0.139	P=0.750	P=0.754	P=0.267
Cochran-Armitage test	P=0.155			
Fisher exact test		P=0.743	P=0.748	P=0.301
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/52 (6%)	3/50 (6%)	1/51 (2%)	2/51 (4%)
Adjusted rate	8.7%	6.9%	3.1%	6.3%
Terminal rate	1/29 (3%)	2/41 (5%)	1/32 (3%)	2/32 (6%)
First incidence (days)	662	581	729 (T)	729 (T)
Life table test	P=0.436N	P=0.544N	P=0.277N	P=0.453N
Logistic regression test	P=0.408N	P=0.656N	P=0.292N	P=0.464N
Cochran-Armitage test	P=0.415N			
Fisher exact test		P=0.642	P=0.316N	P=0.509N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	3/52 (6%)	3/50 (6%)	1/51 (2%)	3/51 (6%)
Adjusted rate	8.7%	6.9%	3.1%	9.4%
Terminal rate	1/29 (3%)	2/41 (5%)	1/32 (3%)	3/32 (9%)
First incidence (days)	662	581	729 (T)	729 (T)
Life table test	P=0.567	P=0.544N	P=0.277N	P=0.613N
Logistic regression test	P=0.600	P=0.656N	P=0.292N	P=0.623N
Cochran-Armitage test	P=0.591			
Fisher exact test		P=0.642	P=0.316N	P=0.652
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/52 (0%)	3/50 (6%)	1/51 (2%)	1/51 (2%)
Adjusted rate	0.0%	7.3%	2.9%	3.1%
Terminal rate	0/29 (0%)	3/41 (7%)	0/32 (0%)	1/32 (3%)
First incidence (days)	–[e]	729 (T)	720	729 (T)
Life table test	P=0.557N	P=0.188	P=0.524	P=0.520
Logistic regression test	P=0.530N	P=0.188	P=0.515	P=0.520
Cochran-Armitage test	P=0.535N			
Fisher exact test		P=0.114	P=0.495	P=0.495
Uterus: Stromal Polyp				
Overall rate	0/52 (0%)	3/50 (6%)	0/51 (0%)	1/51 (2%)
Adjusted rate	0.0%	6.8%	0.0%	3.1%
Terminal rate	0/29 (0%)	2/41 (5%)	0/32 (0%)	1/32 (3%)
First incidence (days)	–	478	–	729 (T)
Life table test	P=0.574N	P=0.170	–	P=0.520
Logistic regression test	P=0.555N	P=0.091	–	P=0.520
Cochran-Armitage test	P=0.555N			
Fisher exact test		P=0.114	–	P=0.495
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	1/52 (2%)	3/50 (6%)	1/51 (2%)	2/51 (4%)
Adjusted rate	3.4%	6.8%	2.3%	6.3%
Terminal rate	1/29 (3%)	2/41 (5%)	0/32 (0%)	2/32 (6%)
First incidence (days)	729 (T)	478	560	729 (T)
Life table test	P=0.584	P=0.408	P=0.747N	P=0.535
Logistic regression test	P=0.608	P=0.280	P=0.758	P=0.535
Cochran-Armitage test	P=0.608			
Fisher exact test		P=0.294	P=0.748	P=0.493
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/52 (2%)	3/50 (6%)	3/51 (6%)	4/51 (8%)
Adjusted rate	2.9%	6.7%	8.1%	11.8%
Terminal rate	0/29 (0%)	1/41 (2%)	1/32 (3%)	3/32 (9%)
First incidence (days)	701	553	654	716
Life table test	P=0.217	P=0.383	P=0.347	P=0.219
Logistic regression test	P=0.231	P=0.286	P=0.314	P=0.213
Cochran-Armitage test	P=0.224			
Fisher exact test		P=0.294	P=0.301	P=0.175

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
All Organs: Malignant Lymphoma and Histiocytic Sarcoma				
Overall rate	9/52 (17%)	3/50 (6%)	6/51 (12%)	6/51 (12%)
Adjusted rate	25.4%	7.0%	17.4%	15.1%
Terminal rate	4/29 (14%)	2/41 (5%)	4/32 (13%)	2/32 (6%)
First incidence (days)	606	653	716	634
Life table test	P=0.565	P=0.025N	P=0.237N	P=0.230N
Logistic regression test	P=0.540N	P=0.043N	P=0.248N	P=0.250N
Cochran-Armitage test	P=0.556N			
Fisher exact test		P=0.070N	P=0.303N	P=0.303N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or Mixed)				
Overall rate	9/52 (17%)	3/50 (6%)	6/51 (12%)	6/51 (12%)
Adjusted rate	25.4%	7.0%	17.4%	15.1%
Terminal rate	4/29 (14%)	2/41 (5%)	4/32 (13%)	2/32 (6%)
First incidence (days)	606	653	716	634
Life table test	P=0.565	P=0.025N	P=0.237N	P=0.230N
Logistic regression test	P=0.540N	P=0.043N	P=0.248N	P=0.250N
Cochran-Armitage test	P=0.556N			
Fisher exact test		P=0.070N	P=0.303N	P=0.303N
All Organs: Benign Neoplasms				
Overall rate	23/52 (44%)	25/50 (50%)	23/51 (45%)	23/51 (45%)
Adjusted rate	62.8%	54.2%	59.8%	56.9%
Terminal rate	16/29 (55%)	20/41 (49%)	17/32 (53%)	15/32 (47%)
First incidence (days)	570	478	465	542
Life table test	P=0.522	P=0.226N	P=0.415N	P=0.398N
Logistic regression test	P=0.422N	P=0.567	P=0.526N	P=0.451N
Cochran-Armitage test	P=0.466N			
Fisher exact test		P=0.350	P=0.544	P=0.544
All Organs: Malignant Neoplasms				
Overall rate	21/52 (40%)	14/50 (28%)	20/51 (39%)	22/51 (43%)
Adjusted rate	50.5%	29.4%	44.5%	48.1%
Terminal rate	9/29 (31%)	8/41 (20%)	8/32 (25%)	9/32 (28%)
First incidence (days)	534	341	424	477
Life table test	P=0.203	P=0.031N	P=0.390N	P=0.481N
Logistic regression test	P=0.191	P=0.108N	P=0.505N	P=0.544
Cochran-Armitage test	P=0.187			
Fisher exact test		P=0.134N	P=0.532N	P=0.467
All Organs: Benign and Malignant Neoplasms				
Overall rate	35/52 (67%)	33/50 (66%)	34/51 (67%)	36/51 (71%)
Adjusted rate	77.8%	66.0%	73.8%	76.4%
Terminal rate	19/29 (66%)	24/41 (59%)	20/32 (63%)	21/32 (66%)
First incidence (days)	534	341	424	477
Life table test	P=0.309	P=0.055N	P=0.325N	P=0.401N
Logistic regression test	P=0.393	P=0.330N	P=0.483N	P=0.550N
Cochran-Armitage test	P=0.359			
Fisher exact test		P=0.528N	P=0.556N	P=0.442

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[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 the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no neoplasms in dose group

TABLE B4
Historical Incidence of Hemangiomas or Hemangiosarcomas in Female B6C3F$_1$ Mice Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls		
	Hemangioma	Hemangiosarcoma	Hemangioma or Hemangiosarcoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	0/50	1/50	1/50
Dichlorvos	0/50	1/50	1/50
Furan	1/50	2/50	3/50
Furfural	0/50	2/50	2/50
γ-Butyrolactone	1/50	2/50	3/50
p-Nitroaniline	0/50	1/50	1/50
Pentachloroanisole	1/50	5/50	6/50
Overall Historical Incidence			
Total	4/698 (0.6%)	17/698 (2.4%)	21/698 (3.0%)
Standard deviation	0.9%	3.0%	3.5%
Range	0%-2%	0%-10%	0%-12%

[a] Data as of 3 April 1991

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	70	70	70	70
9-Month interim evaluation	9	10	9	10
15-Month interim evaluation	9	10	10	9
Early deaths				
Accidental deaths	2		3	1
Moribund	16	5	11	12
Natural deaths	5	4	5	6
Survivors				
Terminal sacrifice	29	41	32	32
Animals examined microscopically	70	70	70	70
9-Month Interim Evaluation				
Alimentary System				
Liver	(9)	(10)	(9)	(10)
Mineralization, focal				1 (10%)
Kupffer cell, pigmentation, hemosiderin				8 (80%)
Pancreas	(9)			(10)
Atrophy, focal	1 (11%)			
Duct, cyst				1 (10%)
Stomach, forestomach	(9)			(10)
Diverticulum	1 (11%)			
Cardiovascular System				
None				
Endocrine System				
Adrenal gland, cortex	(9)			(10)
Capsule, accessory adrenal cortical nodule	2 (22%)			
Thyroid gland	(9)	(10)	(9)	(10)
C-cell, hyperplasia, focal				2 (20%)
Follicle, degeneration, cystic	5 (56%)	7 (70%)	7 (78%)	9 (90%)
General Body System				
None				
Genital System				
Ovary	(9)			(10)
Cyst	2 (22%)			1 (10%)
Mineralization	1 (11%)			
Pigmentation, hemosiderin	1 (11%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
9-Month Interim Evaluation (continued)				
Genital System (continued)				
Uterus	(9)	(10)	(9)	(10)
Hydrometra			1 (11%)	
Hyperplasia, cystic		1 (10%)		
Endometrium, hyperplasia, cystic	9 (100%)	10 (100%)	9 (100%)	10 (100%)
Hematopoietic System				
Spleen	(9)	(10)	(9)	(10)
Congestion			9 (100%)	10 (100%)
Hematopoietic cell proliferation			9 (100%)	10 (100%)
Pigmentation, hemosiderin		1 (10%)	9 (100%)	10 (100%)
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(9)	(10)	(9)	(10)
Infiltration cellular, histiocyte, multifocal		3 (30%)	3 (33%)	2 (20%)
Pigmentation, multifocal, hemosiderin		2 (20%)	3 (33%)	2 (20%)
Pigmentation, multifocal		1 (10%)		
Special Senses System				
None				
Urinary System				
Kidney	(9)			(10)
Casts protein	4 (44%)			5 (50%)
Renal tubule, hyperplasia, focal				1 (10%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
15-Month Interim Evaluation				
Alimentary System				
Liver	(9)	(10)	(10)	(9)
Hematopoietic cell proliferation	1 (11%)	1 (10%)	3 (30%)	1 (11%)
Infiltration cellular, lymphocyte, multifocal	1 (11%)			1 (11%)
Necrosis, multifocal	2 (22%)		2 (20%)	
Kupffer cell, pigmentation	1 (11%)			
Kupffer cell, pigmentation, hemosiderin			1 (10%)	9 (100%)
Cardiovascular System				
None				
Endocrine System				
Adrenal gland, cortex	(9)	(1)		(9)
Accessory adrenal cortical nodule				1 (11%)
Degeneration, fatty, focal				1 (11%)
Hypertrophy, focal				1 (11%)
Pituitary gland	(8)	(1)		(9)
Congestion		1 (100%)		
Thyroid gland	(9)			(9)
Follicle, degeneration, cystic	3 (33%)			4 (44%)
General Body System				
None				
Genital System				
Ovary	(9)		(1)	(9)
Cyst	2 (22%)		1 (100%)	
Uterus	(9)	(8)	(8)	(9)
Hydrometra	4 (44%)	2 (25%)	1 (13%)	2 (22%)
Inflammation, suppurative, acute		1 (13%)		
Endometrium, hyperplasia, cystic	9 (100%)	8 (100%)	8 (100%)	9 (100%)
Hematopoietic System				
Bone marrow	(9)			(9)
Hyperplasia	1 (11%)			
Spleen	(9)	(10)	(10)	(9)
Congestion		2 (20%)	7 (70%)	9 (100%)
Hematopoietic cell proliferation	1 (11%)	3 (30%)	10 (100%)	9 (100%)
Pigmentation, hemosiderin			10 (100%)	9 (100%)
Integumentary System				
None				

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
15-Month Interim Evaluation (continued)				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(9)		(10)	(9)
Hemorrhage, focal			1 (10%)	
Infiltration cellular, lymphocyte, multifocal			2 (20%)	1 (11%)
Infiltration cellular, histiocyte, multifocal	1 (11%)		1 (10%)	1 (11%)
Pigmentation, hemosiderin, multifocal	1 (11%)		1 (10%)	1 (11%)
Alveolar epithelium, hyperplasia, focal				1 (11%)
Nose	(9)			(9)
Foreign body	1 (11%)			1 (11%)
Inflammation, suppurative, acute				2 (22%)
Nasolacrimal duct, exudate				1 (11%)
Nasolacrimal duct, inflammation, subacute				1 (11%)
Special Senses System				
None				
Urinary System				
Kidney	(9)	(1)		(9)
Casts protein	4 (44%)			4 (44%)
Infiltration cellular, lymphocyte, multifocal	4 (44%)			2 (22%)
2-Year Study				
Alimentary System				
Intestine small, duodenum	(52)	(50)	(50)	(51)
Hyperplasia			1 (2%)	
Ulcer				1 (2%)
Intestine small, jejunum	(52)	(50)	(51)	(50)
Hyperplasia				1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Liver	(52)	(50)	(51)	(51)
Angiectasis			1 (2%)	
Clear cell focus	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Cyst			2 (4%)	
Eosinophilic focus	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Hematopoietic cell proliferation			1 (2%)	
Inflammation, granulomatous	2 (4%)			
Mineralization			1 (2%)	
Mixed cell focus	2 (4%)	6 (12%)	5 (10%)	3 (6%)
Mixed cell focus, multiple	1 (2%)			1 (2%)
Necrosis, focal	1 (2%)		2 (4%)	1 (2%)
Vacuolization cytoplasmic	2 (4%)	1 (2%)		
Centrilobular, necrosis	1 (2%)			
Kupffer cell, pigmentation	1 (2%)	1 (2%)	4 (8%)	39 (76%)
Sinusoid, infiltration cellular, polymorphonuclear	1 (2%)			1 (2%)
Mesentery	(8)	(2)	(9)	(6)
Cyst	1 (13%)			1 (17%)
Hemorrhage	1 (13%)			1 (17%)
Inflammation, subacute			1 (11%)	
Fat, necrosis	4 (50%)	1 (50%)	5 (56%)	5 (83%)
Pancreas	(52)	(50)	(51)	(51)
Acinus, atrophy	5 (10%)	4 (8%)	3 (6%)	2 (4%)
Acinus, hyperplasia		2 (4%)		
Duct, dilatation	1 (2%)	2 (4%)	1 (2%)	
Stomach, forestomach	(52)	(50)	(51)	(51)
Hyperplasia	23 (44%)	16 (32%)	10 (20%)	22 (43%)
Stomach, glandular	(52)	(50)	(51)	(51)
Erosion	2 (4%)			
Hyperplasia		1 (2%)		
Artery, inflammation, subacute				1 (2%)
Tongue	(1)			
Congestion	1 (100%)			
Cardiovascular System				
Blood vessel	(1)	(1)		
Mineralization		1 (100%)		
Abdominal, thrombosis	1 (100%)			
Heart	(52)	(50)	(51)	(51)
Inflammation, subacute			2 (4%)	
Mineralization			1 (2%)	
Mineralization, multifocal	1 (2%)			
Epicardium, inflammation, acute			1 (2%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Endocrine System				
Adrenal gland, cortex	(52)	(50)	(51)	(51)
Angiectasis		1 (2%)		
Atrophy				1 (2%)
Cyst				1 (2%)
Hypertrophy, focal			1 (2%)	1 (2%)
Capsule, accessory adrenal cortical nodule	1 (2%)	1 (2%)		
Spindle cell, hyperplasia		1 (2%)		3 (6%)
X-zone, infiltration cellular, lipocyte		1 (2%)	2 (4%)	1 (2%)
Adrenal gland, medulla	(52)	(50)	(51)	(51)
Hyperplasia	2 (4%)		2 (4%)	2 (4%)
Islets, pancreatic	(52)	(50)	(51)	(51)
Hyperplasia			1 (2%)	
Pituitary gland	(50)	(50)	(49)	(48)
Pars distalis, cyst				1 (2%)
Pars distalis, hyperplasia	9 (18%)	13 (26%)	8 (16%)	9 (19%)
Thyroid gland	(52)	(50)	(51)	(51)
Inflammation, subacute			1 (2%)	
Ultimobranchial cyst	1 (2%)			
Follicle, cyst	3 (6%)	2 (4%)	5 (10%)	5 (10%)
Follicle, degeneration, cystic	17 (33%)	22 (44%)	19 (37%)	20 (39%)
Follicular cell, hyperplasia	2 (4%)	1 (2%)	6 (12%)	1 (2%)
General Body System				
None				
Genital System				
Ovary	(49)	(50)	(50)	(49)
Abscess	1 (2%)			1 (2%)
Angiectasis			1 (2%)	
Cyst	9 (18%)	15 (30%)	11 (22%)	8 (16%)
Hemorrhage	2 (4%)		1 (2%)	
Metaplasia, osseous				1 (2%)
Uterus	(52)	(50)	(51)	(51)
Angiectasis	1 (2%)			
Cyst	1 (2%)			
Dilatation	8 (15%)	25 (50%)	12 (24%)	19 (37%)
Hemorrhage	1 (2%)			
Hyperplasia, cystic	2 (4%)			
Inflammation, subacute			1 (2%)	
Endometrium, hyperplasia, cystic	50 (96%)	49 (98%)	49 (96%)	49 (96%)
Hematopoietic System				
Bone marrow	(52)	(50)	(51)	(51)
Hypercellularity	6 (12%)	4 (8%)	8 (16%)	22 (43%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node	(52)	(50)	(51)	(51)
Iliac, ectasia			1 (2%)	
Iliac, hyperplasia	1 (2%)		2 (4%)	1 (2%)
Mediastinal, hyperplasia			1 (2%)	1 (2%)
Mediastinal, inflammation, suppurative, acute	1 (2%)			
Pancreatic, hyperplasia				1 (2%)
Pancreatic, pigmentation				1 (2%)
Renal, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Lymph node, mandibular	(50)	(49)	(51)	(48)
Hyperplasia	2 (4%)		1 (2%)	
Lymph node, mesenteric	(52)	(47)	(50)	(46)
Angiectasis	1 (2%)	1 (2%)		
Hyperplasia	2 (4%)			2 (4%)
Spleen	(52)	(49)	(51)	(51)
Angiectasis				1 (2%)
Atrophy	2 (4%)			1 (2%)
Congestion			1 (2%)	
Ectopic tissue		1 (2%)		
Hematopoietic cell proliferation	45 (87%)	43 (88%)	47 (92%)	48 (94%)
Metaplasia, osseous				4 (8%)
Pigmentation	6 (12%)	23 (47%)	45 (88%)	49 (96%)
Thymus	(51)	(48)	(49)	(49)
Atrophy	2 (4%)			
Cyst		1 (2%)		
Pigmentation, cholesterol				1 (2%)
Mediastinum, foreign body			1 (2%)	
Mediastinum, hemorrhage			1 (2%)	
Mediastinum, inflammation			1 (2%)	
Integumentary System				
None				
Musculoskeletal System				
Bone	(52)	(50)	(51)	(51)
Rib, fracture				1 (2%)
Nervous System				
Brain	(52)	(49)	(51)	(51)
Hemorrhage	1 (2%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice at the 9-Month and 15-Month Interim Evaluations and in the 2-Year Gavage Study of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
2-Year Study (continued)				
Respiratory System				
Lung	(52)	(50)	(51)	(51)
Congestion	1 (2%)			
Foreign body	1 (2%)			
Hemorrhage	2 (4%)		2 (4%)	1 (2%)
Infiltration cellular, histocyte			1 (2%)	
Inflammation, granulomatous	1 (2%)			
Pigmentation		2 (4%)	1 (2%)	4 (8%)
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Mediastinum, foreign body			2 (4%)	
Mediastinum, inflammation, suppurative, acute			2 (4%)	
Pleura, fibrosis		1 (2%)		
Pleura, foreign body		1 (2%)		
Pleura, pigmentation, cholesterol		1 (2%)		
Nose	(52)	(50)	(51)	(51)
Foreign body	20 (38%)	16 (32%)	17 (33%)	10 (20%)
Fungus	1 (2%)			
Inflammation, suppurative, acute	18 (35%)	16 (32%)	12 (24%)	12 (24%)
Glands, cyst	1 (2%)			
Nasolacrimal duct, dilatation			1 (2%)	
Trachea	(52)	(50)	(51)	(51)
Inflammation, subacute		1 (2%)		
Special Senses System				
Ear	(1)			
Pinna, infarct	1 (100%)			
Eye	(1)	(2)	(1)	
Cataract		1 (50%)	1 (100%)	
Cornea, inflammation, subacute		1 (50%)		
Harderian gland	(4)	(3)	(10)	(7)
Hyperplasia			2 (20%)	1 (14%)
Urinary System				
Kidney	(52)	(50)	(51)	(51)
Metaplasia, osseous		3 (6%)		
Nephropathy, chronic	31 (60%)	29 (58%)	37 (73%)	28 (55%)
Glomerulus, amyloid deposition	1 (2%)			
Renal tubule, dilatation	1 (2%)			

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

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983). *p*-Nitroaniline was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, TA1537, or TA97) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver), for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin, and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of *p*-nitroaniline. High dose was limited by toxicity. All positive assays were repeated under the conditions which elicited the positive response.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants which is not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment.

CHINESE HAMSTER OVARY CELL CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1987) and is briefly presented below. *p*-Nitroaniline was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of *p*-nitroaniline; the high dose was limited by toxicity.

In the SCE test without S9, CHO cells were incubated for 26 hours with *p*-nitroaniline in McCoy's 5A medium supplemented with 10% fetal bovine serum, *l*-glutamine (2mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing *p*-nitroaniline was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated for 2 hours with *p*-nitroaniline in a serum-free medium containing S9. The medium was then removed and replaced with medium containing BrdU and no *p*-nitroaniline, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with *p*-nitroaniline for 10 to 12 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with *p*-nitroaniline and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 10 to 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test: if cell cycle delay was anticipated, the incubation period was extended. Cells were selected for scoring on the basis of good morphology and completeness of karyotype

(21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, usually 50 second-division metaphase cells were scored for frequency of SCEs per cell from each dose level; 100 first-division metaphase cells were scored at each dose level for the Abs test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. A single increased dose was considered weak evidence of a positive response (+w); two increased doses were sufficient to evaluate the trial as positive (+). Chromosomal aberration data are presented as percentage of cells with aberrations. Both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P<0.05$) difference for one dose point was considered weak evidence for a positive response (+w); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

Mouse Lymphoma Protocol

The experimental protocol is presented in detail by Myhr *et al.* (1985). *p*-Nitroaniline was supplied as a coded aliquot by Radian Corporation (Austin, TX). The highest dose of *p*-nitroaniline was determined by solubility or toxicity, and did not exceed 5,000 μg/mL. Mouse lymphoma L5178Y cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with 2mM *l*-glutamine, 110 μg/mL sodium pyruvate, 0.05% pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine (TFT)-resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to THG for 1 day, and then to normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added. Freshly prepared S9 metabolic activation factors were obtained from the livers of either Aroclor 1254-induced or noninduced Fischer 344/N male rats.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with *p*-nitroaniline continued for 4 hours, at which time the medium plus *p*-nitroaniline was removed and the cells were resuspended in 20 mL of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells ($TK^{-/-}$), and 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($P<0.05$) for *p*-nitroaniline to be considered capable of inducing TFT-resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988).

Drosophila Protocol

The assays for induction of mutations and chromosomal translocations were performed with adult flies as described by Valencia *et al.* (1985), and with larvae as described in Zimmering *et al.* (1989). *p*-Nitroaniline

was supplied as a coded aliquot from Radian Corporation (Austin, TX). It was assayed in the sex-linked recessive lethal (SLRL) test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, *p*-nitroaniline was retested by injection into adult males.

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

Toxicity tests were performed to set concentrations of *p*-nitroaniline at a level which would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test in adults, oral exposure was achieved by allowing Canton-S males (10 to 20 flies/vial) to feed for 72 hours on a solution of *p*-nitroaniline in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of *p*-nitroaniline dissolved in 0.7% saline and were allowed to recover for 24 hours. For the larval feeding experiment, Canton-S females and males were mated and eggs in vials were exposed to standard cornmeal food containing *p*-nitroaniline in solvent (5% ethanol) or solvent alone (Valencia *et al.*, 1989). Adult emergent males were mated at approximately 24 hours of age with two successive harems of three to five *Basc* females to establish two single-day broods. In the adult exposures, treated males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; in each case, sample sperm from successive matings were treated at successively earlier post-meiotic stages. F_1 heterozygous females were allowed to mate with their siblings and were then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution. If a cluster was identified, all data from the male in question were discarded. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; the females in these vials were retested.

Recessive lethal data were analyzed by the normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered to be positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater than 0.10%, or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15%, or (b) the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

RESULTS

p-Nitroaniline is mutagenic *in vitro*. It was tested (at doses up to 6,666 μg/plate) in two laboratories for induction of gene mutations in several strains of *Salmonella typhimurium* using a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. Both laboratories showed positive results, with and without S9, in strain TA98; a stronger response was seen with S9. Negative results were obtained, with and without S9, in strains TA100, TA1535, TA1537, and TA97 (Table C1; Haworth *et al.*, 1983).

p-Nitroaniline was tested in two laboratories for induction of SCEs (Table C2) and Abs (Table C3) in CHO cells, with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9. In the SCE study, one laboratory (Columbia University) reported negative results in the absence of S9 and positive results with S9, with an effective dose range of 1,600 to 3,000 μg/mL (Galloway *et al.*, 1987). The second laboratory (Environmental Health Research and Testing, Inc.) performed two trials without S9: results of the first trial were weakly positive and the second trial, which showed no significant induction of SCEs, was negative; the results were therefore considered to be equivocal because the initially observed positive response at the high dose did not repeat. In contrast to the results obtained at Columbia in the SCEs study, EHRT reported negative results with *p*-nitroaniline in the presence of S9; the highest dose tested was 5,000 μg/mL.

In the Abs study (Table C3), both testing laboratories obtained positive results with *p*-nitroaniline in the presence of S9. The laboratory at Columbia University reported weakly positive results without S9 at an effective dose of 1,600 μg/mL (Galloway *et al.*, 1987) while EHRT reported negative results without S9 (highest scorable dose, 800 μg/mL).

p-Nitroaniline induced TFT resistance in L5178Y mouse lymphoma cells in the absence of S9; results with S9 were considered to be negative (Table C4). In this assay, *p*-nitroaniline must remain soluble for the duration of the exposure time. Therefore, the positive responses shown for the dose levels at which *p*-nitroaniline precipitation occurred were not included in the evaluation of the experiment (see Trial 1 with S9, for example).

p-Nitroaniline did not induce SLRL mutations in germ cells of male *Drosophila melanogaster* (Table C5) when administered by feeding (5,000 ppm) or by injection (1,000 ppm) to adult males (Valencia *et al.*, 1985), or by feeding (100 ppm) to larvae (Zimmering *et al.*, 1989).

TABLE C1
Mutagenicity of *p*-Nitroaniline in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9	+10% hamster S9	+10% rat S9
Study Performed at SRI, International				
TA100	0	133 ± 1.8	120 ± 2.1	110 ± 3.3
	100	102 ± 3.4	120 ± 11.9	128 ± 6.4
	333	102 ± 17.4	123 ± 12.5	124 ± 17.1
	1,000	112 ± 8.9	138 ± 6.0	132 ± 9.2
	3,333	83 ± 11.0	91 ± 8.3	95 ± 3.7
	6,666	20 ± 8.6	33 ± 14.6	34 ± 5.0
Trial summary		Negative	Negative	Negative
Positive control[c]		379 ± 15.4	1,841 ± 76.4	777 ± 12.8
TA1535	0	22 ± 3.8	10 ± 0.6	14 ± 0.3
	100	22 ± 5.9	9 ± 0.5	11 ± 0.9
	333	22 ± 4.2	11 ± 0.3	9 ± 1.5
	1,000	26 ± 3.0	11 ± 2.3	11 ± 2.0
	3,333	15 ± 3.1	8 ± 1.3	8 ± 1.5
	6,666	8 ± 3.2	5 ± 2.0	3 ± 0.9
Trial summary		Negative	Negative	Negative
Positive control		383 ± 22.4	469 ± 18.1	233 ± 22.2
TA97	0	122 ± 9.2	145 ± 8.0	146 ± 13.6
	100	128 ± 2.5	158 ± 8.4	179 ± 9.4
	333	119 ± 5.0	169 ± 2.8	173 ± 7.0
	1,000	137 ± 7.5	179 ± 3.1	163 ± 4.4
	3,333	105 ± 9.3	168 ± 13.0	117 ± 20.6
	6,666	12 ± 6.0[d]	31 ± 14.8[d]	12 ± 4.7[d]
Trial summary		Negative	Negative	Negative
Positive control		810 ± 8.3	1,190 ± 15.0	1,194 ± 27.5

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	22 ± 1.3	23 ± 2.5	27 ± 2.5	49 ± 0.6	29 ± 0.6	46 ± 6.9
	100	26 ± 3.3	31 ± 3.5	46 ± 2.6	53 ± 5.9	39 ± 0.9	57 ± 3.3
	333	30 ± 1.5	27 ± 3.2	70 ± 3.6	92 ± 8.6	57 ± 6.3	73 ± 0.6
	1,000	51 ± 2.3	41 ± 7.5	105 ± 7.8	140 ± 3.8	95 ± 6.4	104 ± 18.3
	3,333	117 ± 6.4	98 ± 2.5	191 ± 9.9	208 ± 12.7	179 ± 2.4	197 ± 27.2
	6,666	84 ± 19.1	78 ± 7.6	171 ± 15.9	174 ± 36.3	169 ± 35.5	290 ± 18.6
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control		1,007 ± 41.6	497 ± 16.2	770 ± 18.3	1,126 ± 41.6	369 ± 9.0	768 ± 25.8

TABLE C1
Mutagenicity of *p*-Nitroaniline in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	Revertants/plate +10% hamster S9	Revertants/plate +10% rat S9
Study Performed at EG&G Mason Research Institute				
TA100	0	120 ± 1.7	147 ± 9.5	154 ± 3.2
	100	125 ± 3.8	166 ± 9.1	156 ± 4.0
	333	124 ± 2.2	167 ± 3.0	167 ± 4.3
	1,000	111 ± 3.0	161 ± 3.5	166 ± 5.3
	3,333	88 ± 4.2	131 ± 5.9	134 ± 9.4
	6,666	15 ± 2.5	38 ± 4.4	19 ± 0.6
Trial summary		Negative	Negative	Negative
Positive control		1,182 ± 20.9	1,292 ± 40.3	1,165 ± 74.0
TA1535	0	26 ± 1.8	13 ± 4.1	17 ± 2.5
	100	23 ± 2.9	10 ± 2.4	14 ± 1.0
	333	17 ± 0.6	9 ± 1.0	14 ± 1.5
	1,000	24 ± 1.2	8 ± 1.2	8 ± 0.3
	3,333	20 ± 3.7	11 ± 0.7	10 ± 2.9
	6,666	8 ± 0.9	6 ± 3.3	6 ± 1.8
Trial summary		Negative	Negative	Negative
Positive control		910 ± 9.0	73 ± 4.9	69 ± 4.3

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	Revertants/plate -S9 Trial 2	Revertants/plate +10% hamster S9 Trial 1	Revertants/plate +10% hamster S9 Trial 2	Revertants/plate +10% rat S9 Trial 1	Revertants/plate +10% rat S9 Trial 2
TA1537	0	7 ± 1.3	4 ± 1.5	5 ± 0.9	11 ± 1.0	6 ± 0.6	9 ± 2.2
	100	8 ± 0.7		8 ± 1.9		6 ± 1.2	
	333	4 ± 1.2	8 ± 2.6	11 ± 1.3	14 ± 1.3	10 ± 1.5	10 ± 0.9
	1,000	11 ± 1.5	14 ± 1.2	11 ± 2.9	15 ± 2.5	7 ± 1.7	10 ± 1.2
	2,000		14 ± 1.7		16 ± 1.7		8 ± 2.0
	3,333	11 ± 2.0	11 ± 2.2	13 ± 0.9	17 ± 1.3	10 ± 1.0	9 ± 2.7
	4,000		13 ± 0.9		13 ± 0.3		16 ± 1.8
	6,666	9 ± 1.3	10 ± 3.2	4 ± 0.6	6 ± 0.9	7 ± 0.6	8 ± 3.7
Trial summary		Negative	Equivocal	Equivocal	Negative	Negative	Negative
Positive control		369 ± 59.9	511 ± 68.0	57 ± 5.7	92 ± 19.4	74 ± 8.5	68 ± 7.2

TABLE C1
Mutagenicity of *p*-Nitroaniline in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	14 ± 0.9	16 ± 0.6	27 ± 1.5	30 ± 5.4	26 ± 2.0	28 ± 2.2
	10		16 ± 2.4		28 ± 5.6		26 ± 2.7
	100	18 ± 3.3		46 ± 3.5		31 ± 1.0	
	333	18 ± 0.7	24 ± 5.0	61 ± 4.1	54 ± 2.7	44 ± 1.7	37 ± 3.0
	1,000	43 ± 3.0	53 ± 7.3	103 ± 6.4	102 ± 7.9	74 ± 7.5	73 ± 5.9
	3,333	63 ± 7.4	62 ± 7.1	145 ± 7.5	197 ± 6.4	126 ± 10.1	158 ± 5.9
	6,666	33 ± 6.7	24 ± 4.8	53 ± 1.7	34 ± 3.5	58 ± 2.6	58 ± 3.5
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control		1,362 ± 49.8	1,326 ± 50.3	1,209 ± 47.7	1,105 ± 18.2	1,053 ± 9.9	707 ± 42.7

[a] The detailed protocol and the data from the EG&G Mason Research Institute are presented in Haworth *et al.* (1983). Cells and *p*-nitroaniline or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity. 0 μg/plate dose is the solvent control.

[b] Revertants are presented as mean ± standard error from three plates.

[c] 2-Aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537 and TA97.

[d] Slight toxicity

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitroaniline[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[b]
Study Performed at Columbia University								
-S9[c]								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,045	444	0.42	8.9	26.0	
Mitomycin-C	0.005	25	524	572	1.09	22.9	26.0	156.92
p-Nitroaniline	16	50	1,040	448	0.43	9.0	26.0	1.38
	50	50	1,048	457	0.43	9.1	26.0	2.63
	160	58	1,213	534	0.44	9.2	26.0	3.61
								P=0.281[d]
+S9[e]								
Trial 1								
Summary: Weak Positive								
Dimethylsulfoxide		50	1,049	469	0.44	9.4	26.0	
Cyclophosphamide	1	25	523	490	0.93	19.6	26.0	109.56
p-Nitroaniline	160	50	1,048	457	0.43	9.1	26.0	-2.47
	500	50	1,045	488	0.46	9.8	26.0	4.45
	1,600	50	1,046	563	0.53	11.3	26.0	20.39*
								P=0.001
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,048	515	0.49	10.3	26.0	
Cyclophosphamide	1	50	1,051	900	0.85	18.0	26.0	74.26
p-Nitroaniline	2,000	50	1,037	721	0.69	14.4	26.0	41.48*
	2,500	50	1,050	694	0.66	13.9	28.0[f]	34.50*
	3,000	50	1,048	702	0.66	14.0	28.0[f]	36.31*
								P<0.001

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitroaniline (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
Study Performed at Environmental Health Research and Testing								
-S9								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,037	428	0.41	8.6	26.0	
Mitomycin-C	0.01	50	1,036	1,761	1.69	35.2	26.0	311.85
p-Nitroaniline	0.5	50	1,027	392	0.38	7.8	26.0	–7.52
	1.6	50	1,027	414	0.40	8.3	26.0	–2.33
	5	50	1,035	443	0.42	8.9	26.0	3.70
	16	50	1,027	415	0.40	8.3	26.0	–2.09
	50	50	1,032	445	0.43	8.9	26.0	4.48
	160	50	1,046	571	0.54	11.4	26.0	32.26*
								P<0.001
Trial 2								
Summary: Negative								
Dimethylsulfoxide		50	1,041	479	0.46	9.6	26.0	
Mitomycin-C	0.005	50	1,050	1,393	1.32	27.9	26.0	188.32
	0.010	50	1,045	2,049	1.96	41.0	26.0	326.13
p-Nitroaniline	50	50	1,040	440	0.42	8.8	26.0	–8.06
	100	50	1,037	485	0.46	9.7	26.0	1.64
	200	0					26.0	
								P=0.399

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitroaniline (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,043	501	0.48	10.0	26.0	
Cyclophosphamide	2	50	1,045	2,671	2.55	53.4	26.0	432.12
p-Nitroaniline	16	50	1,045	472	0.45	9.4	26.0	–5.97
	50	50	1,040	466	0.44	9.3	26.0	–6.72
	160	50	1,036	458	0.44	9.2	26.0	–7.97
	500	50	1,036	460	0.44	9.2	26.0	–7.56
	1,600	50	1,049	563	0.53	11.3	26.0	11.73
	5,000	50	1,031	550	0.53	11.0	26.0	11.06
								P=0.002
Trial 2								
Summary: Negative								
Dimethylsulfoxide		50	1,046	434	0.41	8.7	26.0	
Cyclophosphamide	1.5	50	1,044	1,894	1.81	37.9	26.0	337.24
	2	50	1,049	3,083	2.93	61.7	26.0	608.34
p-Nitroaniline	160	50	1,048	419	0.39	8.4	26.0	–3.64
	500	50	1,048	436	0.41	8.7	26.0	0.27
	1,000	0					26.0	
								P=0.484
Trial 3								
Summary: Questionable								
Dimethylsulfoxide		50	1,043	513	0.49	10.3	26.0	
Cyclophosphamide	2	50	1,043	2,345	2.24	46.9	26.0	357.11
p-Nitroaniline	250	50	1,038	508	0.48	10.2	31.0[f]	–0.50
	500	50	1,044	540	0.51	10.8	31.0[f]	5.16
	750	50	1,029	604	0.58	12.1	31.0[f]	19.34
	1,000	50	1,039	566	0.54	11.3	31.0[f]	10.76
								P=0.001

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitroaniline (continued)

* Positive (>20% increase over solvent control)
[a] SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and the data from the study performed at Columbia University are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *p*-nitroaniline or solvent (dimethylsulfoxide) as described in [c] and [e] below, and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.
[b] SCEs/chromosome of culture exposed to *p*-nitroaniline relative to those of culture exposed to solvent.
[c] In the absence of S9, cells were incubated with *p*-nitroaniline or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 hours.
[d] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose
[e] In the presence of S9, cells were incubated with *p*-nitroaniline or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with Colcemid present for the final 2 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.
[f] Because *p*-nitroaniline induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *p*-Nitroaniline[a]

-S9[b]					+S9[c]				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Study Performed at Columbia University									
Trial 1 – Harvest time: 14.0 hours Summary: Weak positive					Trial 1 – Harvest time: 14.0 hours Summary: Positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	2	0.02	2.0		100	3	0.03	3.0
Mitomycin-C					Cyclophosphamide				
0.05	100	21	0.21	17.0	150	100	22	0.22	18.0
0.15	100	24	0.24	22.0					
p-Nitroaniline					*p*-Nitroaniline				
50	100	6	0.06	6.0	160	100	8	0.08	8.0
160	100	5	0.05	5.0	500	100	8	0.08	7.0
500	100	7	0.07	7.0	1,600	100	26	0.26	20.0*
1,600	100	11	0.11	10.0*	5,000	100	31	0.31	22.0*
				P=0.012[d]					P<0.001
Study Performed at Environmental Health Research & Testing									
Trial 1 – Harvest time: 12.0 hours Summary: Negative					Trial 1 - Harvest time: 12.0 hours Summary: Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0.00	0.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.25	100	24	0.24	19.0	50	100	136	1.36	61.0
p-Nitroaniline					*p*-Nitroaniline				
16	100	0	0.00	0.0	16	100	0	0.00	0.0
50	100	0	0.00	0.0	50	100	2	0.02	2.0
160	100	0	0.00	0.0	160	100	2	0.02	2.0
500	100	1	0.01	1.0	500	100	3	0.03	3.0
					1,600	100	11	0.11	11.0*
				P=0.079					P<0.001

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *p*-Nitroaniline (continued)

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 2 - Harvest time: 12.8 hours Summary: Negative					**Trial 2** - Harvest time: 12.0 hours Summary: Questionable				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0.0	0.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.5	100	33	0.33	28.0	50	100	183	1.83	71.0
p-Nitroaniline					*p*-Nitroaniline				
100	100	0	0.00	0.0	200	100	1	0.01	1.0
200	100	3	0.03	2.0	400	100	5	0.05	5.0*
400	100	0	0.00	0.0	600	100	2	0.02	2.0
600	100	0	0.00	0.0	800	100	5	0.05	4.0
800	100	2	0.02	2.0	1,200	0			
1,200	0								
				P=0.152					P=0.023
					Trial 3 - Harvest time: 15.0 hours[e] Summary: Weak positive				
					Dimethylsulfoxide				
						100	0	0.00	0.0
					Cyclophosphamide				
					50	100	183	1.83	71.0
					p-Nitroaniline				
					400	100	3	0.03	3.0
					600	100	1	0.01	1.0
					800	100	0	0.00	0.0
					1,200[f]	100	60	0.60	42.0*
									P<0.001

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *p*-Nitroaniline (continued)

-S9 Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	+S9 Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
					Trial 4 - Harvest time: 12.5 hours				
					Summary: Weak positive				
					Dimethylsulfoxide				
						100	3	0.03	3.0
					Cyclophosphamide				
					25	100	76	0.76	45.0
					p-Nitroaniline				
					400	100	0	0.00	0.0
					600	100	3	0.03	3.0
					800	100	2	0.02	2.0
					1,000	100	81	0.81	74.0*
									P<0.001
					Trial 5 - Harvest time: 22.0 hours[e]				
					Summary: Positive				
					Dimethylsulfoxide				
						100	2	0.02	2.0
					Cyclophosphamide				
					50	100	209	2.09	90.0
					p-Nitroaniline				
					400	100	4	0.04	3.0
					600	100	4	0.04	4.0
					800	100	0	0.00	0.0
					1,000	100	1	0.01	1.0
					1,200	100	3	0.03	3.0
					1,600	100	120	1.20	73.0*
					2,000	100	77	0.77	63.0*
									P<0.001

* Positive (P<0.05)

[a] Abs=aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1987). The data from the Columbia University study are presented in Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *p*-nitroaniline or solvent (dimethylsulfoxide) as indicated in [b] and [c]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[b] In the absence of S9, cells were incubated with *p*-nitroaniline or solvent for 10 to 12 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 hours followed by harvest.

[c] In the presence of S9, cells were incubated with *p*-nitroaniline or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 10 to 12 hours. Colcemid was added for the last 2 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[d] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

[e] Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.

[f] Harvest time = 18 hours

TABLE C4
Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitroaniline[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Acetone		101	94	164	54	
		107	106	135	42	48
Ethyl methanesulfonate		70	67	997	475	
	250	89	56	988	369	
		99	67	1,242	418	421[c]
p-Nitroaniline	15.6	91	69	157	58	
		101	78	132	44	
		105	74	152	48	50
	31.3	99	91	140	47	
		98	81	147	50	
		108	87	175	54	50
	62.5	102	96	128	42	
	125	97	65	167	57	
		107	87	163	51	54
	250	102	68	184	60	
		111	80	139	42	51
	500[d]	91	32	285	105	
		105	10	531	169	137[c]
	1,000	Lethal				
		Lethal				
		Lethal				

TABLE C4
Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitroaniline (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Acetone		72	94	46	21	
		104	79	85	27	
		92	124	60	22	
		102	104	62	20	23
Ethyl methanesulfonate		68	60	644	314	
	250	70	52	683	324	
		71	68	711	335	324[c]
p-Nitroaniline	15.6	75	54	88	39	
		57	62	64	38	
		74	81	62	28	35[c]
	31.3	78	80	70	30	
		58	64	56	32	
		59	58	40	23	28
	62.5	57	60	49	29	
		59	52	70	39	
		81	69	61	25	31
	125	65	36	88	45	
		66	58	57	29	
		84	47	71	28	34
	250	65	12	170	87	
		71	21	192	90	
		68	26	135	66	81[c]
	500[d]	63	8	217	115	
		Lethal				
		Lethal				

TABLE C4
Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitroaniline (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 3						
Acetone		67	94	56	28	
		72	97	96	44	
		79	110	96	41	
		76	99	46	20	33
Ethyl methanesulfonate		64	71	803	417	
	250	70	72	839	401	
		56	74	630	375	398[c]
p-Nitroaniline	50	58	64	106	61	
		51	66	58	38	
		60	77	66	37	45
	100	71	78	47	22	
		89	85	91	34	
		72	78	65	30	29
	200	92	61	88	32	
		79	52	127	54	
		80	57	150	63	49
	300	52	20	195	125	
		60	12	132	74	
		54	16	146	90	96[c]
	400	Lethal				
		Lethal				
		Lethal				

TABLE C4
Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitroaniline (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9[e]						
Trial 1						
Acetone		67	99	86	43	
		70	95	60	29	
		88	106	78	30	34
Methylcholanthrene		91	101	464	170	
	2.5	118	100	507	144	
		94	93	465	164	159[c]
p-Nitroaniline	25	77	97	72	31	
		68	111	69	34	
		60	109	67	37	34
	50	73	83	108	49	
		88	88	107	41	
		64	96	98	51	47
	100	62	55	104	56	
		71	67	74	35	
		92	101	166	60	50
	200	71	70	96	45	
		74	67	107	48	
		70	82	99	47	47
	300[d]	79	48	122	51	
		51	35	102	67	
		74	57	126	57	58[c]
	500	77	53	162	71	
		80	15	245	102	
		82	12	346	141	105[c]

[a] Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr *et al.* (1985). The highest dose of *p*-nitroaniline is determined by solubility or toxicity and may not exceed 5,000 μg/mL. All doses are tested in triplicate; the average of the three tests is presented in the table. Cells (6×10^5/mL) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

[b] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 $\times 10^6$ cells treated).

[c] Positive response ($P<0.05$)

[d] Precipitate formed at this and all higher doses. Responses at these doses are presented, but are not used for statistical evaluation.

[e] Tests conducted with metabolic activation were performed as described in [a] except that S9, prepared from the livers of Aroclor 1254-induced Fischer 344 rats, was added at the same time as *p*-nitroaniline and/or solvent.

TABLE C5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by *p*-Nitroaniline[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethal/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[b]
Injection	500	0	0	0/1,378	0/1,315	2/1,205	2/3,898 (0.05%)
	0			0/1,993	3/1,926	1/1,766	4/5,685 (0.07%)
Injection	1,000	6	9	2/2,197	0/1,885	1/1,257	3/5,339 (0.06%)
	0			0/2,334	1/2,179	1/1,669	2/6,182 (0.03%)
Feeding	3,333	0	3	1/1,087	0/1,069	1/1,092	2/3,248 (0.06%)
	0			0/1,084	3/1,082	0/1,082	3/3,248 (0.09%)
Feeding	5,000	0	3	0/1,114	2/1,003	0/986	2/3,103 (0.06%)
	0			0/1,148	0/1,097	0/1,075	0/3,320 (0.00%)
Larval feeding	100	60	0	3/2,561	3/2,551	0/000	6/5,112 (0.12%)
	0			2/2,511	2/2,538	0/000	4/5,049 (0.08%)

[a] Study performed at University of Wisconsin, Madison. A detailed protocol of the sex-linked recessive lethal assay with adult flies and these data are presented in Valencia *et al.* (1985). The protocol and data from the larva feeding study are presented in Zimmering *et al.* (1989). Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; clusters were removed from the solvent control trials in the injection and larval feeding experiments. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were not significant at the 5% level (Margolin *et al.*, 1983).

[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials

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

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Male					
n	5	4	5	3	5
Necropsy body wt	27.0 ± 0.7	27.0 ± 0.5	28.1 ± 0.7	26.5 ± 0.7	26.4 ± 0.5
Brain					
Absolute	0.464 ± 0.012	0.490 ± 0.011	0.476 ± 0.012	0.453 ± 0.032	0.474 ± 0.009
Relative	17.20 ± 0.61	18.17 ± 0.59	16.97 ± 0.53	17.13 ± 1.40	17.96 ± 0.57
Heart					
Absolute	0.146 ± 0.003	0.152 ± 0.006	0.155 ± 0.007	0.152 ± 0.013	0.168 ± 0.004*
Relative	5.40 ± 0.21	5.61 ± 0.21	5.50 ± 0.16	5.71 ± 0.43	6.35 ± 0.16**
R. Kidney					
Absolute	0.253 ± 0.012	0.262 ± 0.008	0.263 ± 0.012	0.248 ± 0.010	0.262 ± 0.015
Relative	9.36 ± 0.30	9.70 ± 0.19	9.37 ± 0.47	9.34 ± 0.32	9.91 ± 0.59
Liver					
Absolute	1.542 ± 0.049	1.553 ± 0.061	1.548 ± 0.053	1.433 ± 0.067	1.400 ± 0.061
Relative	57.09 ± 1.65	57.48 ± 1.89	55.13 ± 1.66	53.98 ± 1.44	52.95 ± 2.12
Lungs					
Absolute	0.252 ± 0.018	0.253 ± 0.019	0.254 ± 0.007	0.253 ± 0.008	0.237 ± 0.007
Relative	9.30 ± 0.57	9.37 ± 0.63	9.08 ± 0.36	9.57 ± 0.47	8.95 ± 0.28
Spleen					
Absolute	0.121 ± 0.013	0.118 ± 0.009	0.143 ± 0.012	0.191 ± 0.026**	0.359 ± 0.015**
Relative	4.46 ± 0.38	4.37 ± 0.27	5.06 ± 0.31	7.16 ± 0.81**	13.58 ± 0.41**
R. Testis					
Absolute	0.104 ± 0.006	0.105 ± 0.004	0.113 ± 0.003	0.104 ± 0.008	0.110 ± 0.005
Relative	3.84 ± 0.13	3.89 ± 0.20	4.02 ± 0.09	3.93 ± 0.42	4.16 ± 0.22
Thymus					
Absolute	0.064 ± 0.002	0.057 ± 0.007	0.071 ± 0.006	0.052 ± 0.003	0.051 ± 0.005
Relative	2.37 ± 0.14	2.11 ± 0.30	2.51 ± 0.17	1.95 ± 0.10	1.93 ± 0.21

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Female					
n	5	4	4	5	4
Necropsy body wt	22.1 ± 0.2	21.0 ± 0.3	22.9 ± 0.2	22.0 ± 0.4	23.0 ± 0.5
Brain					
Absolute	0.474 ± 0.019	0.468 ± 0.013	0.505 ± 0.013	0.482 ± 0.016	0.493 ± 0.014
Relative	21.47 ± 0.93	22.32 ± 0.60	22.10 ± 0.78	21.93 ± 0.44	21.42 ± 0.56
Heart					
Absolute	0.132 ± 0.005	0.134 ± 0.011	0.139 ± 0.009	0.145 ± 0.012	0.133 ± 0.004
Relative	5.98 ± 0.24	6.37 ± 0.48	6.06 ± 0.33	6.62 ± 0.59	5.76 ± 0.07
R. Kidney					
Absolute	0.190 ± 0.007	0.190 ± 0.002	0.193 ± 0.007	0.187 ± 0.014	0.193 ± 0.003
Relative	8.62 ± 0.31	9.07 ± 0.15	8.42 ± 0.37	8.51 ± 0.55	8.41 ± 0.20
Liver					
Absolute	1.136 ± 0.047	1.100 ± 0.053	1.190 ± 0.032	1.138 ± 0.044	1.173 ± 0.059
Relative	51.36 ± 1.74	52.49 ± 2.29	52.00 ± 0.94	51.78 ± 1.46	50.96 ± 2.17
Lungs					
Absolute	0.235 ± 0.011[b]	0.254 ± 0.005	0.243 ± 0.008	0.232 ± 0.011	0.242 ± 0.011
Relative	10.65 ± 0.55[b]	12.13 ± 0.31	10.63 ± 0.40	10.56 ± 0.35	10.55 ± 0.66
Spleen					
Absolute	0.109 ± 0.018	0.118 ± 0.011	0.131 ± 0.013	0.184 ± 0.015**	0.300 ± 0.020**
Relative	4.91 ± 0.81	5.61 ± 0.47	5.74 ± 0.56	8.34 ± 0.57**	13.06 ± 0.90**
Thymus					
Absolute	0.062 ± 0.006	0.069 ± 0.005	0.065 ± 0.010	0.071 ± 0.007	0.069 ± 0.004
Relative	2.78 ± 0.24	3.28 ± 0.20	2.82 ± 0.43	3.25 ± 0.30	3.01 ± 0.17

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=4

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 7-Week Interim Evaluations in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	9	8	8	9	9	8
Necropsy body wt	28.7 ± 0.6	29.9 ± 0.5	29.8 ± 0.6	29.5 ± 0.5	29.2 ± 0.8	28.4 ± 0.5
Brain						
Absolute	0.466 ± 0.010	0.479 ± 0.011	0.472 ± 0.007	0.469 ± 0.008	0.468 ± 0.009	0.462 ± 0.009
Relative	16.27 ± 0.39	16.03 ± 0.27	15.90 ± 0.32	15.93 ± 0.34	16.10 ± 0.55	16.29 ± 0.28
Heart						
Absolute	0.146 ± 0.005	0.163 ± 0.006	0.164 ± 0.007	0.151 ± 0.006	0.157 ± 0.006	0.159 ± 0.007
Relative	5.06 ± 0.10	5.43 ± 0.13	5.52 ± 0.28	5.12 ± 0.18	5.37 ± 0.16	5.58 ± 0.17
R. Kidney						
Absolute	0.233 ± 0.012	0.276 ± 0.009*	0.274 ± 0.007*	0.259 ± 0.008	0.260 ± 0.015	0.245 ± 0.009
Relative	8.10 ± 0.29	9.24 ± 0.18*	9.19 ± 0.09*	8.80 ± 0.30	8.90 ± 0.43	8.62 ± 0.21
Liver						
Absolute	1.404 ± 0.043	1.374 ± 0.044	1.564 ± 0.078	1.460 ± 0.028	1.576 ± 0.046	1.488 ± 0.049
Relative	48.92 ± 1.10	45.98 ± 1.00	52.63 ± 2.69	49.58 ± 0.95	53.96 ± 0.84*	52.39 ± 1.28*
Lungs						
Absolute	0.228 ± 0.009	0.233 ± 0.005	0.253 ± 0.006	0.251 ± 0.009	0.246 ± 0.007	0.226 ± 0.005
Relative	7.96 ± 0.25	7.81 ± 0.13	8.52 ± 0.20	8.53 ± 0.31	8.46 ± 0.31	7.97 ± 0.11
Spleen						
Absolute	0.087 ± 0.004	0.084 ± 0.003	0.087 ± 0.004[b]	0.106 ± 0.009	0.142 ± 0.008**	0.200 ± 0.010**
Relative	3.02 ± 0.14	2.82 ± 0.11	2.91 ± 0.17[b]	3.64 ± 0.37	4.88 ± 0.28**	7.04 ± 0.30**
R. Testis						
Absolute	0.103 ± 0.004	0.111 ± 0.002	0.106 ± 0.005[b]	0.106 ± 0.003	0.108 ± 0.003	0.108 ± 0.005
Relative	3.59 ± 0.11	3.73 ± 0.05	3.53 ± 0.11[b]	3.59 ± 0.10	3.70 ± 0.11	3.80 ± 0.20
Thymus						
Absolute	0.052 ± 0.004	0.055 ± 0.003	0.047 ± 0.004	0.049 ± 0.004	0.046 ± 0.003	0.049 ± 0.004
Relative	1.83 ± 0.16	1.84 ± 0.09	1.58 ± 0.14	1.66 ± 0.14	1.58 ± 0.10	1.72 ± 0.12

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 7-Week Interim Evaluations in the 13-Week Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Female						
n	10	10	9	10	10	10
Necropsy body wt	24.7 ± 0.2	25.0 ± 0.2	24.8 ± 0.2	24.5 ± 0.3	25.3 ± 0.2	25.7 ± 0.4
Brain						
Absolute	0.479 ± 0.007	0.488 ± 0.009	0.482 ± 0.007	0.474 ± 0.007	0.487 ± 0.011	0.484 ± 0.010
Relative	19.36 ± 0.26	19.55 ± 0.36	19.42 ± 0.22	19.41 ± 0.37	19.31 ± 0.48	18.86 ± 0.33
Heart						
Absolute	0.135 ± 0.002	0.147 ± 0.005	0.134 ± 0.004	0.140 ± 0.005	0.138 ± 0.003	0.146 ± 0.004
Relative	5.46 ± 0.07	5.88 ± 0.17	5.41 ± 0.14	5.73 ± 0.22	5.46 ± 0.12	5.69 ± 0.10
R. Kidney						
Absolute	0.197 ± 0.003	0.196 ± 0.004	0.197 ± 0.004	0.193 ± 0.004	0.199 ± 0.004	0.205 ± 0.006
Relative	7.96 ± 0.12	7.85 ± 0.14	7.91 ± 0.14	7.89 ± 0.15	7.88 ± 0.17	7.99 ± 0.18
Liver						
Absolute	1.179 ± 0.029	1.227 ± 0.018	1.248 ± 0.033	1.265 ± 0.036	1.306 ± 0.035**	1.384 ± 0.038**
Relative	47.64 ± 1.04	49.18 ± 0.82	50.19 ± 1.09	51.67 ± 1.13**	51.65 ± 1.20**	53.89 ± 0.96**
Lungs						
Absolute	0.224 ± 0.002	0.218 ± 0.006	0.218 ± 0.005	0.216 ± 0.004	0.214 ± 0.014	0.219 ± 0.007
Relative	9.07 ± 0.09	8.73 ± 0.26	8.77 ± 0.18	8.84 ± 0.21	8.45 ± 0.54	8.53 ± 0.23
Spleen						
Absolute	0.105 ± 0.005	0.106 ± 0.002	0.113 ± 0.004	0.117 ± 0.003	0.177 ± 0.012**	0.233 ± 0.011**
Relative	4.24 ± 0.19	4.23 ± 0.07	4.56 ± 0.18	4.78 ± 0.16	7.00 ± 0.47**	9.08 ± 0.45**
Thymus						
Absolute	0.051 ± 0.002	0.050 ± 0.003	0.046 ± 0.002	0.053 ± 0.003	0.055 ± 0.003	0.051 ± 0.003
Relative	2.06 ± 0.08	2.00 ± 0.10	1.86 ± 0.08	2.18 ± 0.11	2.19 ± 0.13	1.98 ± 0.10

° Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
°° P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=7

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	9	11	8	9	10	9
Necropsy body wt	32.9 ± 0.8	34.0 ± 0.6	31.9 ± 0.7	35.0 ± 0.6	32.4 ± 0.6	33.0 ± 0.7
Brain						
Absolute	0.485 ± 0.008	0.482 ± 0.005	0.475 ± 0.010	0.465 ± 0.010	0.472 ± 0.009	0.468 ± 0.006
Relative	14.77 ± 0.31	14.20 ± 0.27	14.93 ± 0.45	13.32 ± 0.33*	14.59 ± 0.39	14.24 ± 0.29
Heart						
Absolute	0.181 ± 0.006	0.180 ± 0.005	0.163 ± 0.009	0.183 ± 0.006	0.184 ± 0.007	0.164 ± 0.006
Relative	5.51 ± 0.17	5.29 ± 0.10	5.08 ± 0.21	5.24 ± 0.13	5.67 ± 0.20	4.97 ± 0.09
R. Kidney						
Absolute	0.298 ± 0.014	0.282 ± 0.010	0.308 ± 0.015	0.321 ± 0.008	0.307 ± 0.010	0.274 ± 0.010
Relative	9.03 ± 0.31	8.27 ± 0.21	9.67 ± 0.50	9.19 ± 0.23	9.46 ± 0.24	8.31 ± 0.18
Liver						
Absolute	1.614 ± 0.058	1.469 ± 0.033	1.508 ± 0.041	1.712 ± 0.046	1.649 ± 0.033	1.483 ± 0.047
Relative	49.01 ± 1.20	43.15 ± 0.53**	47.26 ± 0.79	48.93 ± 0.72	50.92 ± 1.04	44.91 ± 0.73**
Lungs						
Absolute	0.264 ± 0.013	0.256 ± 0.010	0.270 ± 0.007	0.328 ± 0.020*	0.303 ± 0.021	0.245 ± 0.006
Relative	7.99 ± 0.28	7.53 ± 0.25	8.47 ± 0.24	9.35 ± 0.49	9.35 ± 0.63	7.43 ± 0.14
Spleen						
Absolute	0.091 ± 0.002[b]	0.075 ± 0.003	0.084 ± 0.004	0.105 ± 0.004	0.147 ± 0.007**	0.239 ± 0.008**
Relative	2.82 ± 0.07[b]	2.21 ± 0.09	2.64 ± 0.13	3.00 ± 0.11	4.53 ± 0.25**	7.27 ± 0.26**
R. Testis						
Absolute	0.112 ± 0.004	0.109 ± 0.002	–[c]	0.112 ± 0.002	–	0.108 ± 0.003
Relative	3.40 ± 0.12	3.22 ± 0.08	–	3.20 ± 0.08	–	3.28 ± 0.11
Thymus						
Absolute	0.040 ± 0.002	0.040 ± 0.002	0.050 ± 0.003*	0.050 ± 0.004*	0.038 ± 0.002	0.043 ± 0.003
Relative	1.22 ± 0.07	1.18 ± 0.05	1.59 ± 0.12*	1.44 ± 0.11	1.16 ± 0.06	1.29 ± 0.06

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Female						
n	10	10	10	8	10	10
Necropsy body wt	26.5 ± 0.4	26.9 ± 0.5	27.4 ± 0.3	27.7 ± 0.8	28.2 ± 0.4*	28.0 ± 0.5*
Brain						
Absolute	0.495 ± 0.009	0.489 ± 0.002	0.463 ± 0.007*	0.460 ± 0.010*	0.483 ± 0.009	0.478 ± 0.012
Relative	18.70 ± 0.37	18.25 ± 0.34	16.89 ± 0.32**	16.67 ± 0.53**	17.10 ± 0.26**	17.16 ± 0.58**
Heart						
Absolute	0.160 ± 0.004	0.150 ± 0.006	0.145 ± 0.003	0.148 ± 0.004	0.156 ± 0.004	0.166 ± 0.005
Relative	6.04 ± 0.16	5.58 ± 0.19	5.29 ± 0.11**	5.34 ± 0.17*	5.53 ± 0.15	5.95 ± 0.18
R. Kidney						
Absolute	0.229 ± 0.007	0.221 ± 0.006	0.219 ± 0.006	0.214 ± 0.006	0.232 ± 0.007	0.231 ± 0.006
Relative	8.65 ± 0.23	8.24 ± 0.19	8.00 ± 0.25	7.72 ± 0.18*	8.21 ± 0.18	8.28 ± 0.25
Liver						
Absolute	1.354 ± 0.037	1.307 ± 0.030	1.364 ± 0.039	1.411 ± 0.062	1.432 ± 0.054	1.428 ± 0.026
Relative	51.07 ± 1.03	48.74 ± 1.10	49.72 ± 1.33	50.74 ± 0.82	50.64 ± 1.38	51.16 ± 0.96
Lungs						
Absolute	0.250 ± 0.011	0.250 ± 0.011	0.242 ± 0.007	0.249 ± 0.014	0.264 ± 0.008	0.259 ± 0.009
Relative	9.43 ± 0.43	9.30 ± 0.40	8.83 ± 0.28	9.02 ± 0.51	9.37 ± 0.24	9.31 ± 0.40
Spleen						
Absolute	0.097 ± 0.007	0.093 ± 0.004	0.101 ± 0.004	0.114 ± 0.010[d]	0.141 ± 0.006**	0.220 ± 0.009**
Relative	3.65 ± 0.25	3.46 ± 0.14	3.69 ± 0.12	4.07 ± 0.27[d]	5.00 ± 0.17**	7.92 ± 0.39**
Thymus						
Absolute	0.055 ± 0.004	0.047 ± 0.003	0.043 ± 0.002**	0.046 ± 0.002	0.043 ± 0.002**	0.048 ± 0.002
Relative	2.07 ± 0.14	1.77 ± 0.11*	1.56 ± 0.06**	1.67 ± 0.07**	1.54 ± 0.07**	1.72 ± 0.07**

* Significantly different ($P \leq 0.05$) from the control group by William's or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=8
[c] n=0; no organs weighed
[d] n=7

TABLE D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 9-Month Interim Evaluations in the 2-Year Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
n	10	10	10	10
Necropsy body wt	47.0 ± 1.6	50.0 ± 0.9	47.9 ± 1.0	50.5 ± 0.8
Brain				
Absolute	0.481 ± 0.008	0.472 ± 0.003	0.474 ± 0.005	0.468 ± 0.006
Relative	10.31 ± 0.31	9.46 ± 0.16	9.94 ± 0.27	9.28 ± 0.12**
R. Kidney				
Absolute	0.358 ± 0.018	0.385 ± 0.009	0.365 ± 0.010	0.363 ± 0.008
Relative	7.61 ± 0.26	7.71 ± 0.21	7.62 ± 0.15	7.21 ± 0.21
Liver				
Absolute	1.795 ± 0.121	1.956 ± 0.071	2.038 ± 0.076	2.202 ± 0.098**
Relative	37.97 ± 1.74	39.02 ± 1.00	42.45 ± 0.94*	43.49 ± 1.37**
Spleen				
Absolute	0.085 ± 0.008	0.077 ± 0.003	0.103 ± 0.006	0.178 ± 0.009**
Relative	1.81 ± 0.15	1.54 ± 0.05	2.14 ± 0.11	3.52 ± 0.15**
Female				
n	9	10	9	10
Necropsy body wt	43.6 ± 1.8	47.2 ± 1.5	44.9 ± 1.6	45.4 ± 1.7
Brain				
Absolute	0.484 ± 0.004	0.483 ± 0.005	0.480 ± 0.005	0.487 ± 0.005
Relative	11.26 ± 0.47	10.33 ± 0.35	10.81 ± 0.41	10.84 ± 0.38
R. Kidney				
Absolute	0.234 ± 0.005	0.228 ± 0.004	0.232 ± 0.009	0.241 ± 0.008
Relative	5.43 ± 0.19	4.87 ± 0.14	5.21 ± 0.22	5.34 ± 0.17
Liver				
Absolute	1.466 ± 0.042	1.558 ± 0.051	1.580 ± 0.036	1.671 ± 0.037**
Relative	33.88 ± 1.22	33.11 ± 0.84	35.58 ± 1.45	37.12 ± 1.26
Spleen				
Absolute	0.082 ± 0.005	0.092 ± 0.003	0.123 ± 0.009**	0.186 ± 0.004**
Relative	1.89 ± 0.09	1.96 ± 0.07	2.80 ± 0.24**	4.16 ± 0.21**

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

TABLE D5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
n	10	10	10	10
Necropsy body wt	50.9 ± 1.6	52.4 ± 0.9	49.7 ± 1.2	48.5 ± 2.0
Brain				
Absolute	0.464 ± 0.005	0.461 ± 0.005	0.465 ± 0.005	0.468 ± 0.006
Relative	9.21 ± 0.34	8.82 ± 0.15	9.39 ± 0.19	9.78 ± 0.35
R. Kidney				
Absolute	0.402 ± 0.014	0.440 ± 0.011	0.380 ± 0.013	0.391 ± 0.016
Relative	7.93 ± 0.26	8.41 ± 0.25	7.64 ± 0.20	8.10 ± 0.24
Liver				
Absolute	1.998 ± 0.115[b]	2.286 ± 0.097[b]	1.974 ± 0.122[c]	2.041 ± 0.132[b]
Relative	39.37 ± 1.27[b]	43.48 ± 1.63[b]	38.51 ± 1.69[c]	41.63 ± 1.21[b]
Spleen				
Absolute	0.078 ± 0.007[b]	0.084 ± 0.006	0.136 ± 0.036	0.167 ± 0.009**
Relative	1.54 ± 0.13[b]	1.61 ± 0.12	2.85 ± 0.86	3.44 ± 0.13**
Female				
n	9	10	10	9
Necropsy body wt	48.2 ± 2.7	49.6 ± 1.8	50.7 ± 1.8	52.5 ± 1.4
Brain				
Absolute	0.473 ± 0.006	0.480 ± 0.004	0.487 ± 0.004	0.478 ± 0.010
Relative	10.09 ± 0.61	9.78 ± 0.36	9.72 ± 0.37	9.15 ± 0.29
R. Kidney				
Absolute	0.246 ± 0.006	0.247 ± 0.005	0.251 ± 0.005	0.249 ± 0.008
Relative	5.23 ± 0.34	5.01 ± 0.13	5.00 ± 0.19	4.75 ± 0.13
Liver				
Absolute	1.483 ± 0.058[c]	1.601 ± 0.065	1.676 ± 0.036*	1.774 ± 0.061**
Relative	29.95 ± 1.24[c]	32.28 ± 0.68	33.30 ± 0.91*	33.80 ± 0.57**
Spleen				
Absolute	0.117 ± 0.025	0.103 ± 0.004	0.118 ± 0.006	0.199 ± 0.008**
Relative	2.66 ± 0.79	2.10 ± 0.12	2.34 ± 0.12	3.80 ± 0.15
Uterus				
Absolute	0.698 ± 0.191	0.531 ± 0.148	0.367 ± 0.052	0.513 ± 0.138
Relative	14.78 ± 4.49	10.93 ± 3.25	7.17 ± 0.91	9.75 ± 2.71

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=8

APPENDIX E
HEMATOLOGY AND CLINICAL CHEMISTRY

TABLE E1
Hematology and Clinical Chemistry Data for Mice in the 14-Day Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Male					
n	5	4	5	3	5
Hematology					
Hematocrit (%)	43.0 ± 0.6	41.9 ± 0.7	39.0 ± 1.3*	42.7 ± 0.2	35.9 ± 1.7**
Hemoglobin (g/dL)	15.4 ± 0.2	15.0 ± 0.0	14.6 ± 0.5	19.0 ± 0.6	15.6 ± 0.8
Erythrocytes (10^6/μL)	9.17 ± 0.15	9.00 ± 0.19	8.21 ± 0.29*	8.44 ± 0.06*	6.75 ± 0.32**
Reticulocytes (10^6/μL)	2.90 ± 0.27	2.45 ± 0.70	3.32 ± 0.66	4.37 ± 1.78	18.04 ± 1.34**
Leukocytes (10^3/μL)	4.22 ± 0.35	4.08 ± 0.34	4.22 ± 0.24	12.03 ± 4.63*	16.50 ± 3.38**
Segmented neutrophils (10^3/μL)	0.90 ± 0.10	0.90 ± 0.29	0.62 ± 0.15	4.37 ± 1.42	3.67 ± 0.87*
Lymphocytes (10^3/μL)	3.05 ± 0.35	3.00 ± 0.12	3.47 ± 0.14	7.25 ± 2.90	12.34 ± 2.50**
Monocytes (10^3/μL)	0.12 ± 0.04	0.07 ± 0.05	0.05 ± 0.02	0.24 ± 0.20	0.08 ± 0.05
Eosinophils (10^3/μL)	0.15 ± 0.04	0.11 ± 0.04	0.09 ± 0.02	0.17 ± 0.13	0.42 ± 0.25
Total bone marrow cellularity (10^6/femur)	17.3 ± 1.9	18.7 ± 0.9	20.8 ± 1.2	17.9 ± 2.0	20.5 ± 1.6
Clinical Chemistry					
Methemoglobin (%)	1.70 ± 0.22	3.03 ± 0.56*	5.74 ± 0.55**	13.77 ± 2.10**	11.92 ± 3.15**
Female					
n	5	4	4	5	4
Hematology					
Hematocrit (%)	43.4 ± 0.5	41.9 ± 0.9	42.6 ± 0.4	42.0 ± 1.2	36.2 ± 1.4**
Hemoglobin (g/dL)	15.4 ± 0.2	15.0 ± 0.4	15.5 ± 0.3	16.0 ± 0.3	17.5 ± 0.3**
Erythrocytes (10^6/μL)	9.10 ± 0.09	8.78 ± 0.13*	8.80 ± 0.11	8.34 ± 0.24**	7.09 ± 0.25**
Reticulocytes (10^6/μL)	0.80 ± 0.15	2.03 ± 0.67	2.73 ± 0.69*	4.92 ± 0.88**	5.95 ± 1.49**
Leukocytes (10^3/μL)	2.90 ± 0.39	2.90 ± 0.35	3.00 ± 0.12	4.58 ± 0.13**	41.90 ± 4.21**
Segmented neutrophils (10^3/μL)	0.55 ± 0.13	0.79 ± 0.28	0.42 ± 0.08	1.51 ± 0.19*	12.60 ± 3.19**
Lymphocytes (10^3/μL)	2.21 ± 0.21	2.05 ± 0.16	2.48 ± 0.12	2.95 ± 0.27	27.91 ± 2.60**
Monocytes (10^3/μL)	0.10 ± 0.06	0.00 ± 0.00	0.01 ± 0.01	0.06 ± 0.02	0.77 ± 0.28
Eosinophils (10^3/μL)	0.05 ± 0.01	0.06 ± 0.02	0.03 ± 0.02	0.06 ± 0.03	0.62 ± 0.11**
Total bone marrow cellularity (10^6/femur)	18.8 ± 1.1	15.7 ± 1.1	18.8 ± 0.3	19.1 ± 0.4	21.7 ± 1.3
Clinical Chemistry					
Methemoglobin (%)	0.00 ± 0.00	1.35 ± 0.17**	3.20 ± 0.68**	6.16 ± 0.67**	16.73 ± 1.38**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. All mice receiving 1,000 mg/kg died before terminal sacrifice.

TABLE E2
Hematology and Clinical Chemistry Data for Mice at the 7-Week Interim Evaluations in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	9	8	8	9	9	8
Hematology						
Hematocrit (%)	44.0 ± 0.7	45.6 ± 0.7	42.7 ± 1.0	44.0 ± 0.6	42.1 ± 0.9	41.3 ± 0.6*
Hemoglobin (g/dL)	13.9 ± 0.2	14.6 ± 0.2	13.6 ± 0.3	14.1 ± 0.2	14.4 ± 0.3	17.3 ± 0.2**
Erythrocytes ($10^6/\mu L$)	7.84 ± 0.12	8.15 ± 0.12	7.55 ± 0.14	7.89 ± 0.10	7.30 ± 0.14*	7.08 ± 0.10**
Mean cell volume (fL)	56.0 ± 0.5	56.0 ± 0.4	56.6 ± 0.7	55.9 ± 0.4	57.7 ± 0.4*	58.4 ± 0.4**
Mean cell hemoglobin (pg)	17.7 ± 0.2	17.9 ± 0.1	18.0 ± 0.3	17.8 ± 0.2	19.7 ± 0.2**	24.5 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	31.5 ± 0.3	32.0 ± 0.1	31.8 ± 0.2	32.0 ± 0.2	34.2 ± 0.3**	42.0 ± 0.5**
Reticulocytes (%)	2.64 ± 0.20	2.16 ± 0.25	1.88 ± 0.20	2.60 ± 0.31	4.58 ± 0.76	5.44 ± 0.41**
Leukocytes ($10^3/\mu L$)	4.70 ± 0.38[b]	4.80 ± 0.40	5.43 ± 0.78	4.18 ± 0.37	5.70 ± 0.35	70.61 ± 8.02**
Segmented neutrophils ($10^3/\mu L$)	1.99 ± 0.47[b]	0.99 ± 0.14	3.08 ± 0.61	1.08 ± 0.24	1.87 ± 0.33	16.58 ± 2.71**
Lymphocytes ($10^3/\mu L$)	2.76 ± 0.29	3.71 ± 0.31*	2.22 ± 0.27	2.98 ± 0.30	3.69 ± 0.34	52.51 ± 5.62**
Monocytes ($10^3/\mu L$)	0.05 ± 0.03	0.02 ± 0.01	0.09 ± 0.05	0.02 ± 0.02	0.07 ± 0.02	0.38 ± 0.24
Eosinophils ($10^3/\mu L$)	0.03 ± 0.01	0.07 ± 0.03	0.04 ± 0.01	0.09 ± 0.02	0.07 ± 0.03	1.14 ± 0.58
Nucleated erythrocytes (/100 leukocytes)	0.00 ± 0.00	0.00 ± 0.00[c]	0.50 ± 0.27*	0.44 ± 0.24	0.56 ± 0.24*	0.25 ± 0.16
Total bone marrow cellularity (10^6/femur)	17.2 ± 1.1[b]	18.4 ± 0.7	16.6 ± 1.6	17.7 ± 1.0	19.0 ± 1.1	19.3 ± 0.9
Clinical Chemistry						
Methemoglobin (%)	4.17 ± 1.07	5.56 ± 1.02	5.28 ± 1.31	4.70 ± 0.87	12.53 ± 0.93**	30.70 ± 3.10**

TABLE E2
Hematology and Clinical Chemistry Data for Mice at the 7-Week Interim Evaluations in the 13-Week Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Female						
n	10	10	9	10	10	10
Hematology						
Hematocrit (%)	49.0 ± 0.6	48.2 ± 0.3	47.6 ± 0.7	47.5 ± 0.4*	42.4 ± 0.8**	44.2 ± 0.7**
Hemoglobin (g/dL)	15.0 ± 0.2	14.7 ± 0.1	14.6 ± 0.2	14.6 ± 0.1	13.7 ± 0.3**	15.4 ± 0.2
Erythrocytes (10^6/μL)	8.39 ± 0.11	8.25 ± 0.09	8.25 ± 0.09	8.23 ± 0.07	7.42 ± 0.13**	7.62 ± 0.11**
Mean cell volume (fL)	58.5 ± 0.3	58.5 ± 0.3	57.8 ± 0.5	57.6 ± 0.5	57.1 ± 0.2**	58.0 ± 0.7
Mean cell hemoglobin (pg)	17.9 ± 0.1	17.8 ± 0.1	17.7 ± 0.1	17.7 ± 0.2	18.5 ± 0.1*	20.2 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	30.7 ± 0.1	30.5 ± 0.1	30.7 ± 0.1	30.6 ± 0.1	32.3 ± 0.2**	34.9 ± 0.3**
Reticulocytes (%)	2.02 ± 0.22	2.28 ± 0.32	1.81 ± 0.18	2.26 ± 0.22	4.64 ± 0.52**	5.93 ± 0.39**
Leukocytes (10^3/μL)	3.26 ± 0.40	3.23 ± 0.20	3.56 ± 0.49	3.83 ± 0.47	3.84 ± 0.44	6.79 ± 0.45**
Segmented neutrophils (10^3/μL)	1.05 ± 0.25	0.97 ± 0.08	1.10 ± 0.26	1.51 ± 0.30	1.39 ± 0.36	2.33 ± 0.38**
Lymphocytes (10^3/μL)	2.10 ± 0.20	2.20 ± 0.16	2.40 ± 0.29	2.24 ± 0.21	2.37 ± 0.19	4.27 ± 0.27**
Monocytes (10^3/μL)	0.06 ± 0.03	0.01 ± 0.01	0.03 ± 0.03	0.04 ± 0.02	0.05 ± 0.03	0.09 ± 0.03
Eosinophils (10^3/μL)	0.05 ± 0.02	0.05 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.09 ± 0.03
Nucleated erythrocytes (/100 leukocytes)	0.00 ± 0.00	0.20 ± 0.20	0.44 ± 0.18*	0.10 ± 0.10	0.50 ± 0.22*	2.50 ± 0.75**
Total bone marrow cellularity (10^6/femur)	16.4 ± 0.6	14.6 ± 1.1	14.1 ± 0.8	14.9 ± 1.0[c]	17.5 ± 1.2	17.8 ± 0.8
Clinical Chemistry						
Methemoglobin (%)	0.61 ± 0.27	0.31 ± 0.27	0.43 ± 0.39	1.13 ± 0.30	4.20 ± 0.35**[c]	10.56 ± 1.12**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error
[b] n=8
[c] n=9

TABLE E3
Hematology and Clinical Chemistry Data for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male						
n	9	11	8	9	10	9
Hematology						
Hematocrit (%)	40.5 ± 0.7	45.8 ± 0.5	46.8 ± 1.1	41.2 ± 0.7	41.9 ± 0.5	39.7 ± 0.4
Hemoglobin (g/dL)	13.4 ± 0.3	15.0 ± 0.2**	15.6 ± 0.3**	13.4 ± 0.3*	15.0 ± 0.3**	18.4 ± 0.4**
Erythrocytes (10^6/μL)	8.10 ± 0.14	8.89 ± 0.10	9.08 ± 0.18	8.03 ± 0.14	7.79 ± 0.10	7.56 ± 0.08*
Mean cell volume (fL)	50.1 ± 1.1	51.5 ± 0.3	51.6 ± 0.4	51.3 ± 0.2	53.8 ± 0.3**	52.6 ± 0.2**
Mean cell hemoglobin (pg)	16.5 ± 0.4	16.9 ± 0.2	17.2 ± 0.1	16.6 ± 0.2	19.3 ± 0.2**	24.3 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.4	32.9 ± 0.3	33.4 ± 0.2	32.4 ± 0.3	35.8 ± 0.4**	46.2 ± 0.6**
Reticulocytes (%)	2.56 ± 0.20	1.25 ± 0.19	1.80 ± 0.16	2.46 ± 0.28	5.86 ± 0.62*	9.67 ± 0.86**
Leukocytes (10^3/μL)	3.91 ± 0.53	2.26 ± 0.21	2.96 ± 0.72	3.02 ± 0.39	2.93 ± 0.41	57.41 ± 9.94*
Segmented neutrophils (10^3/μL)	1.87 ± 0.45	0.73 ± 0.17	1.60 ± 0.62	1.33 ± 0.31	1.00 ± 0.41	8.78 ± 1.40*
Lymphocytes (10^3/μL)	1.95 ± 0.31	1.51 ± 0.13	1.29 ± 0.14	1.62 ± 0.27	1.88 ± 0.15	47.53 ± 9.18**
Monocytes (10^3/μL)	0.05 ± 0.02	0.01 ± 0.00	0.04 ± 0.02	0.05 ± 0.04	0.02 ± 0.01	0.20 ± 0.14
Eosinophils (10^3/μL)	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.90 ± 0.40
Nucleated erythrocytes (/100 leukocytes)	0.10 ± 0.10[b]	0.55 ± 0.21	0.13 ± 0.13	0.67 ± 0.67	0.50 ± 0.17	2.22 ± 0.49**
Total bone marrow cellularity (10^6/femur)	16.2 ± 1.2	17.8 ± 1.0	19.6 ± 0.9*	22.3 ± 0.9**	21.5 ± 1.2**	23.2 ± 0.9**
Clinical Chemistry						
Methemoglobin (%)	3.62 ± 0.20	2.57 ± 0.23[b]	2.86 ± 0.21	7.16 ± 0.31*	7.40 ± 0.38**	17.01 ± 2.00**

TABLE E3
Hematology and Clinical Chemistry Data for Mice in the 13-Week Gavage Studies of *p*-Nitroaniline
(continued)

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Female						
n	10	10	10	8	10	10
Hematology						
Hematocrit (%)	40.8 ± 1.0	42.5 ± 0.4	43.7 ± 0.5	43.7 ± 0.5	44.2 ± 0.8*	39.9 ± 0.9
Hemoglobin (g/dL)	13.2 ± 0.4	13.7 ± 0.1	14.4 ± 0.2**	14.2 ± 0.2*	14.3 ± 0.2*	15.6 ± 0.4**
Erythrocytes (10^6/μL)	7.76 ± 0.18	8.14 ± 0.07	8.33 ± 0.09*	8.33 ± 0.11	8.41 ± 0.14*	7.70 ± 0.15
Mean cell volume (fL)	52.5 ± 0.4	52.2 ± 0.1	52.6 ± 0.2	52.4 ± 0.2	52.5 ± 0.2	51.6 ± 0.3
Mean cell hemoglobin (pg)	17.0 ± 0.2	16.9 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.0 ± 0.1	20.3 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	32.4 ± 0.3	32.3 ± 0.1	32.9 ± 0.1*	32.5 ± 0.1	32.3 ± 0.2	39.3 ± 0.6**
Reticulocytes (%)	1.64 ± 0.17	1.31 ± 0.19	1.39 ± 0.22	2.11 ± 0.36	4.44 ± 0.49**	6.33 ± 0.41**
Leukocytes (10^3/μL)	2.02 ± 0.28[c]	2.08 ± 0.16[c]	2.67 ± 0.32	1.93 ± 0.20[d]	2.14 ± 0.31	5.43 ± 0.73**
Segmented neutrophils (10^3/μL)	0.76 ± 0.13[c]	0.53 ± 0.09[c]	0.93 ± 0.08	0.57 ± 0.08[d]	0.70 ± 0.22	1.00 ± 0.22
Lymphocytes (10^3/μL)	1.38 ± 0.25	1.73 ± 0.30	1.68 ± 0.27	1.92 ± 0.63	1.36 ± 0.15	4.32 ± 0.61**
Monocytes (10^3/μL)	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.03 ± 0.00	0.04 ± 0.01
Eosinophils (10^3/μL)	0.06 ± 0.03	0.07 ± 0.02	0.05 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.07 ± 0.03
Nucleated erythrocytes (/100 leukocytes)	0.60 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.38 ± 0.26	0.70 ± 0.34	1.30 ± 0.26*
Total bone marrow cellularity (10^6/femur)	17.6 ± 1.0	18.8 ± 1.2	19.2 ± 0.5	19.7 ± 1.5	20.3 ± 0.8	19.1 ± 0.6[c]
Clinical Chemistry						
Methemoglobin (%)	3.67 ± 0.11	3.71 ± 0.39	2.29 ± 0.11	3.38 ± 0.21	10.09 ± 0.32**	14.69 ± 0.31**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error
[b] n=10
[c] n=9
[d] n=7

TABLE E4
Hematology and Clinical Chemistry Data for Mice at the 9-Month Interim Evaluations in the 2-Year Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
n	9	9	10	10
Hematology				
Hematocrit (%)	34.7 ± 1.0	34.0 ± 0.9	32.7 ± 0.6	31.8 ± 0.7*
Hemoglobin (g/dL)	14.9 ± 0.3	14.8 ± 0.3	15.2 ± 0.2	16.4 ± 0.3**
Erythrocytes (10^6/μL)	9.16 ± 0.12	8.97 ± 0.12	8.89 ± 0.11	8.16 ± 0.09**
Mean cell volume (fL)	37.7 ± 0.8	37.2 ± 0.7	36.7 ± 0.4	39.1 ± 0.8
Mean cell hemoglobin (pg)	16.2 ± 0.1	16.2 ± 0.2	17.1 ± 0.2**	20.1 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	43.0 ± 0.5	43.4 ± 0.4	46.5 ± 0.3**	51.7 ± 1.3**
Platelets (10^3/μL)	907.7 ± 21.4	980.2 ± 28.2	977.8 ± 26.4	955.8 ± 39.8
Reticulocytes (10^6/μL)	0.12 ± 0.01	0.11 ± 0.02	0.23 ± 0.03**	0.38 ± 0.04**
Leukocytes (10^3/μL)	0.67 ± 0.09	0.53 ± 0.06	1.13 ± 0.16	1.54 ± 0.21**
Segmented neutrophils (10^3/μL)	0.15 ± 0.03	0.11 ± 0.02	0.28 ± 0.10	0.25 ± 0.03
Lymphocytes (10^3/μL)	0.49 ± 0.06	0.41 ± 0.05	0.82 ± 0.12*	1.24 ± 0.17**
Atypical lymphocytes (10^3/μL)	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.04 ± 0.01
Monocytes (10^3/μL)	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/μL)	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Clinical Chemistry				
Methemoglobin (g/dL)	0.20 ± 0.05	0.23 ± 0.02	0.58 ± 0.06**	1.49 ± 0.16**
Sulfhemoglobin (g/dL)	0.39 ± 0.05	0.46 ± 0.05	1.21 ± 0.17**	4.01 ± 0.56**

TABLE E4
Hematology and Clinical Chemistry Data for Mice at the 9-Month Interim Evaluations in the 2-Year Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Female				
n	9	10	9	10
Hematology				
Hematocrit (%)	33.7 ± 0.6	33.7 ± 0.7	34.0 ± 0.8	32.6 ± 0.7
Hemoglobin (g/dL)	14.6 ± 0.2	14.6 ± 0.2	15.0 ± 0.2	15.1 ± 0.3
Erythrocytes (10^6/μL)	8.97 ± 0.10	8.94 ± 0.09	8.96 ± 0.10	8.44 ± 0.13*
Mean cell volume (fL)	37.6 ± 0.8	37.7 ± 0.6	37.9 ± 0.6	38.6 ± 0.7
Mean cell hemoglobin (pg)	16.3 ± 0.2	16.3 ± 0.1	16.8 ± 0.1*	17.9 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	43.5 ± 0.6	43.4 ± 0.7	44.4 ± 0.7	46.5 ± 0.9*
Platelets (10^3/μL)	831.4 ± 21.0	757.8 ± 32.4	855.2 ± 64.4	849.3 ± 32.2
Reticulocytes (10^6/μL)	0.12 ± 0.02	0.13 ± 0.01	0.21 ± 0.02**	0.40 ± 0.04**
Leukocytes (10^3/μL)	0.70 ± 0.10	0.59 ± 0.11	0.74 ± 0.18	0.75 ± 0.12
Segmented neutrophils (10^3/μL)	0.20 ± 0.04	0.11 ± 0.02	0.17 ± 0.05	0.12 ± 0.02
Lymphocytes (10^3/μL)	0.48 ± 0.07	0.45 ± 0.10	0.53 ± 0.12	0.61 ± 0.10
Atypical lymphocytes (10^3/μL)	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
Monocytes (10^3/μL)	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/μL)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Clinical Chemistry				
Methemoglobin (g/dL)	0.18 ± 0.06	0.20 ± 0.03	0.49 ± 0.12**	0.83 ± 0.12**
Sulfhemoglobin (g/dL)	0.44 ± 0.05	0.46 ± 0.07	0.81 ± 0.09**	1.78 ± 0.25**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error

TABLE E5
Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of *p*-Nitroaniline[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
Hematology				
n	10	10	10	10
Hematocrit (%)	33.3 ± 0.9	34.5 ± 1.2	30.0 ± 1.6	30.7 ± 0.8*
Hemoglobin (g/dL)	13.2 ± 0.4	13.6 ± 0.4	12.7 ± 0.7	14.6 ± 0.3**
Erythrocytes (10^6/μL)	8.80 ± 0.29	8.86 ± 0.30	8.11 ± 0.50	7.79 ± 0.12**
Mean cell volume (fL)	37.9 ± 0.6	39.0 ± 0.5	37.0 ± 0.7	39.4 ± 0.8
Mean cell hemoglobin (pg)	15.0 ± 0.2	15.4 ± 0.2	15.7 ± 0.3**	18.8 ± 0.4**
Mean cell hemoglobin concentration (g/dL)	39.5 ± 0.5	39.7 ± 0.5	42.3 ± 0.4**	47.7 ± 0.8**
Platelets (10^3/μL)	982.7 ± 85.3	1085.1 ± 55.8	991.6 ± 66.6	973.2 ± 90.7
Reticulocytes (10^6/μL)	0.35 ± 0.06	0.30 ± 0.03	0.42 ± 0.05	0.85 ± 0.06**
Leukocytes (10^3/μL)	1.78 ± 0.37	1.66 ± 0.27	1.40 ± 0.28	12.75 ± 2.05**
Segmented neutrophils (10^3/μL)	0.53 ± 0.13	0.44 ± 0.08	0.57 ± 0.19	2.79 ± 0.61**
Lymphocytes (10^3/μL)	1.19 ± 0.29	1.16 ± 0.20	0.78 ± 0.11	9.39 ± 1.57**
Atypical lymphocytes (10^3/μL)	0.04 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.27 ± 0.11
Monocytes (10^3/μL)	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.03 ± 0.02
Eosinophils (10^3/μL)	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.19 ± 0.06**
Clinical Chemistry				
n	9	10	10	10
Methemoglobin (g/dL)	0.18 ± 0.03	0.18 ± 0.04	0.34 ± 0.05*	0.82 ± 0.14**
Sulfhemoglobin (g/dL)	0.43 ± 0.12	0.35 ± 0.13	0.46 ± 0.16	1.26 ± 0.49

TABLE E5
Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of *p*-Nitroaniline (continued)

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Female				
Hematology				
n	8	10	10	9
Hematocrit (%)	35.0 ± 0.7	33.7 ± 0.5	32.6 ± 0.7*	30.8 ± 0.5**
Hemoglobin (g/dL)	14.1 ± 0.3	13.5 ± 0.2	13.2 ± 0.2*	13.7 ± 0.2
Erythrocytes ($10^6/\mu L$)	9.09 ± 0.12	8.72 ± 0.12	8.44 ± 0.12**	7.81 ± 0.07**
Mean cell volume (fL)	38.5 ± 0.6	38.7 ± 0.6	38.6 ± 0.5	39.6 ± 0.5
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.4 ± 0.1	15.7 ± 0.1	17.5 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	40.3 ± 0.5	40.0 ± 0.5	40.6 ± 0.5	44.4 ± 0.6**
Platelets ($10^3/\mu L$)	849.7 ± 30.6	765.8 ± 58.6	877.9 ± 27.9	817.8 ± 50.8
Reticulocytes ($10^6/\mu L$)	0.26 ± 0.02	0.30 ± 0.02	0.34 ± 0.03	0.78 ± 0.05**[b]
Leukocytes ($10^3/\mu L$)	0.66 ± 0.12	1.76 ± 0.58**	1.01 ± 0.17*	1.47 ± 0.31**
Segmented neutrophils ($10^3/\mu L$)	0.17 ± 0.03	0.43 ± 0.14	0.25 ± 0.05	0.35 ± 0.06
Lymphocytes ($10^3/\mu L$)	0.46 ± 0.09	1.25 ± 0.43**	0.73 ± 0.13*	1.04 ± 0.25**
Atypical lymphocytes ($10^3/\mu L$)	0.01 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Eosinophils ($10^3/\mu L$)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
Clinical Chemistry				
n	8	10	10	9
Methemoglobin (g/dL)	0.11 ± 0.03	0.31 ± 0.10	0.24 ± 0.04*	0.55 ± 0.07**
Sulfhemoglobin (g/dL)	0.09 ± 0.05	0.24 ± 0.07	0.52 ± 0.17	0.86 ± 0.34*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error
[b] n=8

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF *p*-NITROANILINE

p-Nitroaniline was obtained from the American Color and Chemical Corporation (Charlotte, NC) in one lot (990-002), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO). MRI reports on analyses performed in support of the *p*-nitroaniline studies are on file at the National Institute of Environmental Health Sciences.

Lot 990-002, a yellow, amorphous powder, was identified as *p*-nitroaniline by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with those expected for the structure and with those reported in the literature for *p*-nitroaniline (*Sadtler Standard Spectra*), as shown in Figures F1 and F2.

The purity of the lot was determined by Karl Fischer water analysis, elemental analyses, titration of the nitro group, thin-layer chromatography (TLC), and gas chromatography. Titration of the nitro group was performed with 0.5 N titanium (III) chloride and the sample was dissolved in ethanol/aqueous sodium citrate. TLC was performed on silica gel plates with two solvent systems: A) chloroform:acetone (85:15) and B) ethyl acetate:anhydrous ethanol:acetic acid (89:9:2). Plates were examined under shortwave (254 nm) and long wave (366 nm) ultraviolet light. Gas chromatographic analysis was performed using a flame ionization detector (FID) with a nitrogen carrier gas at a flow rate of 70 mL/minute and an oven temperature program of 50° C for 5 minutes, then 50° to 250° C at 10° C per minute. Two systems were used: A) a 3% OV-225 on 100/120 mesh Supelcoport column and B) a 3% SP-2401 (DB) on 100/120 mesh Supelcoport column.

Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for *p*-nitroaniline. Karl Fischer water analysis of the lot revealed less than 0.04% water. Reduction of the nitro group indicated a purity of greater than 99%. Each TLC system indicated one major spot and two trace impurities. Gas chromatography using the first column indicated a major peak and one impurity with a total area of 0.30% relative to the major peak. A major peak and one impurity with a total area of 0.18% relative to the major peak was observed with the second column.

Stability studies were performed by the analytical chemistry laboratory on lot TD101987 (Aldrich Chemical Co., Milwaukee, WI), which was of similar purity but was not used during the studies. Gas chromatography was performed with system A described above, but with a solution of 0.5% *p*-nitroaniline in chloroform containing 0.27% *p*-terphenyl added as an internal standard. These studies indicated that *p*-nitroaniline was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when protected from light. The stability of the bulk chemical was monitored periodically at the study laboratory with infrared and ultraviolet/visible spectroscopy and gas chromatography methods similar to those described above. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulation solutions and suspensions were prepared by mixing appropriate amounts of *p*-nitroaniline and corn oil (w/v) to give the required concentrations (Table F1). Dose formulation concentrations greater than 10 mg/mL were suspensions. The dose formulations, which were stored at 5° C, were agitated by hand before administration. Dose formulations were prepared once for the 14-day studies and every 2 weeks during the 13-week and 2-year studies. Formulations were discarded 20 days after the date of preparation.

Homogeneity and stability analyses were performed on lot TD101987 by the analytical chemistry laboratory. For homogeneity analysis of 50 mg/mL formulations, aliquots were extracted and diluted with methanol, and the absorbance of the samples was measured versus methanol by ultraviolet spectroscopy at 369 nm. For the stability studies, aliquots were diluted with acetone in beakers containing docosone (7 mg/mL in methylene chloride) as an internal standard. Gas chromatographic analysis was then performed with the second system described for the bulk purity analyses, but with a carrier gas flow rate of 30 mL/minute, an oven temperature program of 180° C, isothermal, and an internal standard of docosane. Homogeneity was confirmed, and the stability of the dose formulations was established for at least 2 weeks at 5° C and room temperature when stored in the dark, as well as for at least 3 hours when exposed to air and light. The study laboratory also conducted and confirmed the stability of dose formulations (Table F3).

Periodic analyses of the dose formulations of *p*-nitroaniline were conducted at the study laboratory and the analytical chemistry laboratory using ultraviolet spectroscopy. During the 14-day studies all formulations were analyzed (Table F2). During the 13-week studies, the dose formulations were analyzed at the initiation, midpoint, and termination of the studies (Table F3). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks using ultraviolet spectroscopy (Table F4). In the 2-year studies, 98% (45/46) of the dose formulations were within 10% of the target concentrations. Periodic peroxide analyses of the corn oil vehicle by the study laboratory indicated that peroxide levels were within the acceptable limit of 10 mEq/kg. Results of the periodic referee analyses performed by the analytical chemistry laboratory were in good agreement with the results obtained by the study laboratory (Table F5).

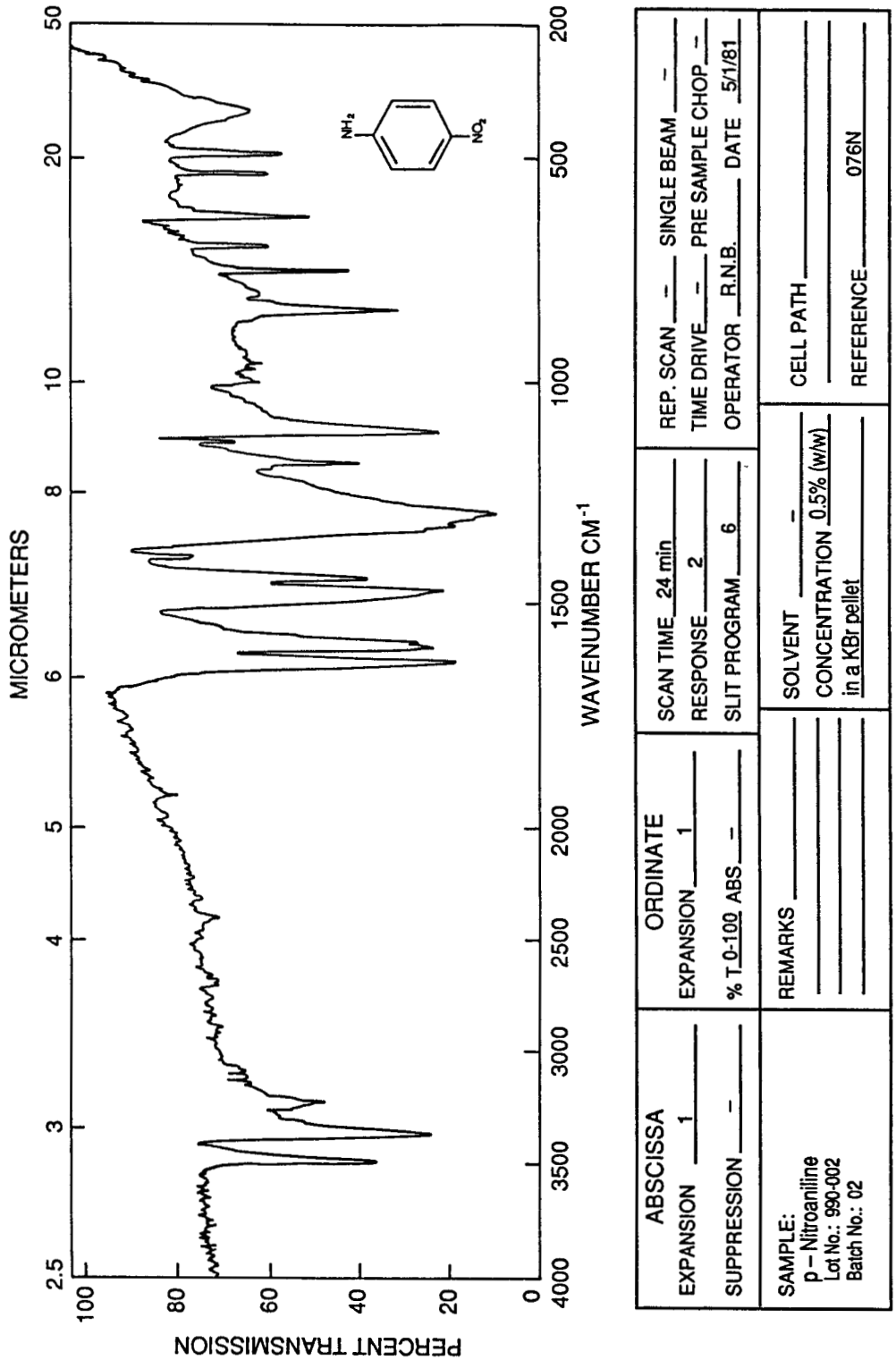


FIGURE F1
Infrared Absorption Spectrum of *p*-Nitroaniline

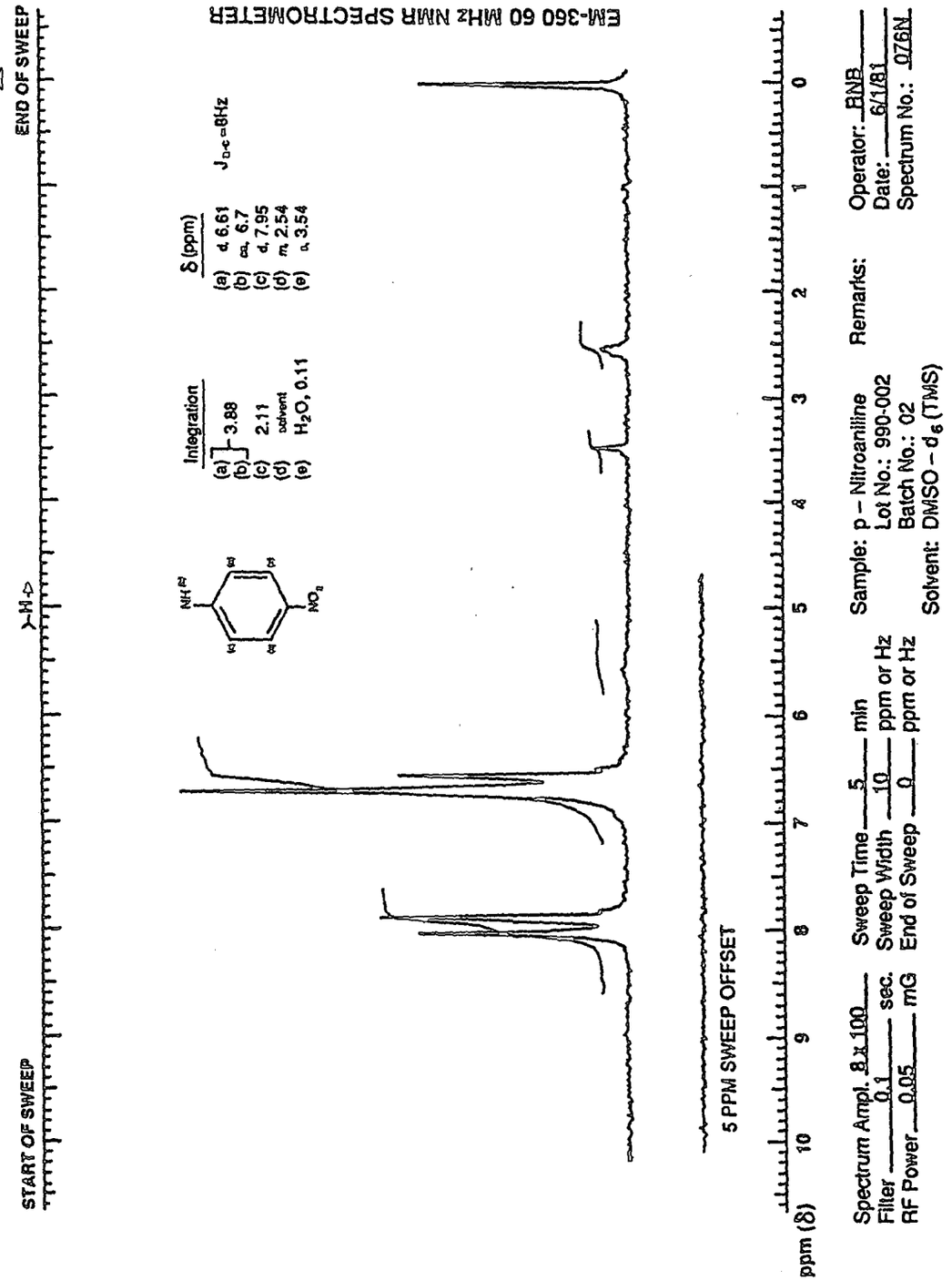


FIGURE F2
Nuclear Magnetic Resonance Spectrum of *p*-Nitroaniline

TABLE F1
Preparation and Storage of Dose Formulations in the Gavage Studies of *p*-Nitroaniline

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
p-Nitroaniline was mixed with corn oil (w/v) while stirring. Stirring continued for 20 minutes. Any visible clumps were crushed manually and stirring continued until a solution was obtained or a homogeneous suspension was achieved. Formulations were transferred to aspirator bottles, and dispensed into labeled serum bottles for storage while stirring continued. Doses were prepared once and agitated before administration.	Same as 14-day studies, except all formulations were solutions.	Same as 13-week studies
Chemical Lot Number		
990-002	Same as 14-day studies	Same as 14-day studies
Maximum Storage Time		
14 days after mixing	20 days after mixing	Same as 13-week studies
Storage Conditions		
Stored in amber glass bottles in the dark at 5° C.	Same as 14-day studies	Same as 14-day studies
Study Laboratory		
Hazleton Raltech, Inc., Madison, WI	Same as 14-day studies	Southern Research Institute, Birmingham, AL
Referee Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 14-day studies	Same as 14-day studies

TABLE F2
Results of Analysis of Dose Formulations Administered to Mice in the 14-Day Gavage Studies of *p*-Nitroanilline[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	% Difference from Target
22 April 1982	22 April 1982	1.0	1.39	+39
		3.0	2.98	–1
		10.0	9.10	–9
		30[c]	19.15	–36
		100[c]	68.20	–32
	28 April 1982[d]	30	22.75	–24
		100	112.00	+12
	12 May 1982[d]	1.0	1.33	+33
		3.0	2.91	–3
		10.0	8.55	–15
		30	32.65	+9
		100	105.00	+5

[a] Target concentrations expressed as mg/kg body weight: 1 mg/mL = 10 mg/kg, 3 mg/mL = 30 mg/kg, 10 mg/mL = 100 mg/kg, 30 mg/mL = 300 mg/kg, 100 mg/mL = 1,000 mg/kg.
[b] Results of duplicate analyses
[c] Dose formulations were suspensions.
[d] Animal room sample

TABLE F3
Results of Analysis of Dose Formulations Administered to Mice in the 13-Week Gavage Studies of *p*-Nitroaniline[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	% Difference from Target
15 November 1982	15 November 1982	0.1	0.10	0
		0.3	0.32	+7
		1.0	1.07	+7
		3.0	3.21	+7
		10.0	10.70	+7
17 December 1982	17 December 1982	0.1	0.10	0
		0.3	0.30	0
		1.0	1.03	+3
		3.0	3.01	0
		10.0	10.25	+3
	10 January 1983[c]	0.1	0.10	0
		0.3	0.30	0
		1.0	0.98	–2
		3.0	2.87	–4
		10.0	10.11	+1
7 February 1983	7 February 1983	0.1	0.10	0
		0.3	0.30	0
		1.0	1.04	+4
		3.0	2.98	–1
		10.0	10.48	+5
	20-24 February 1983[d]	0.1	0.09	–10
		0.3	0.28	–7
		1.0	1.03	+3
		3.0	3.00	0
		10.0	10.25	+3

[a] Target concentrations expressed as mg/kg body weight: 0.1 mg/mL = 1 mg/kg, 0.3 mg/mL = 3 mg/kg, 1 mg/mL = 10 mg/kg, 3 mg/mL = 30 mg/kg, 10 mg/mL = 100 mg/kg.
[b] Results of duplicate analyses
[c] Stability study of dose formulations prepared on 17 December 1982 and stored at room temperature for 24 days
[d] Animal room sample

TABLE F4
Results of Analysis of Dose Formulations Administered to Mice in the 2-Year Gavage Studies of *p*-Nitroaniline

Date Prepared	Date Analyzed	Target Concentration[a] (w/w %)	Determined Concentration[b] (w/w %)	% Difference from Target
17 September 1984	19-20 September 1984	0.0326	0.0331	+2
		0.0326	0.0355	+9
		0.0326	0.0324	–1
		0.325	0.325	0
		1.08	1.05	–3
		1.08	1.08	0
		1.08	1.08	0
11 October 1984	12, 15 October 1984	0.0326	0.0332	+2
		0.325	0.314	–3
		1.08	1.08	0
	1 November 1984[c]	0.0326	0.0316	–3
		0.325	0.312	–4
		1.08	1.06	–2
8 November 1984	11-12 November 1984	0.0326	0.0320	–2
		0.325	0.324	0
		1.08	1.08	0
17 January 1985	18 January 1985	0.0326	0.0305	–6
		0.325	0.304	–6
		1.08	1.08	0
28 February 1985	1 March 1985	0.0326	0.0266	–18[d]
		0.325	0.320	–2
		1.08	1.08	0
	21 March 1985[c]	0.325	0.318	–2
		1.08	1.06	–2
4 March 1985	5 March 1985	0.0326	0.0333	+2
	21 March 1985[c]	0.0326	0.0306	–6
11 April 1985	12 April 1985	0.0326	0.0316	–3
		0.325	0.329	+1
		1.08	1.06	–2
6 June 1985	7 June 1985	0.0326	0.0329	+1
		0.325	0.318	–2
		1.08	1.07	–1
18 July 1985	19 July 1985	0.0326	0.0340	+4
		0.325	0.317	–2
		1.08	1.10	+2
	8 August 1985[c]	0.0326	0.0323	–1
		0.325	0.320	–2
		1.08	1.04	–4

TABLE F4
Results of Analysis of Dose Formulations Administered to Mice in the 2-Year Gavage Studies of *p*-Nitroaniline (continued)

Date Prepared	Date Analyzed	Target Concentration (w/w %)	Determined Concentration (w/w %)	% Difference from Target
26 September 1985	27 September 1985	0.0326	0.0314	–4
		0.325	0.320	–2
		1.08	1.10	+2
21 November 1985	22 November 1985	0.0326	0.0326	0
		0.325	0.312	–4
		1.08	1.08	0
16 January 1986	17 January 1986	0.0326	0.0314	–4
		0.325	0.322	–1
		1.08	1.12	+4
	10 February 1986[c]	0.0326	0.0299	–8
		0.325	0.322	–1
		1.08	1.16	+7
27 February 1986	28 February 1986	0.0326	0.0308	–6
		0.325	0.316	–3
		1.08	1.08	0
8 May 1986	8-9 May 1986	0.0326	0.0314	–4
		0.325	0.319	–2
		1.08	1.06	–2
17 July 1986	17 July 1986	0.0326	0.0332	+2
		0.325	0.316	–3
		1.08	1.04	–4
	7-8 August 1986[c]	0.0326	0.0294	–10
		0.325	0.318	–2
		1.08	1.05	–3
28 August 1986	29 August 1986	0.0326	0.0324	–1
		0.325	0.322	–1
		1.08	1.08	0
	16 September 1986[c]	0.0326	0.0318	–2
		0.325	0.320	–2
		1.08	1.66	+54[e]

[a] Target concentrations expressed as mg/kg body weight: 0.0326% = 3 mg/kg, 0.325% = 30 mg/kg, and 1.08% = 100 mg/kg.
[b] Results of duplicate analyses
[c] Animal room sample
[d] Sample remixed
[e] Chemical formulation probably not stirred properly in animal room.

TABLE F5
Results of Referee Analysis of Dose Formulations in the 2-Year Gavage Studies of *p*-Nitroaniline

Date Prepared	Target Concentration (w/w %)	Determined Concentration (w/w %)	
		Study Laboratory[a]	Referee Laboratory[b]
13 September 1984	0.0326	0.0332	0.0306 ± 0.0001
17 September 1984	1.08	1.08	1.08 ± 0.00
11 April 1985	0.325	0.329	0.339 ± 0.001
26 September 1985	0.0326	0.0314	0.0313 ± 0.0002
27 February 1986	0.325	0.316	0.316 ± 0.002
28 August 1986	0.0326	0.0324	0.0323 ± 0.001

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard deviation)

APPENDIX G
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE G1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE G2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE G3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.13 ± 0.49	21.1 – 23.1	24
Crude Fat (% by weight)	5.68 ± 0.47	4.7 – 6.5	24
Crude Fiber (% by weight)	3.46 ± 0.47	2.7 – 5.4	24
Ash (% by weight)	6.45 ± 0.25	6.1 – 7.0	24
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.060	1.210 – 1.390	8
Cystine	0.306 ± 0.084	0.181 – 0.400	8
Glycine	1.150 ± 0.047	1.060 – 1.210	8
Histidine	0.576 ± 0.024	0.531 – 0.607	8
Isoleucine	0.917 ± 0.029	0.881 – 0.944	8
Leucine	1.946 ± 0.055	1.850 – 2.040	8
Lysine	1.270 ± 0.058	1.200 – 1.370	8
Methionine	0.448 ± 0.128	0.306 – 0.699	8
Phenylalanine	0.987 ± 0.140	0.665 – 1.110	8
Threonine	0.877 ± 0.042	0.824 – 0.940	8
Tryptophan	0.236 ± 0.176	0.107 – 0.671	8
Tyrosine	0.676 ± 0.105	0.564 – 0.794	8
Valine	1.103 ± 0.040	1.050 – 1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830 – 2.570	7
Linolenic	0.280 ± 0.040	0.210 – 0.320	7
Vitamins			
Vitamin A (IU/kg)	8,908 ± 2,513	4,700 – 15,000	24
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	37.95 ± 9.41	22.5 – 48.9	8
Thiamine (ppm)	20.42 ± 1.64	17.0 – 23.0	24
Riboflavin (ppm)	7.92 ± 0.87	6.10 – 9.00	8
Niacin (ppm)	103.4 ± 26.59	65.0 – 150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0 – 34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60 – 14.0	8
Folic acid (ppm)	2.25 ± 0.73	1.80 – 3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19 – 0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6 – 65.0	8
Choline (ppm)	3,089 ± 328.69	2,400 – 3,430	8
Minerals			
Calcium (%)	1.14 ± 0.10	0.95 – 1.41	24
Phosphorus (%)	0.92 ± 0.05	0.73 – 0.99	24
Potassium (%)	0.883 ± 0.078	0.772 – 0.971	6
Chloride (%)	0.526 ± 0.092	0.380 – 0.635	8
Sodium (%)	0.313 ± 0.390	0.258 – 0.371	8
Magnesium (%)	0.168 ± 0.010	0.151 – 0.181	8
Sulfur (%)	0.280 ± 0.064	0.208 – 0.420	8
Iron (ppm)	360.5 ± 100	255.0 – 523.0	8
Manganese (ppm)	92.0 ± 6.01	81.70 – 99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10 – 64.50	8
Copper (ppm)	11.06 ± 2.50	8.090 – 15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52 – 4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04 – 2.09	8
Cobalt (ppm)	0.681 ± 0.14	0.490 – 0.780	4

TABLE G4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.76 ± 0.17	0.32 – 1.07	24
Cadmium (ppm)	<0.1		24
Lead (ppm)	0.52 ± 0.26	0.05 – 1.27	24
Mercury (ppm)	<0.05		24
Selenium (ppm)	0.39 ± 0.09	0.17 – 0.48	24
Aflatoxins (ppb)	<5.0		24
Nitrate nitrogen (ppm)[b]	15.00 ± 4.63	2.80 – 22.0	24
Nitrite nitrogen (ppm)[b]	0.38 ± 0.73	<0.10 – 2.60	24
BHA (ppm)[c]	2.58 ± 1.06	<2.00 – 5.00	24
BHT (ppm)[c]	1.86 ± 1.08	<1.00 – 4.00	24
Aerobic plate count (CFU/g)[d]	36,945 ± 41,938	770 – 130,000	24
Coliform (MPN/g)[e]	15.67 ± 48.48	<3.00 – 240	24
Coliform (MPN/g)[f]	5.91 ± 8.40	<3.00 – 43.0	23
E. coli (MPN/g)[g]	3.04 ± 0.20	<3.00 – 4.00	24
Total nitrosoamines (ppb)[h]	7.70 ± 3.28	3.80 – 16.0	24
N-Nitrosodimethylamine (ppb)[h]	6.55 ± 3.10	2.80 – 15.0	24
N-Nitrosopyrrolidine (ppb)[h]	1.15 ± 0.55	1.00 – 3.40	24
Pesticides			
α-BHC[i]	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.1		24
Estimated PCBs	<0.2		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.1		24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion[j]	0.23 ± 0.07	0.05 – 3.20	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

TABLE G4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU = colony forming unit
[e] MPN = most probable number
[f] Excludes one high value of 240 MPN/g obtained in the lot milled 10/17/84.
[g] Includes one value of 4.0 MPN/g from the lot milled 10/17/84.
[h] All values were correct for % recovery.
[i] BHC = hexachlorocyclohexane or benzene hexachloride
[j] Nine lots contained more than 0.05 ppm, including one lot milled on 05/07/85 containing 3.20 ppm.

APPENDIX H
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. The sentinel animals come from the same production source and weanling groups as animals used for the studies of chemical compounds, and these animals and the study animals are subject to identical environmental conditions.

During the 13-week studies, five male and five female B6C3F$_1$ mice were maintained with the study animals to serve as sentinel animals. At termination of the 13-week studies, blood samples were taken from the sentinel mice. The blood was allowed to clot, and the serum was separated. The serum was cooled and sent to Microbiological Associates, Incorporated (Bethesda, MD), for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus	Study termination
ELISA	
MHV (mouse hepatitis virus)	Study termination
Hemagglutination Inhibition	
Ectromelia virus (mouse pox)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
MVM (minute virus of mice)	Study termination
Polyoma virus	Study termination
PVM (pneumonia virus of mice)	Study termination
Reovirus 3	Study termination
Sendai	Study termination

During the 2-year studies, 15 B6C3F$_1$ mice of each sex were maintained with the study animals to serve as sentinel animals. Blood was drawn from five mice of each sex at 6, 12, and 18 months following study initiation. Five randomly selected control animals of each sex were bled at study termination (24 months). Blood collected from each animal was allowed to clot and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Incorporated, for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
LCM	6, 12, 18, and 24 months
ELISA	
CARB	24 months
Ectromelia virus	6, 12, 18, and, 24 months
GDVII	6, 12, 18, and, 24 months
MHV	6, 12, 18, and, 24 months
Mouse adenoma virus	6, 12, 18, and, 24 months
Mycoplasma arthritidis	6, 12, 18, and, 24 months
Mycoplasma pulmonis	6, 12, 18, and, 24 months
Reovirus 3	6, 12, 18, and, 24 months
PVM	6, 12, 18, and, 24 months
Sendai	6, 12, 18, and, 24 months
Hemagglutination Inhibition	
K (papovavirus)	6, 12, 18, and 24 months
MVM	6, 12, 18, and 24 months
Polyoma virus	6, 12, 18, and 24 months
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	6, 12, 18, and 24 months

RESULTS

The serology results for sentinel animals are presented in Table H1.

TABLE H1
Murine Virus Antibody Determinations for Mice in the 13-Week and 2-Year Gavage Studies of *p*-Nitroaniline

	Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
13-Week Studies			
	13 weeks	1/10	Reovirus 3
2-Year Studies			
	6 months	0/10	None positive
	12 months	0/10	None positive
	18 months	0/9	None positive
	24 months	0/10	None positive

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF MAY 1993

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl 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
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-Methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
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 & Tetrakis(hydroxymethyl) phosponium 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 (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Pariffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
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	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PRINTED AS OF MAY 1993 (CONT.)

TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3′-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3′-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4′-Diamino-2,2′-Stilbenedisulfonic Acid
413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
419	HC Hellow 4

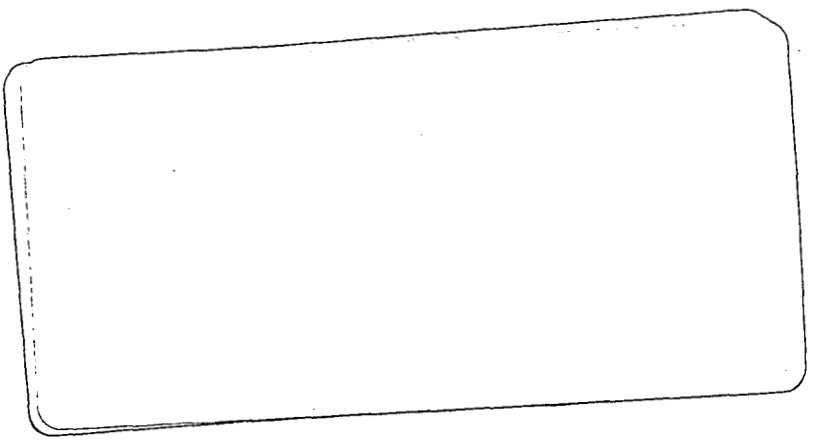
These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709.

DEPARTMENT OF
HEALTH & HUMAN SERVICES

Public Health Service
National Toxicology Program
Central Data Management
P.O. Box 12233, MD A0-01
Research Triangle Park, NC 27709

SPECIAL FOURTH-CLASS RATE
POSTAGE AND FEES PAID
DHHS/NIH
Permit No. G-763

Official Business
Penalty for Private Use - $300



NIH Publication No. 93-3149
May 1993