

NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 442



TOXICOLOGY AND CARCINOGENESIS

STUDIES OF *p*-NITROBENZOIC ACID

(CAS NO. 62-23-7)

IN F344/N RATS AND B6C3F₁ MICE

(FEED STUDIES)

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

FOREWORD

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

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

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

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

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NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

December 1994

NTP TR 442

NIH Publication No. 95-3358

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

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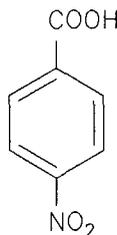
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ABSTRACT



p-NITROBENZOIC ACID

CAS No. 62-23-7

Chemical Formula: $C_7H_5NO_4$

Molecular Weight: 167.12

Synonyms: 4-Nitrobenzoic acid; nitrodracrylic acid; *p*-nitrobenzenecarboxylic acid; *p*-carboxynitrobenzene

p-Nitrobenzoic acid is produced in large volumes for organic synthesis and as an intermediate in the manufacture of pesticides, dyes, and industrial solvents. Groups of male and female F344/N rats and B6C3F₁ mice were exposed to *p*-nitrobenzoic acid (>99% pure) in feed for 14 days, 13 weeks, or 2 years for toxicity and carcinogenicity studies. Genetic toxicology studies were conducted in *in vitro* assays with *Salmonella typhimurium* and cultured Chinese hamster ovary cells, and in *in vivo* studies of erythrocyte micronucleus formation in mice in the 13-week study.

14-DAY STUDY IN RATS

Groups of five male and five female rats were given 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm *p*-nitrobenzoic acid in feed for 14 days. All rats survived until the end of the study. Male and female rats given 20,000 and 40,000 ppm lost weight. The final mean body weights of 10,000, 20,000, and 40,000 ppm males were 82%, 60%, or 52% that of the controls, and the final mean body weights of 10,000, 20,000, and 40,000 ppm females were 87%, 68%, and 65% that of the controls. There were no clinical findings that were characteristic of organ-specific toxicity.

Absolute and relative spleen weights were significantly increased in rats exposed to 10,000, 20,000, and 40,000 ppm. There were decreases in erythrocyte count and hemoglobin and hematocrit values and increases in reticulocyte count, nucleated erythrocytes, and methemoglobin concentration that were most pronounced in the 20,000 and 40,000 ppm groups. Congestion of the spleen occurred in 10,000 ppm males and in 20,000 and 40,000 ppm females. Hypertrophy of the follicular epithelium of the thyroid gland was present in male and female rats exposed to 10,000, 20,000, or 40,000 ppm *p*-nitrobenzoic acid, while follicular hyperplasia was observed in the 40,000 ppm males and females. Atrophy of the testis was observed in 20,000 and 40,000 ppm males. Other lesions observed in 20,000 and 40,000 ppm rats included atrophy of the thymus in males and atrophy of the ovary, bone marrow, and thymus in females.

14-DAY STUDY IN MICE

Groups of five male and five female mice were given 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm *p*-nitrobenzoic acid in feed for 14 days. Three males and two females given 40,000 ppm died during the study. All other animals survived until the end of the

study. Male mice given 20,000 and 40,000 ppm and females given 20,000 ppm lost weight. Mean body weight gains of 20,000 and 40,000 ppm males and 10,000, 20,000, and 40,000 ppm females were significantly lower than those of the controls. There were no clinical findings related to organ-specific toxicity although lethargy and ataxia were observed in 40,000 ppm mice.

Relative liver weights were significantly increased in 20,000 and 40,000 ppm males and females and in 10,000 ppm females. Absolute and relative thymus weights of 20,000 and 40,000 ppm males and of 10,000, 20,000, and 40,000 ppm females were reduced. No significant differences in hematology parameters occurred in exposed mice. Testicular degeneration was observed in three 20,000 ppm and two 40,000 ppm males. Bone marrow hemorrhage and atrophy occurred in 40,000 ppm females.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were given 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm *p*-nitrobenzoic acid in feed for 13 weeks resulting in approximate daily doses of 40, 70, 160, 310, or 660 mg/kg to males and 40, 80, 170, 340, or 680 mg/kg to females. All rats survived until the end of the study. Mean body weight gains and final mean body weights were significantly less than those of the controls in 2,500, 5,000, and 10,000 ppm males and in 5,000 and 10,000 ppm females. There were no clinical findings related to organ-specific toxicity.

Differences in spleen weights and hematology parameters characteristic of regenerative anemia were observed in males and females, primarily in groups given 10,000 ppm. The absolute and relative spleen weights were significantly increased in 10,000 ppm males and females and the relative spleen weights were significantly increased in 5,000 ppm males and females. Methemoglobin, Heinz bodies, and reticulocyte counts were increased and erythrocyte counts, hemoglobin, and hematocrit values were decreased in 10,000 ppm males and females.

Congestion, pigmentation, and accumulation of macrophages in the spleen and pigmentation in the kidney occurred in 2,500, 5,000, and 10,000 ppm males. Congestion and pigmentation of the spleen occurred in 10,000 ppm females. A yellowish brown pigment (hemosiderin) in the spleen and kidney was

associated with hemolytic anemia. Mild cytoplasmic hyaline droplet accumulation was present in renal tubule epithelial cells in 10,000 ppm males while karyomegaly was present in male and female rats exposed to 2,500, 5,000, and 10,000 ppm *p*-nitrobenzoic acid. A chemical-related testicular lesion, consisting of atrophy of the seminiferous tubules, occurred in 10,000 ppm males.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice were given 0, 1,250, 5,000, 10,000, or 20,000 ppm *p*-nitrobenzoic acid in feed for 13 weeks resulting in approximate daily doses of 170, 330, 670, 1,900, or 4,000 mg/kg body weight to males and 240, 460, 970, 2,500, or 4,900 mg/kg to females. All mice survived until the end of the study, except one 1,250 ppm female that was killed accidentally. Final mean body weights and mean body weight gains of all exposed males and of 5,000, 10,000, and 20,000 ppm females were significantly lower than those of the controls. No clinical findings or differences in organ weights or histopathology related to organ-specific toxicity were observed in exposed mice.

2-YEAR STUDY IN RATS

Groups of 60 male and 60 female rats were given 0, 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid in feed for 2 years. Ten males and 10 females from each exposure group were evaluated at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Two-year survival rates of 1,250 and 2,500 ppm males were similar to that of the controls. Two-year survival of 5,000 ppm males was marginally greater than that of the controls and was attributed in part to a decrease in the severity of nephropathy and a decrease in the incidence of mononuclear cell leukemia. Survival of exposed females was similar to that of the controls. Mean body weights of 5,000 ppm males were 2% to 8% lower than those of the controls through week 80. Final mean body weights of exposed males were similar to that of the controls. Mean body weights of 5,000 ppm females were 2% to 9% lower than those of the controls during the first year of the study and were 10% to 16% lower during the second year of the study. Final mean body weights of exposed females were 97% (1,250 ppm), 92% (2,500 ppm), and 84% (5,000 ppm) that of the

controls. Feed consumption by exposed males and females was similar to that by the controls. Dietary levels of 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid delivered approximately 50, 100, or 210 mg/kg body weight per day to males and 60, 125, or 250 mg/kg per day to females. There were no clinical findings attributable to organ-specific toxicity.

Pathology Findings

There were increases in the incidences of clitoral gland adenoma and of clitoral gland adenoma or carcinoma (combined) (4/50, 14/49, 15/49, 15/50) in exposed females. The incidences of clitoral gland adenoma or carcinoma (combined) in the exposed groups (29% to 31%) exceeded the historical control mean incidence (11%) and range (2% to 21%) in female F344/N rats in recent 2-year NTP feed studies. The increased incidences of clitoral gland neoplasms were considered to be some evidence of carcinogenic activity in female rats exposed to *p*-nitrobenzoic acid. The incidences of hyperplasia of the clitoral gland in exposed females were marginally lower than that of the controls (10/50, 6/49, 6/49, 7/50).

There was a chemical-related decrease in the severity of nephropathy in male rats. Male rat kidneys were examined using both single and step-section analyses, and the incidences of renal tubule neoplasms were not statistically greater than those of the controls. Mild hyaline droplet accumulation was observed in renal tubule epithelial cells in 10,000 ppm males in the 13-week study, but this effect was not severe enough to lead to a chemical-related neoplastic response in the 2-year study as has been observed with other chemicals.

At the 15-month interim evaluation, hematologic parameters characteristic of a mild regenerative anemia and significant differences in spleen weights were noted in 5,000 ppm females. These differences included decreases in erythrocyte count, hemoglobin, and hematocrit, increases in spleen weights, and hemosiderin accumulation in splenic macrophages.

At 2 years, significant decreases in the incidences of mononuclear cell leukemia were observed in 5,000 ppm males and 2,500 and 5,000 ppm females (males: 29/50, 35/50, 26/50, 2/50; females: 17/50, 11/50, 3/50, 0/50). While the mechanism for this

decrease is unknown, decreases in the incidence of mononuclear cell leukemia have also been observed in 2-year studies with other amine/nitro compounds.

2-YEAR STUDY IN MICE

Groups of 60 male and 60 female mice were given 0, 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid in feed for 2 years. Ten males and 10 females from each exposure group were evaluated at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Two-year survival rates of exposed mice were similar to those of the controls. Mean body weights of 5,000 ppm males were 6% to 12% lower than those of the controls after week 17, and mean body weights of 5,000 ppm females were 12% to 24% lower than those of the controls after week 16. The final mean body weight of 5,000 ppm females was 19% less than that of the controls; final mean body weights of males were similar to that of the controls. Feed consumption by exposed mice was similar to that by the controls. Dietary levels of 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid delivered approximately 150, 300, or 675 mg/kg per day to males and 170, 365, or 905 mg/kg per day to females. There were no clinical findings of organ-specific toxicity. No chemical-related effects on hematology parameters were noted at the 15-month interim evaluation.

Pathology Findings

There were no increases or decreases in neoplasms in male or female mice that were considered to be related to chemical administration.

GENETIC TOXICOLOGY

p-Nitrobenzoic acid was mutagenic in *Salmonella typhimurium* strain TA100 with and without S9. No mutagenic activity was noted in strains TA98, TA1535, or TA1537, with or without S9. *p*-Nitrobenzoic acid induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells in the absence of S9; with S9, results of both tests were negative. *In vivo*, no increase in micronuclei was observed in peripheral blood erythrocytes of male or female mice administered *p*-nitrobenzoic acid in dosed feed for 13 weeks.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of *p*-nitrobenzoic acid in male F344/N rats exposed to 1,250, 2,500, or 5,000 ppm. There was *some evidence of carcinogenic activity* of *p*-nitrobenzoic acid in female F344/N rats based on increases in the incidences of clitoral gland adenoma and of clitoral gland

adenoma or carcinoma (combined). There was *no evidence of carcinogenic activity* of *p*-nitrobenzoic acid in male or female B6C3F₁ mice exposed to 1,250, 2,500, or 5,000 ppm.

There were chemical-related decreases in the incidences of mononuclear cell leukemia in exposed male and female rats. *p*-Nitrobenzoic acid caused mild hematologic toxicity in female rats.

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

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of p-Nitrobenzoic Acid

| | Male F344/N Rats | Female F344/N Rats | Male B6C3F ₁ Mice | Female B6C3F ₁ Mice |
|--|---|--|--|--|
| Doses | 0, 1,250, 2,500, or 5,000 ppm in feed (approximately 50, 100, or 210 mg/kg/day) | 0, 1,250, 2,500, or 5,000 ppm in feed (approximately 60, 125, or 250 mg/kg/day) | 0, 1,250, 2,500, or 5,000 ppm in feed (approximately 150, 300, or 675 mg/kg/day) | 0, 1,250, 2,500, or 5,000 ppm in feed (approximately 170, 365, or 905 mg/kg/day) |
| Body weights | Dosed groups similar to control | High- and mid-dose groups lower than control | High-dose group lower than control | High-dose group lower than control |
| 2-Year survival rates | 12/50, 13/50, 13/50, 21/50 | 27/50, 23/50, 21/50, 21/50 | 39/50, 36/50, 39/50, 44/50 | 38/50, 36/49, 33/50, 30/50 |
| Nonneoplastic effects | None | Mild hematologic toxicity | None | None |
| Neoplastic effects | None | Clitoral gland: adenoma (4/50, 12/49, 10/49, 12/50), carcinoma (1/50, 2/49, 5/49, 4/50), adenoma or carcinoma (combined) (4/50, 14/49, 15/49, 15/50) | None | None |
| Decreased incidences | Mononuclear cell leukemia (29/50, 35/50, 26/50, 2/50) | Mononuclear cell leukemia (17/50, 11/50, 3/50, 0/50) | None | None |
| Level of evidence of carcinogenic activity | No evidence | Some evidence | No evidence | No evidence |
| Genetic toxicology | | | | |
| <i>Salmonella typhimurium</i> gene mutation: | | Positive in strain TA100 with and without S9; negative in strains TA98, TA1535, and TA1537, with and without S9 | | |
| Sister chromatid exchanges | | | | |
| Cultured Chinese hamster ovary cells <i>in vitro</i> : | | Positive without S9; negative with S9 | | |
| Chromosomal aberrations | | | | |
| Cultured Chinese hamster ovary cells <i>in vitro</i> : | | Positive without S9; negative with S9 | | |
| Micronuclei in mouse peripheral blood cells: | | Negative at 13 weeks | | |

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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*-nitrobenzoic acid on June 22, 1993, 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.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 22, 1993, the draft Technical Report on the toxicology and carcinogenesis studies of *p*-nitrobenzoic acid 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. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of *p*-nitrobenzoic acid by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on chemical-related neoplastic lesions in female rats and nonneoplastic lesions in male (nephropathy) and female (hematologic toxicity) rats. Additional step-sections of the kidney were performed in male rats. The proposed conclusions were *no evidence of carcinogenic activity* in male F344/N rats, *some evidence of carcinogenic activity* in female F344/N rats, and *no evidence of carcinogenic activity* in male or female B6C3F₁ mice.

Dr. Brown, a principal reviewer, agreed with the proposed conclusions. He asked for comment on the seemingly paradoxical decrease in the incidence of mononuclear cell leukemia in exposed rats and the increased weight of the spleen. Dr. Ward noted that there was hematopoietic toxicity associated with the chemical and speculated that the stem cell in the bone marrow or spleen from which the leukemia derives may be one of the targets of the chemical resulting in an inhibition of leukemogenesis.

Dr. van Zwieten, the second principal reviewer, agreed with the proposed conclusions. He asked for substantiation of the conclusion that preputial gland and clitoral gland neoplasms were potentially lethal, because, in his experience, these neoplasms tend to be quite small and well circumscribed. Dr. S.L. Eustis, NIEHS, responded that the preputial gland neoplasms are not lethal in the sense of causing the animal's death, but as they get quite large with some becoming ulcerated, the animals are killed. Dr. J.K. Haseman, NIEHS, added that if a neoplasm were incidental, one would expect it to be more or less

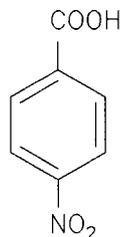
evenly distributed among the animals that died naturally and those that survived. However, in this study, the likelihood of observing a preputial gland neoplasm in an animal that died early was almost three times as high as in a surviving animal.

Dr. Ryan, the third principal reviewer, deferred her opinion of the proposed conclusions pending further discussion of exposure-related effects on clitoral gland and preputial gland lesions. She said there were inconsistencies in how body weight differences were discussed. For instance, decreased body weight in rats is offered as a possible explanation for the exposure-related decrease in leukemia. On the other hand, lack of an exposure-response for clitoral gland neoplasms was the main reason for some evidence rather than clear evidence for female rats, but was likely due, in her opinion, also to decreased body weight. Dr. Dunnick said the conclusion in female rats was based primarily on there being increases in neoplasms, mostly adenomas, at all three exposure levels. She agreed that body weight can affect the incidence of neoplasms, but the decrease in leukemia was believed to be more of a chemical effect than a body weight effect. Based on preputial gland neoplasms, Dr. Haseman said it was a close call between no evidence and equivocal evidence of carcinogenic activity in male rats. Dr. Eustis noted that the incidence of preputial gland carcinoma at the highest exposure level was within the historical control range.

Dr. Ward asked for comment on the presence of hyaline droplets in the kidneys of rats in subchronic studies and whether they were associated with $\alpha_2\mu$ -globulin accumulation. Dr. Eustis said there was no evidence for accumulation of $\alpha_2\mu$ -globulin in this study.

Dr. Brown moved that the Technical Report on *p*-nitrobenzoic acid be accepted with the revision discussed and with the conclusions as written for male rats and male and female mice, *no evidence of carcinogenic activity*, and for female rats, *some evidence of carcinogenic activity*. Dr. Taylor seconded the motion, which was accepted unanimously with ten votes.

INTRODUCTION



p-NITROBENZOIC ACID

CAS No. 62-23-7

Chemical Formula: $C_7H_5NO_4$

Molecular Weight: 167.12

Synonyms: 4-Nitrobenzoic acid; nitrodraclic acid; *p*-nitrobenzenecarboxylic acid; *p*-carboxynitrobenzene

CHEMICAL AND PHYSICAL PROPERTIES

p-Nitrobenzoic acid is a yellow-to-white crystalline material with no odor. It has a density of 1.61 at 20° C, and a melting point of 242° C. It is insoluble in water and petroleum ether; slightly soluble in acetone, benzene, and carbon disulfide; and soluble in methyl alcohol (1 g/110 mL) and ethanol (1 g/110 mL). The pK_a of *p*-nitrobenzoic acid is 3.4 (Sax, 1979; *Merck Index*, 1983; Lide, 1992).

USE AND HUMAN EXPOSURE

p-Nitrobenzoic acid is used in organic synthesis, in the manufacture of intermediates, and as a reagent for alkaloids and thorium (*Merck Index*, 1983). Nitrobenzoates are used in the manufacture of pesticides, dyes, explosives, and industrial solvents (Groenewegen *et al.*, 1992). Exposure to *p*-nitrobenzoic acid may occur through exposure to other chemicals that are metabolized or hydrolyzed to *p*-nitrobenzoic acid, including *p*-nitrobenzoyl chloride (Radding, 1977), *p*-nitrotoluene (Chism *et al.*, 1984), and 5-(4-nitrophenyl)-2,4-pentadienal (spy dust) (Burka *et al.*, 1987).

p-Nitrobenzoic acid was not found in a survey of 717 hazardous waste sites (USEPA, 1987). *p*-Nitrobenzoic acid is metabolized under aerobic and anaerobic conditions by bacteria, as well as when mixed with a representative municipal sewage

sample, suggesting that under natural conditions bacteria would metabolize the chemical (Hallas and Alexander, 1983).

The U.S. International Trade Commission (USITC) did not report the production volume for *p*-nitrobenzoic acid or *p*-nitrobenzoyl chloride for 1988 (USITC, 1989). Other sources estimate the production of *p*-nitrobenzoic acid at 450 to 900 tons per year (4 to 8×10^6 kg/year), the production of *p*-aminobenzoic acid, a major metabolite of *p*-nitrobenzoic acid, at 250 tons/year (2×10^6 kg/year) (Kirk-Othmer, 1978), and the production of *p*-nitrobenzoyl chloride at 3×10^6 kg/year (NCI, 1980). The National Institute for Occupational Safety and Health (NIOSH) has estimated that there are 42,700 workers potentially exposed to *p*-nitrobenzoic acid in 16 different industries (NIOSH, 1993).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

The metabolism of nitroaromatic compounds varies with species of animal and with the isomeric configuration of the chemical, as has been noted for some representative nitroaromatic chemicals such as nitrobenzene and *o*-, *m*-, and *p*-nitrotoluene (Rickert, 1987; NTP, 1992). One common pathway for metabolism of nitroaromatic compounds is reduction of the nitro groups (Rickert, 1987).

Nitroreductase activities are found both in mammalian tissues and in gastrointestinal microflora, and both can contribute to the eventual metabolism of nitroaromatic compounds (Zachariah and Juchau, 1974). In mammalian species, two nitro-reducing systems have been characterized in the liver, including one associated with the endoplasmic reticulum of the liver and one with the soluble cellular fractions (Carlson and Dubois, 1970; Mitchard, 1971). *Escherichia coli* and other bacteria contain various enzymes capable of reducing *p*-nitrobenzoic acid to *p*-aminobenzoic acid (Saz and Martinez, 1956; Thijssen and Henderson, 1973). Intestinal microbial flora appear to be responsible for at least some of the *in vivo* reduction of *p*-nitrobenzoic acid because the intestinal contents of rats receiving antibiotics indicated diminished reduction of *p*-nitrobenzoic acid (Zachariah and Juchau, 1974; Gardner and Renwick, 1978). Germ-free rats converted about 1% of *p*-nitrobenzoic acid to *p*-aminobenzoic acid, while conventional rats converted 25% to *p*-aminobenzoic acid (Wheeler *et al.*, 1975).

In addition to reduction of the nitro group, *p*-nitrobenzoic acid metabolism may occur by a number of pathways involving conjugation of the carboxylic acid

group with glycine or glucuronic acid and reduction to *p*-aminobenzoic acid, which may then be conjugated at the carboxylic acid group or acetylated at the amino substituent (Williams, 1959).

Nitroaromatic compounds are characteristically toxic to the hematopoietic system (NTP, 1992). Aromatic and heterocyclic nitro compounds require reduction of the nitro group for the expression of these toxicologic activities (McCalla and Voutsinos, 1974; Wheeler *et al.*, 1975; Reddy *et al.*, 1976). A common feature of chemicals that cause hematopoietic toxicity characterized by methemoglobin formation is a free aromatic amine functional group. Hydroxylation of this aromatic amine group to a phenylhydroxylamine is thought to account for the formation of methemoglobinemia and the subsequent hematologic toxicity (Bus and Popp, 1987). This is supported by studies that show that hydroxylamino compounds produce methemoglobin *in vivo* and *in vitro*, and arylamines or nitrobenzenes themselves cannot oxidize hemoglobin *in vitro* (Facchini and Griffiths, 1981). Using liver homogenates from Wistar rats, Kato *et al.* (1969) identified *p*-hydroxylamino benzoic acid as a metabolite of *p*-nitrobenzoic acid, and a metabolic scheme (Figure 1) for *p*-nitrobenzoic acid has been proposed (Gillette *et al.*, 1968; Mitchard, 1971).

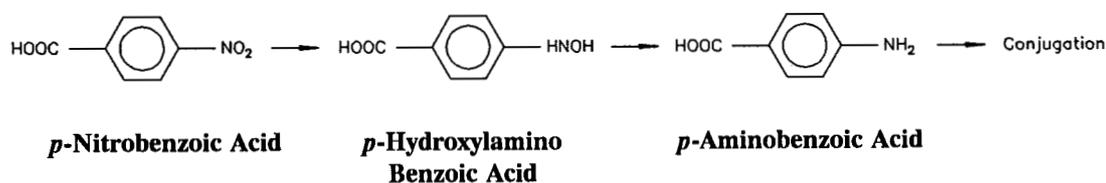


FIGURE 1
Metabolic Pathway of *p*-Nitrobenzoic Acid
[Proposed by Gillette *et al.* (1968) and Mitchard (1971)]

Quantitative information on pharmacokinetics, distribution, and elimination of metabolites of *p*-nitrobenzoic acid *in vivo* is limited. After an oral or intraperitoneal dose of 25 mg [¹⁴C]-*p*-nitrobenzoic acid, female Wistar rats excreted 83% to 94% of the radiolabel in the urine within 24 hours. Urine metabolites were 2% free *p*-aminobenzoic acid, 18% conjugated aminobenzoic acid, 42% *p*-nitrobenzoic acid, and 13% conjugated *p*-nitrobenzoic acid (Gardner and Renwick, 1978). Studies to quantify the urinary metabolites of *p*-nitrobenzoic acid in the F344 rat and B6C3F₁ mouse have not been conducted.

Pharmacokinetic studies in marmosets receiving oral doses of 4-nitro[carboxy-¹⁴C]benzoic acid (0.4 mmol/kg) found that peak blood levels were reached in 30 to 40 minutes and the terminal half-life of the chemical in the blood was estimated at 1 hour. Distribution and elimination of metabolites could not be determined from these studies (Kuzniar and James, 1981).

Humans

No information on the absorption, distribution, metabolism, or excretion of *p*-nitrobenzoic acid in humans was found in the literature.

TOXICITY

Experimental Animals

Methemoglobin formation and hematopoietic toxicity are found after administration of aromatic nitro and amino compounds, and this toxicity is often more severe in rats than in mice (Beard and Noe, 1981; Rickert, 1987; NTP, 1992). For example, in studies of *o*-, *m*-, and *p*-nitrotoluene hematologic toxicity was characterized by increased methemoglobin, Heinz body formation, and hematopoiesis and by hemosiderin deposition and congestion in the spleen of rats.

p-Nitrobenzoic acid was reported to have LD₅₀ values of 1.47, 0.88, and 0.77 g/kg in female Swiss mice after oral, intraperitoneal, and intravenous administration, respectively. The oral LD₅₀ was 1.96 g/kg and the intravenous LD₅₀ was 1.21 g/kg in female Wistar rats. Rats receiving LD₅₀ levels of *p*-nitrobenzoic acid had liver infiltration with red blood cells and myeloid metaplasia of the red pulp of the spleen (Caujolle *et al.*, 1966).

Humans

No information on the toxicity of *p*-nitrobenzoic acid in humans was found in the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Chapin *et al.* (1987) evaluated the effects of *p*-nitrobenzoic acid on sperm morphology and vaginal cytology in rats and mice exposed to 0, 630 (rats), 1,250, 2,500, 5,000, 10,000, or 20,000 (mice) ppm *p*-nitrobenzoic acid in feed for 13 weeks. Final body weights of 5,000 and 10,000 ppm male rats were 8% to 16% lower than that of the controls. The sperm count and right caudal, epididymal, and testis weights were reduced in 10,000 ppm male rats. Final body weights of 10,000 and 20,000 ppm male mice were 14% to 31% lower than those of the controls. In mice receiving 20,000 ppm, the right caudal, epididymal, and testis weights were reduced, but the sperm count was not significantly reduced. At lower exposure levels, there were no chemical-related effects in male rats and only minor effects in male mice. Significantly reduced body weights were observed in 10,000 ppm female rats and in 20,000 ppm female mice. Some of these females had prolonged estrous cycles that were apparently related to the reduced body weights (Chapin *et al.*, 1987).

Continuous breeding studies were conducted in Swiss (CD-1[®]) mice exposed to 7,500 or 15,000 ppm *p*-nitrobenzoic acid *ad libitum* in feed for a 7-day pre-cohabitation period followed by a 98-day cohabitation period (Hope *et al.*, 1990). Final body weights of 15,000 ppm males and females were 93% and 88% that of the corresponding control groups, respectively. Feed consumption was similar between exposed and control groups. Pairs of mice exposed to 7,500 and 15,000 ppm had fewer litters and fewer live pups per litter and their pups weighed less than those of pairs receiving control feed. Crossover matings of exposed F₁ females to control males also resulted in fewer live pups per litter and lower pup weights, indicating that reproductive toxicity was primarily due to effects in females. Hope *et al.* (1990) concluded that the general toxic effects of *p*-nitrobenzoic acid (as measured by decreases in body weight of exposed animals) were not severe enough to cause impairment of fertility and reproduction, and the studies did not identify the mechanism responsible for this toxicity.

Humans

No information on reproductive and developmental toxicity of *p*-nitrobenzoic acid in humans was found in the literature.

CARCINOGENICITY

Experimental Animals

No studies describing the carcinogenic potential of *p*-nitrobenzoic acid in experimental animals were found in the literature.

Humans

No published information on carcinogenic potential of *p*-nitrobenzoic acid in humans is available.

GENETIC TOXICITY

p-Nitrobenzoic acid was positive, in the absence of S9 activation, in the *Bacillus subtilis* rec assay for growth inhibition due to DNA damage (Shimizu and Yano, 1986), and it induced gene mutations in *Salmonella typhimurium*, with and without S9 (Chiu *et al.*, 1978; Sundvall *et al.*, 1984; Shimizu and Yano, 1986; Zeiger *et al.*, 1987; Dellarco and Prival, 1989). No induction of unscheduled DNA synthesis was noted in rat hepatocytes treated *in vitro* with up to 1,000 nmol *p*-nitrobenzoic acid/mL (Probst *et al.*, 1981). Unpublished NTP data show that *p*-nitrobenzoic acid induces sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. However, no increase in the frequency of micronucleated erythrocytes was observed in the peripheral blood of male and female mice administered *p*-nitrobenzoic acid in feed for 13 weeks (Appendix E).

The structural analogue, *m*-nitrobenzoic acid, was also positive in the *B. subtilis* rec assay (Shimizu and

Yano, 1986) and *S. typhimurium* gene mutation assays (Chiu *et al.*, 1978; Sundvall *et al.*, 1984; Shimizu and Yano, 1986; Zeiger *et al.*, 1987). Unpublished NTP data show no induction of chromosomal aberrations or sister chromatid exchanges in cultured Chinese hamster ovary cells and no increase in the frequency of micronucleated erythrocytes in the blood of male or female mice receiving *m*-nitrobenzoic acid in feed for 13 weeks. *o*-Nitrobenzoic acid did not induce chromosomal aberrations in cultured Chinese hamster ovary cells, but it did induce sister chromatid exchanges (NTP, unpublished data) and, like the *p*- and *m*-isomers, it was mutagenic in the *S. typhimurium* assay (Zeiger *et al.*, 1987).

STUDY RATIONALE

p-Nitrobenzoic acid is a hydrolysis product of *p*-nitrobenzoyl chloride. *p*-Nitrobenzoyl chloride was originally nominated for testing by the National Cancer Institute because it is an acyl chloride and aromatic nitro compound with a large import volume (10,000 kg/year). However, because *p*-nitrobenzoyl chloride is unstable in feed and undergoes rapid hydrolysis to *p*-nitrobenzoic acid, *p*-nitrobenzoic acid was selected for study. *p*-Nitrobenzoic acid was selected because of workplace exposure through its use in manufacturing chemical intermediates, because it is a metabolite of other chemicals, and because of the lack of existing information on its chronic toxic and carcinogenic effects.

Interest in the potential carcinogenicity of *p*-nitrobenzoic acid is also based on its structure as a single, aromatic, nitro compound and the finding that other members of this chemical class are carcinogenic in rodents (Clayson and Garner, 1976; Ashby and Tennant, 1991).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *p*-NITROBENZOIC ACID

p-Nitrobenzoic acid was obtained from E.I. du Pont de Nemours and Company, Inc. (Wilmington, DE), in one lot (40). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix H). Reports on the analyses performed in support of the *p*-nitrobenzoic acid studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a light yellow, crystalline solid, was identified as *p*-nitrobenzoic acid by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopies. Purity was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography, and high-performance liquid chromatography. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for *p*-nitrobenzoic acid. Karl Fischer analysis indicated 0.08% water. Functional group titration indicated a purity of 100.1%. Thin-layer chromatography using two systems detected one major spot and one trace impurity. No impurities with areas greater than 0.1% relative to the major peak area were observed using high-performance liquid chromatography. The overall purity was determined to be greater than 99%.

Stability studies performed using high-performance liquid chromatography indicated that *p*-nitrobenzoic acid was stable when stored in the dark for 2 weeks at temperatures up to 60° C. The study laboratory stored the bulk chemical in sealed containers, protected from light, at room temperature. Purity and stability were monitored during the 2-year study by high-performance liquid chromatography and functional group titration. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once in the 14-day studies, every 2 weeks in the 13-week studies, and weekly in the 2-year studies by mixing *p*-nitrobenzoic acid and feed (Table H1). Homogeneity and stability studies of the 400 ppm concentration were performed by Midwest Research Institute using high-performance liquid chromatography. Homogeneity was confirmed, and the stability of the dose formulations when stored in the dark at room temperature was confirmed for at least 3 weeks. Dose formulations open to air and light were stable for 1 week.

Periodic analyses of the dose formulations of *p*-nitrobenzoic acid were conducted at the study laboratory and analytical chemistry laboratory using high-performance liquid chromatography. Dose formulations were analyzed once during the 14-day studies and were within 10% of the target concentrations (Table H2). Dose formulations for the 13-week studies were analyzed pre-study, during week 1, at study mid-point, and at the final mix (Table H3). During the 2-year studies, the dose formulations were analyzed approximately every two months (Table H4). All dose formulations were within 10% of the target concentrations during the 13-week studies; 95% (160/168) of the formulations were within 10% of the target concentrations during the 2-year studies. 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 H5).

14-DAY STUDIES

The 14-day studies were conducted to evaluate the cumulative toxic effects of repeated exposure to *p*-nitrobenzoic acid and to determine the appropriate doses to be used in the 13-week studies.

Male and female F344/N rats and B6C3F₁ mice were obtained from Frederick Cancer Research Facility

(Frederick, MD). At receipt, the animals were 4 weeks old. The rats were quarantined for 16 days before dosing began; the mice were quarantined for 15 days. Before the beginning of the studies, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Groups of five male and five female rats and mice received 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm *p*-nitrobenzoic acid in feed for 14 days. Water and feed were available *ad libitum*. Feed consumption was measured twice weekly for rats and weekly for mice. Clinical observations were recorded twice daily. Animals were weighed at the beginning of the studies and weekly thereafter. Rats were housed five per cage; mice were housed individually.

At the end of the studies, blood was collected from the orbital sinus for hematology analyses. Automated hematologic determinations, except platelet counts, were performed using a Baker Series 7000 cell counter; platelet counts were determined using a Baker Series 810 whole blood platelet analyzer (Baker Instruments, Allentown, PA). Reagents were obtained from Baker Instruments. The clinical pathology parameters measured are listed in Table 1. The brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus of all surviving animals were weighed. A necropsy was performed on all animals. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 6 μ m, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all controls and all 40,000 ppm animals at the end of the studies. Table 1 lists the tissues and organs routinely examined.

13-WEEK STUDIES

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

Male and female F344/N rats and B6C3F₁ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). At receipt, the animals were 3 to

4 weeks old. The rats were quarantined for 13 days before dosing began; the mice were quarantined for 11 days. Before the beginning of the studies, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats received 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm *p*-nitrobenzoic acid in feed for 13 weeks. Groups of 10 male and 10 female mice received 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm *p*-nitrobenzoic acid in feed for 13 weeks. The brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus of all surviving animals were weighed.

Water and feed were available *ad libitum*. Feed consumption was measured weekly. Clinical observations were recorded twice daily. Animals were weighed at the beginning of the studies and weekly thereafter. Rats were housed five per cage; mice were housed individually.

Special study groups of 10 male and 10 female rats received 0, 630, 2,500, or 10,000 ppm *p*-nitrobenzoic acid for 13 weeks. On days 7, 30, 60, and 90, blood samples were collected from the orbital sinus for hematology and clinical chemistry analyses. Hematology analyses were performed as in the 14-day studies. Clinical chemistry parameters were measured using a Centrifichem-400 chemistry analyzer (Baker Instruments). Reagents were obtained from Baker Instruments or Sigma Diagnostics (St. Louis, MO). The clinical pathology parameters measured are listed in Table 1.

A necropsy was performed on all core study animals. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 6 μ m, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all controls, all animals dying before the end of the studies, and all 10,000 ppm rats and 20,000 ppm mice surviving to the end of the studies. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 60 male and 60 female rats and mice received 0, 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid in feed for 103 weeks. Ten male and 10 female rats and mice from each group were evaluated at 15 months.

Source and Specification of Animals

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. The animals were quarantined for 12 days before the beginning of the studies. Five male and five female rats and mice were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. Rats and mice were approximately 6 weeks old at the beginning of the 2-year studies. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats were housed five per cage; mice were housed individually. Feed and water were available *ad libitum*. Feed consumption was measured every 4 weeks. Cages were rotated twice a week for rats and once a week for mice; racks were rotated every two weeks during the studies. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical observations and body weights were recorded at study initiation, weekly for 13 weeks, and monthly thereafter. Blood samples were collected from the retro-orbital sinus at the 15-month interim evaluations for hematology analyses. Automated determinations were performed using an Ortho ELT-8 hematology analyzer (Ortho Instruments, Westwood, MA). Methemoglobin was measured using the Roche Cobas Fara (Roche Diagnostic Systems, Inc., Montclair, NJ). Reagents were obtained from the instrument manufacturer. The clinical pathology parameters measured are listed in Table 1. The right kidney, liver, and spleen were weighed at the 15-month interim evaluations.

A 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. Complete histopathologic examinations were performed on all animals. 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 microscope 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 pathology 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 by the quality assessment laboratory. The quality assessment pathologist microscopically reviewed the clitoral gland, kidney, liver, preputial gland, spleen, stomach, and uterus of all rats to confirm the incidences of neoplasms and nonneoplastic lesions. For mice, the quality assessment pathologist reviewed the forestomach, kidney, liver, lung, and thyroid gland to confirm the incidences of neoplasms and nonneoplastic lesions. In addition, each tissue with a neoplasm diagnosis from all rats and mice was microscopically reviewed.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed representative examples of potential chemical-related lesions including neoplasms of the clitoral gland, kidney, liver, preputial gland, spleen, and thyroid gland from rats; the forestomach, kidney, liver, lung, and thyroid gland from mice; and any other tissues when there was disagreement in diagnosis between the laboratory and quality assessment pathologist. Examples of disagreements in diagnoses between the laboratory and quality assessment pathologist 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 knowledge of dose groups or previously rendered diagnoses. When the PWG

consensus differed from the opinion 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 analysis 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 found dead of other than natural causes or found to be missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and of all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, hardierian gland, and mammary gland) before microscopic evaluation or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

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 the fit of the model was not significantly enhanced. The neoplasm incidences of exposed 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 methods 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 exposed group with controls, and a test for an overall dose-related 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).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and

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

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of lesion incidence in certain instances. 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. Step-section historical data is taken from other NTP technical reports.

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, these studies 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 staff 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 discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicology of *p*-nitrobenzoic acid was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of *p*-nitrobenzoic acid are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of *p*-Nitrobenzoic Acid

| 14-Day Studies | 13-Week Studies | 2-Year Studies |
|---|--|---|
| Study Laboratory Microbiological Associates, Inc. (Bethesda, MD) | Microbiological Associates, Inc. (Bethesda, MD) | Southern Research Institute (Birmingham, AL) |
| Strain and Species Rats: F344/N Mice: B6C3F ₁ | Rats: F344/N Mice: B6C3F ₁ | Rats: F344/N Mice: B6C3F ₁ |
| Animal Source Frederick Cancer Research Facility (Frederick, MD) | Simonsen Laboratories, Inc. (Gilroy, CA) | Taconic Laboratory Animals and Services (Germantown, NY) |
| Size of Study Groups 5 males and 5 females | Core study group: 10 male and 10 female rats and mice Special study group: 10 male and 10 female rats | 60 males and 60 females |
| Time Held Before Studies Rats: 16 days Mice: 15 days | Rats: 13 days Mice: 11 days | 12 days |
| Average Age When Studies Began 6 weeks | 5-6 weeks | 44 days |
| Date of First Dose Rats: 13 December 1985 Mice: 12 December 1985 | Rats: 22 May 1986 Mice: 20 May 1986 | Rats: 11 May 1988 Mice: 25 May 1988 |
| Duration of Dosing 14 days | 13 weeks | 103 weeks |
| Date of Last Dose Rats: 26 December 1985 Mice: 25 December 1985 | Rats: August 1986 Mice: August 1986 | Rats: 1 May 1990 Mice: 15 May 1990 |
| Method of Sacrifice CO ₂ and exsanguination | CO ₂ and exsanguination | CO ₂ and exsanguination |

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of *p*-Nitrobenzoic Acid (continued)

| 14-Day Studies | 13-Week Studies | 2-Year Studies |
|--|---|---|
| Necropsy Dates | | |
| Rats: 27 December 1985 Mice: 26 December 1985 | Rats: August 1986 Mice: August 1986 | Rats: 9-10 May 1990 (males), 10-11 May 1990 (females) Mice: 23-24 May 1990 (males) 24-29 May 1990 (females) |
| Average Age at Necropsy | | |
| 8 weeks | 18-19 weeks | 772-778 days |
| Method of Animal Distribution | | |
| Animals were randomized by weight with a computer randomization program. | Same as 14-day studies | Same as 14-day studies |
| Animals per Cage | | |
| Rats: 5 Mice: 1 | Rats: 5 Mice: 1 | Rats: 5 Mice: 1 |
| Method of Animal Identification | | |
| Ear clip and toe clip | Ear clip and toe clip | Toe clip |
| Diet | | |
| NIH-07 Open Formula Diet (powdered) (Zeigler Brothers, Inc., Gardners, PA), available <i>ad libitum</i> | Same as 14-day studies | NIH-07 Open Formula Mash (Zeigler Brothers, Inc., Gardners, PA), available <i>ad libitum</i> |
| Maximum Storage Time for Feed | | |
| 120 days after milling | 120 days after milling | 120 days after milling |
| Feeders | | |
| Rats: Stainless steel (Hahns Roofing and Sheet Metal Company, Birmingham, AL), changed twice weekly Mice: Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed weekly | Stainless steel (Hahns Roofing and Sheet Metal Company, Birmingham, AL), changed weekly | Stainless steel (Lab Products, Maywood, NY; Hoeltge, Inc., Cincinnati, OH; or Automated Precision, Madison, AL), changed weekly |
| Water | | |
| Automatic watering system (Edstrom Industries, Inc., Waterford, WI), available <i>ad libitum</i> | Same as 14-day studies | Same as 14-day studies |

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of *p*-Nitrobenzoic Acid (continued)

| 14-Day Studies | 13-Week Studies | 2-Year Studies |
|---|---|---|
| Cages Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed weekly for rats and twice weekly for mice | Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed twice weekly for rats and weekly for mice | Polycarbonate (Lab Products, Maywood, NJ), changed twice weekly for rats and weekly for mice |
| Bedding BetaChips® (Northeastern Product Corporation, Warrensburg, NY), changed weekly for rats and twice weekly for mice | BetaChips® (Northeastern Product Corporation, Warrensburg, NY), changed twice weekly for rats and weekly for mice | Sani-Chips (P.J. Murphy Forest Products Corporation, Montville, NJ), changed twice weekly for rats and weekly for mice |
| Cage Filters Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH), changed once every 2 weeks | Same as 14-day studies | Remay® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks |
| Racks Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed once every 2 weeks | Same as 14-day studies | Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks |
| Animal Room Environment Average temperature: 22° C (rats), 21° C (mice) Relative humidity: 59% (rats), 45% (mice) Fluorescent light: 12 hours/day Room air changes: minimum of 12 changes/hour | Average temperature: 22° C Relative humidity: 71% (rats), 72% (mice) Fluorescent light: 12 hours/day Room air changes: minimum of 12 changes/hour | Average temperature: 22° C Relative humidity: 50%-51% Fluorescent light: 12 hours/day Room air changes: minimum of 10 changes/hour |
| Doses 0, 2,500, 5,000, 10,000, 20,000, or 40,000 <i>p</i> -nitrobenzoic acid in feed, available <i>ad libitum</i> | Rats: 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm <i>p</i> -nitrobenzoic acid in feed, available <i>ad libitum</i> Mice: 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm <i>p</i> -nitrobenzoic acid in feed, available <i>ad libitum</i> | 0, 1,250, 2,500, or 5,000 ppm <i>p</i> -nitrobenzoic acid in feed, available <i>ad libitum</i> |
| Type and Frequency of Observation Animals were observed and clinical observations were recorded twice daily; animals were weighed on days 1, 8, and 15. Feed consumption was measured twice weekly for rats and once weekly for mice. | Animals were observed and clinical observations were recorded twice daily; animals were weighed initially and weekly thereafter. Feed consumption was measured weekly. | Animals were observed twice daily. Clinical observations and body weights were recorded initially, weekly during first 13 weeks, and monthly thereafter. Feed consumption was measured every 4 weeks. |

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of *p*-Nitrobenzoic Acid (continued)

| 14-Day Studies | 13-Week Studies | 2-Year Studies |
|--|---|---|
| <p>Necropsy Necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus.</p> | <p>Necropsy was performed on all core animals. Organs weighed were brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus.</p> | <p>Necropsy was performed on all animals. Organs weighed at 15 months were right kidney, liver, and spleen.</p> |
| <p>Clinical Pathology Blood samples were collected from the orbital sinus of all animals at necropsy. Hematology: hematocrit, hemoglobin, erythrocytes, reticulocytes, mean erythrocyte volume, leukocyte count and differential, and methemoglobin</p> | <p>Blood samples were collected from the orbital sinus of special study rats on days 7, 30, 60, and 90 at exposure levels of 0, 630, 2,500, and 10,000 ppm. Hematology: hematocrit, hemoglobin, erythrocytes, reticulocytes, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, leukocyte count and differential, Heinz bodies, and methemoglobin Clinical Chemistry: alkaline phosphatase, alanine aminotransferase, and sorbitol dehydrogenase</p> | <p>Blood samples were collected from the retroorbital sinus of all animals at the 15-month interim evaluation. Hematology: hematocrit, hemoglobin, erythrocytes, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, platelets, reticulocytes, leukocyte count and differential, and methemoglobin</p> |
| <p>Histopathology Complete histopathologic examinations were performed on all controls and all 40,000 ppm animals at the end of the studies. In addition to gross lesions, the tissues examined included: adrenal gland, bone and marrow, brain, epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Selected organs and gross lesions were examined in lower exposure groups until a no-effect level was observed. Selected organs were bone marrow, ovary (rats), testis, thymus (rats), thyroid gland (rats), and spleen (rats).</p> | <p>Except for special study rats, complete histopathologic examinations were performed on all controls, all animals dying before the end of the studies, and all 10,000 ppm rats and 20,000 ppm mice surviving to the end of the studies. In addition to gross lesions, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland (rats), epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.</p> | <p>Complete histopathologic examinations were performed on all animals. In addition to gross lesions, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.</p> |

RESULTS

RATS

14-DAY STUDY

All rats survived to the end of the study (Table 2). Mean body weight gains were significantly reduced in males exposed to 10,000 ppm, and males and females exposed to 20,000 and 40,000 ppm lost weight. Males and females exposed to 10,000, 20,000, and 40,000 ppm had final mean body weights significantly lower than those of the controls. There were no clinical findings relating to organ-specific toxicity, although animals in the 40,000 ppm groups were lethargic during the second week on study. In most

of the groups that lost weight, there was a reduction in feed consumption (Table 2). Feed spillage was not measured. Feed consumption by the other exposure groups was similar to that by the controls. Dietary levels of 2,500, 5,000, 10,000, 20,000, and 40,000 ppm *p*-nitrobenzoic acid resulted in average daily doses of 240, 450, 810, 1,170, and 2,260 mg/kg body weight to males and 230, 430, 840, 930, and 2,840 mg/kg to females. The estimate for 40,000 ppm females may exceed the actual value because of scattering of feed.

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 14-Day Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) | Feed Consumption ^c | |
|---------------|-----------------------|-----------------------------------|-----------|-----------|---------------------------------------|-------------------------------|--------|
| | | Initial | Final | Change | | Week 1 | Week 2 |
| Male | | | | | | | |
| 0 | 5/5 | 152 ± 5 | 210 ± 4 | 58 ± 4 | | 16.3 | 16.4 |
| 2,500 | 5/5 | 145 ± 5 | 205 ± 6 | 60 ± 2 | 98 | 16.2 | 17.4 |
| 5,000 | 5/5 | 145 ± 3 | 198 ± 3 | 53 ± 2 | 94 | 14.6 | 16.0 |
| 10,000 | 5/5 | 148 ± 4 | 173 ± 4** | 25 ± 2** | 82 | 12.0 | 13.9 |
| 20,000 | 5/5 | 143 ± 4 | 127 ± 5** | -16 ± 2** | 60 | 6.8 | 9.0 |
| 40,000 | 5/5 | 141 ± 5 | 110 ± 6** | -31 ± 4** | 52 | 3.8 | 10.5 |
| Female | | | | | | | |
| 0 | 5/5 | 117 ± 3 | 140 ± 3 | 23 ± 1 | | 10.8 | 11.6 |
| 2,500 | 5/5 | 116 ± 4 | 139 ± 4 | 24 ± 1 | 99 | 11.1 | 12.5 |
| 5,000 | 5/5 | 117 ± 2 | 134 ± 3 | 18 ± 1 | 96 | 10.1 | 11.4 |
| 10,000 | 5/5 | 102 ± 4** | 123 ± 3** | 21 ± 4 | 87 | 8.3 | 10.5 |
| 20,000 | 5/5 | 114 ± 3 | 95 ± 4** | -19 ± 2** | 68 | 4.7 | 5.0 |
| 40,000 | 5/5 | 116 ± 3 | 91 ± 4** | -25 ± 4** | 65 | 3.4 | 11.3 |

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test

^a Number of animals surviving/number initially in group

^b Weights and weight changes are given as mean ± standard error.

^c Feed consumption is expressed as grams of feed consumed per animal per day and was not corrected for feed spillage (scattering).

The absolute and relative thymus weights of 20,000 and 40,000 ppm males and females and the absolute thymus weights of 10,000 ppm males and females were significantly lower than those of the controls (Table F1). The thymus weight effects were considered to be related to lower body weight, lower feed intake, and stress. Decreases in absolute weights and increases in relative weights of other organs except the spleen were attributed to decreased body weights.

There were statistically significant increases in the absolute and relative spleen weights of 10,000, 20,000, and 40,000 ppm males and females and differences in the hematology parameters characteristic of a regenerative anemia, which was probably hemolytic in nature. There were statistically significant reductions in erythrocyte count, hemoglobin, and hematocrit values and statistically significant increases in reticulocyte count, nucleated erythrocytes, and methemoglobin concentrations (except in 10,000 ppm males) in the 10,000, 20,000, and 40,000 ppm groups (Table G1). Less pronounced differences in these parameters were observed in the other exposure groups. A significant leukocytosis with lymphocytes was present in 20,000 and 40,000 ppm males.

There were no gross lesions observed at necropsy that were considered to be related to chemical administration. Hypertrophy of the follicular epithelium of the thyroid gland was present in all male and female rats receiving 10,000, 20,000, and 40,000 ppm *p*-nitrobenzoic acid, while follicular cell hyperplasia was observed in four 40,000 ppm males and two

40,000 ppm females (Table 3). Atrophy (degeneration) of the testis was observed in 20,000 and 40,000 ppm males. Congestion of the spleen was observed in one 5,000 ppm male, in all 10,000 ppm males, and in all 20,000 and 40,000 ppm males and females. Other microscopic findings observed in the 20,000 and 40,000 ppm groups, including atrophy in the thymus of 20,000 and 40,000 ppm males and atrophy in the ovary, bone marrow, and thymus of 20,000 and 40,000 ppm females, were considered secondary to stress and inanition.

Hypertrophy of the follicular epithelium was diffuse and ranged from minimal to moderate in severity, in a dose-related manner, across exposure groups. Thyroid glands in exposed animals had a predominance of large follicles. The follicular epithelium was tall columnar to low cuboidal, the cytoplasm was slightly basophilic, nuclei were vesiculate, and the colloid was pale eosinophilic. Minimal thyroid follicular hyperplasia was characterized by focal stacking of epithelial cells that did not maintain contact with the basement membranes. Testicular atrophy was characterized by reduced seminiferous tubule size and fewer spermatogenic cells. Affected seminiferous tubules contained multinucleated giant cells that represented fused spermatid nuclei.

Based on the decreased mean body weights and clinical pathologic and histopathologic findings at 20,000 and 40,000 ppm, the high dose selected for the 13-week study was 10,000 ppm.

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Day Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 2,500 | 5,000 | 10,000 | 20,000 | 40,000 |
|---|---|----------------|-------|------------------------|-----------|-----------|
| Male | | | | | | |
| Thyroid Gland ^a | 5 | — ^b | — | 5 | 5 | 5 |
| Hypertrophy, Follicular Epithelium ^c | 0 | — | — | 5** (1.0) ^d | 5** (1.5) | 5** (2.5) |
| Hyperplasia, Follicular Epithelium | 0 | — | — | 0 | 0 | 4* |
| Testis | 5 | — | — | 5 | 5 | 5 |
| Atrophy, Germinal Epithelium | 0 | — | — | 0 | 1 | 4* |
| Spleen | 5 | 5 | 5 | 5 | 5 | 5 |
| Congestion | 0 | 0 | 1 | 5** | 5** | 5** |
| Thymus | 5 | — | — | 5 | 5 | 5 |
| Atrophy, Cortex | 0 | — | — | 0 | 2 | 2 |
| Bone Marrow | 5 | — | — | — | — | 5 |
| Atrophy | 0 | — | — | — | — | 0 |
| Female | | | | | | |
| Thyroid Gland | 5 | — | — | 5 | 5 | 5 |
| Hypertrophy, Follicular Epithelium | 0 | — | — | 5** (1.0) | 5** (1.5) | 5** (2.5) |
| Hyperplasia, Follicular Epithelium | 0 | — | — | 0 | 0 | 2 |
| Spleen | 5 | — | — | 5 | 5 | 5 |
| Congestion | 0 | — | — | 0 | 5** | 5** |
| Thymus | 5 | — | — | 5 | 5 | 5 |
| Atrophy, Cortex | 0 | — | — | 0 | 1 | 4* |
| Bone Marrow | 5 | — | — | 5 | 5 | 5 |
| Atrophy | 0 | — | — | 0 | 1 | 5** |
| Ovary | 5 | — | — | 5 | 5 | 5 |
| Atrophy | 0 | — | — | 0 | 2 | 4* |

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test

** $P \leq 0.01$

^a Number of animals with organ examined microscopically

^b Organ not examined in this exposure group

^c Number of animals with lesion

^d Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe

13-WEEK STUDY

All rats in the 13-week study survived until the end of the study (Table 4). Mean body weight gain and final mean body weights were significantly lower than those of the controls in 2,500 ppm males and in 5,000 and 10,000 ppm males and females. There were no clinical findings that could be clearly related to *p*-nitrobenzoic acid exposure. Feed consumption by males and females was similar to that by the controls. Dietary levels of 630, 1,250, 2,500, 5,000, or 10,000 ppm *p*-nitrobenzoic acid delivered average

daily doses of 40, 70, 160, 210, or 660 mg/kg to males and 40, 80, 170, 340, or 680 mg/kg to females.

The absolute and relative spleen weights were significantly increased in males and females exposed to 10,000 ppm, and the relative spleen weights were significantly increased in males and females exposed to 5,000 ppm (Table F2). These differences were probably a result of a hemolytic anemia. Differences in the absolute and relative weights of other organs were considered to be related to decreased body weights.

TABLE 4
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) | Feed Consumption ^c | |
|---------------|-----------------------|-----------------------------------|-----------|-----------|---------------------------------------|-------------------------------|---------|
| | | Initial | Final | Change | | Week 1 | Week 13 |
| Male | | | | | | | |
| 0 | 10/10 | 116 ± 4 | 354 ± 6 | 238 ± 5 | | 11.8 | 15.6 |
| 630 | 10/10 | 119 ± 4 | 358 ± 5 | 239 ± 5 | 101 | 12.3 | 16.8 |
| 1,250 | 10/10 | 114 ± 4 | 341 ± 6 | 227 ± 5 | 96 | 10.5 | 15.3 |
| 2,500 | 10/10 | 114 ± 3 | 335 ± 4* | 221 ± 4* | 95 | 11.1 | 16.8 |
| 5,000 | 10/10 | 107 ± 5 | 322 ± 6** | 216 ± 4** | 91 | 10.4 | 15.8 |
| 10,000 | 10/10 | 117 ± 4 | 261 ± 3** | 144 ± 3** | 74 | 10.3 | 14.6 |
| Female | | | | | | | |
| 0 | 10/10 | 97 ± 2 | 204 ± 4 | 106 ± 4 | | 9.5 | 10.0 |
| 630 | 10/10 | 100 ± 3 | 202 ± 3 | 102 ± 3 | 99 | 9.7 | 9.7 |
| 1,250 | 10/10 | 97 ± 2 | 200 ± 3 | 103 ± 3 | 98 | 9.0 | 10.0 |
| 2,500 | 10/10 | 98 ± 3 | 195 ± 3 | 98 ± 2 | 96 | 9.5 | 10.3 |
| 5,000 | 10/10 | 95 ± 2 | 182 ± 2** | 87 ± 3** | 89 | 8.6 | 10.0 |
| 10,000 | 10/10 | 99 ± 2 | 169 ± 2** | 70 ± 2** | 83 | 8.5 | 9.8 |

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

** $P \leq 0.01$

^a Number of animals surviving/number initially in group

^b Weights and weight changes are given as mean ± standard error.

^c Feed consumption is expressed as grams of feed consumed per animal per day.

Differences in hematology and clinical chemistry parameters were observed primarily in the 10,000 ppm rats (Table G2). These differences included increases in methemoglobin and Heinz bodies as early as day 7 and increased reticulocyte counts, decreased erythrocyte counts, and decreased hemoglobin and hematocrit values as early as day 30 of the study. In addition, slight increases in mean erythrocyte hemoglobin at days 30 and 60 in male rats and increases in mean erythrocyte volume in male and female rats at days 30 and 60 were supportive of a regenerative anemia. The slight increases in leukocyte count in 10,000 ppm females at days 7 and 30 may have been associated with a hemolytic anemia. Slight increases in alanine aminotransferase were present in 10,000 ppm males and females at days 7 and 30.

Chemical-related histopathologic lesions were observed in the testis, spleen, and kidney. As in the 14-day study, the testicular lesion in 10,000 ppm males consisted of atrophy of the seminiferous tubules characterized by mild to severe depletion of spermatogenic cells and by pyknotic cells and multinucleated cells in the lumen of the seminiferous tubules.

Yellow-brown pigmentation of the red pulp was present in the spleen of 2,500, 5,000, and 10,000 ppm males and females, and congestion was observed in the spleen of 2,500, 5,000, and 10,000 ppm males and 10,000 ppm females (Table 5). Splenic sinusoids were ectatic, and macrophages contained yellow-brown pigment consistent with hemosiderin. Mild pigmentation was also present in the kidney of 5,000 and 10,000 ppm males. The pigmentation was present in the cytoplasm of renal tubule epithelial cells. The yellow-brown pigment, consistent with

hemosiderin, in the spleen and kidney was associated with the hemolytic anemia.

Mild karyomegaly of renal tubule epithelial cells was observed in the kidney of 5,000 ppm females and 10,000 ppm males and females. The mild karyomegaly was more prominent in the outer cortex and was scattered in renal tubule epithelial cells. Karyomegaly was characterized by nuclei that were 4 to 6 times larger than normal. Occasional nuclei were pleomorphic and contained two prominent nucleoli.

Male rats exposed to 10,000 ppm had mild cytoplasmic hyaline droplet accumulation in renal tubule epithelial cells within the outer renal cortex. Multiple hyaline droplets were most commonly present within the cytoplasm of cells, but the droplets were also observed protruding from the cell, and were often observed within renal tubule lumens. Hyaline droplets were eosinophilic, crystalline-shaped or amorphous to spherical, and variable in size. The droplets were more frequent and larger than the smaller, more uniform protein "reabsorption droplets" typically present in the kidney of male control rats. Minimal hyaline droplet accumulation was also observed in 630, 1,250, 2,500, and 5,000 ppm males. Using the Mallory-Heidenhain stain for proteins, the cytoplasmic hyaline droplets appeared intensely eosinophilic, similar to the staining observed for the protein "reabsorption droplets." There was no degeneration or necrosis of the renal tubule epithelial cells and no mineralization or granular casts was observed.

Dose Selection Rationale: Based on lower mean body weights and increased severity of splenic lesions, dietary levels of *p*-nitrobenzoic acid selected for the 2-year feed study in rats were 0, 1,250, 2,500, and 5,000 ppm.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 630 | 1,250 | 2,500 | 5,000 | 10,000 |
|--|----|----------------|------------|----------------------|------------|------------|
| Male | | | | | | |
| Spleen ^a | 10 | — ^b | 10 | 10 | 10 | 10 |
| Congestion ^c | 0 | — | 0 | 2 (1.0) ^d | 10** (1.0) | 10** (1.5) |
| Pigmentation | 0 | — | 0 | 6** (1.0) | 10** (1.0) | 10** (1.6) |
| Kidney | 10 | 10 | 10 | 10 | 10 | 10 |
| Pigmentation | 0 | 0 | 0 | 10** (1.0) | 10** (2.0) | 10** (2.0) |
| Cytoplasmic Hyaline Droplet Accumulation ^e | 0 | 10** (1.0) | 10** (1.0) | 10** (1.0) | 10** (1.0) | 10** (2.0) |
| Karyomegaly | 0 | 0 | 0 | 6** (1.0) | 10** (1.0) | 10** (2.0) |
| Female | | | | | | |
| Spleen | 10 | — | 10 | 10 | 10 | 10 |
| Congestion | 0 | — | 0 | 0 | 0 | 10** (1.5) |
| Pigmentation | 0 | — | 0 | 10** (1.0) | 10** (1.2) | 10** (1.5) |
| Kidney | 10 | 10 | 10 | 10 | 10 | 10 |
| Karyomegaly | 0 | 0 | 0 | 10** (1.0) | 10** (2.0) | 10** (2.0) |

** Significantly different ($P < 0.01$) from the control group by the Fisher exact test

^a Number of animals with organ examined microscopically

^b Organ not examined in this exposure group

^c Number of animals with lesion

^d Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe

^e Diagnosed as cytoplasmic change by the study pathologist

2-YEAR STUDY

Survival

Estimates of survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier curves in Figure 2. Two-year survival of males receiving 1,250 and 2,500 ppm was similar to that of the controls at the end of the study. The survival of 5,000 ppm males was marginally greater than that of the controls, which was attributed to a decrease in the severity of nephropathy and a decrease in the incidence of mononuclear cell leukemia in this exposure group. Survival of exposed females was similar to that of the controls.

Body Weights, Feed Consumption, and Clinical Findings

The mean body weights of 5,000 ppm males were 2% to 8% lower than those of the controls through week 80 (Table 7 and Figure 3). Final mean body weights of exposed males were similar to that of the controls. The mean body weights of 5,000 ppm females were 2% to 9% lower than those of the controls during the first year of the study and 10% to 16% lower during the second year of the study

(Table 8 and Figure 3). Feed consumption by exposed groups was similar to that by the controls (Tables I1 and I2). Dietary levels of 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid delivered approximately 50, 100, or 210 mg/kg body weight per day to males and 60, 125, or 250 mg/kg per day to females. There were no clinical findings attributable to organ-specific toxicity.

Hematology

Marginal differences were noted between the hematologic profile of 5,000 ppm females and that of the controls. These differences did not appear to affect the well-being of the animals. At the 15-month interim evaluation, leukocyte count was significantly increased in 5,000 ppm males and females (Table G3). In 5,000 ppm females, erythrocyte count, hemoglobin, hematocrit, mean erythrocyte hemoglobin, and mean erythrocyte hemoglobin concentration values were significantly lower than those of the controls. Nucleated erythrocyte, segmented neutrophil, lymphocyte, and platelet values were significantly greater than those of the controls in 5,000 ppm females.

TABLE 6
Survival of Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|--|----------|----------|----------|----------|
| Male | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Moribund | 32 | 34 | 34 | 25 |
| Natural deaths | 6 | 3 | 3 | 4 |
| Animals surviving to study termination | 12 | 13 | 13 | 21 |
| Percent probability of survival at end of study ^b | 25 | 26 | 26 | 42 |
| Mean survival (days) ^c | 587 | 607 | 618 | 618 |
| Survival analyses ^d | P=0.038N | P=0.651N | P=0.563N | P=0.053N |
| Female | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Moribund | 21 | 23 | 27 | 26 |
| Natural deaths | 2 | 4 | 2 | 3 |
| Animals surviving to study termination | 27 | 23 | 21 | 21 |
| Percent probability of survival at end of study | 54 | 46 | 43 | 42 |
| Mean survival (days) | 659 | 633 | 616 | 635 |
| Survival analyses | P=0.250 | P=0.400 | P=0.137 | P=0.231 |

^a Censored from survival analyses

^b Kaplan-Meier determinations based on the number of animals alive on first day of terminal sacrifice

^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 exposure columns. A negative trend or a lower mortality in an exposure group is indicated by N.

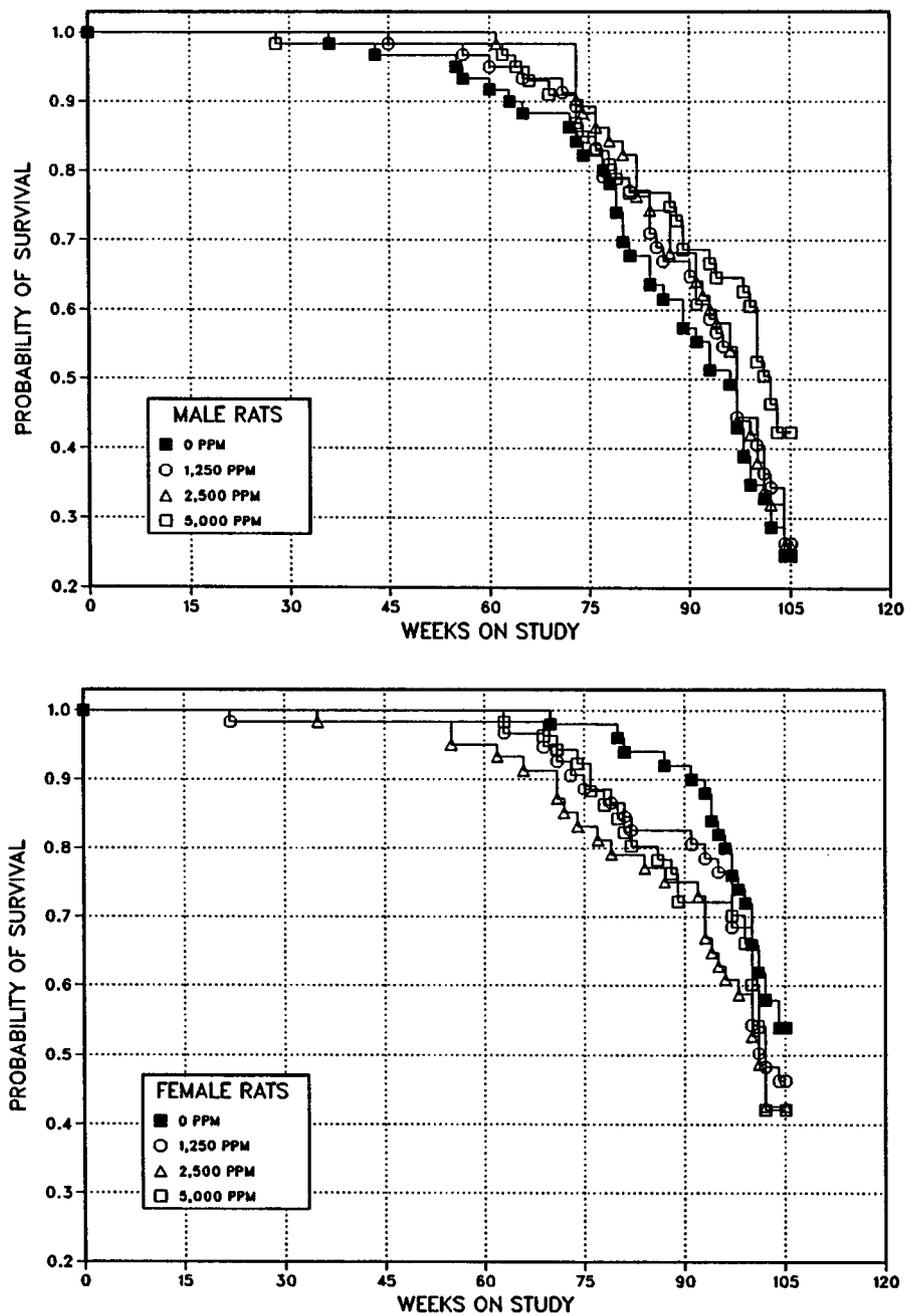


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered
***p*-Nitrobenzoic Acid in Feed for 2 Years**

TABLE 7
 Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Weeks on Study | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|----------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 128 | 60 | 126 | 98 | 60 | 124 | 96 | 60 | 126 | 98 | 60 |
| 2 | 169 | 60 | 168 | 100 | 60 | 166 | 98 | 60 | 164 | 97 | 60 |
| 3 | 207 | 60 | 206 | 99 | 60 | 200 | 97 | 60 | 198 | 96 | 60 |
| 4 | 235 | 60 | 231 | 99 | 60 | 227 | 97 | 60 | 221 | 94 | 60 |
| 5 | 262 | 60 | 259 | 99 | 60 | 258 | 99 | 60 | 240 | 92 | 60 |
| 6 | 283 | 60 | 281 | 100 | 60 | 280 | 99 | 60 | 262 | 93 | 60 |
| 7 | 300 | 60 | 299 | 100 | 60 | 298 | 99 | 60 | 278 | 93 | 60 |
| 8 | 313 | 60 | 310 | 99 | 60 | 311 | 99 | 60 | 294 | 94 | 60 |
| 9 | 321 | 60 | 320 | 100 | 60 | 322 | 100 | 60 | 304 | 94 | 60 |
| 10 | 338 | 60 | 337 | 100 | 60 | 337 | 100 | 60 | 315 | 93 | 60 |
| 11 | 347 | 60 | 350 | 101 | 60 | 350 | 101 | 60 | 329 | 95 | 60 |
| 12 | 352 | 60 | 355 | 101 | 60 | 356 | 101 | 60 | 334 | 95 | 60 |
| 13 | 362 | 60 | 360 | 99 | 60 | 359 | 99 | 60 | 338 | 93 | 60 |
| 17 | 387 | 60 | 381 | 99 | 60 | 391 | 101 | 60 | 366 | 95 | 60 |
| 21 | 409 | 60 | 409 | 100 | 60 | 412 | 101 | 60 | 389 | 95 | 60 |
| 25 | 426 | 60 | 421 | 99 | 60 | 427 | 100 | 60 | 400 | 94 | 60 |
| 28 | 435 | 60 | 431 | 99 | 60 | 435 | 100 | 60 | 410 | 94 | 59 |
| 33 | 450 | 60 | 450 | 100 | 60 | 447 | 99 | 60 | 426 | 95 | 59 |
| 37 | 460 | 59 | 461 | 100 | 60 | 464 | 101 | 60 | 442 | 96 | 59 |
| 41 | 469 | 59 | 470 | 100 | 60 | 466 | 99 | 60 | 446 | 95 | 59 |
| 45 | 475 | 58 | 475 | 100 | 60 | 480 | 101 | 60 | 459 | 97 | 59 |
| 49 | 478 | 58 | 485 | 102 | 59 | 484 | 101 | 60 | 464 | 97 | 59 |
| 53 | 480 | 58 | 488 | 102 | 59 | 485 | 101 | 60 | 463 | 97 | 59 |
| 57 | 482 | 56 | 491 | 102 | 58 | 486 | 101 | 60 | 464 | 96 | 59 |
| 61 | 484 | 55 | 484 | 100 | 57 | 480 | 99 | 60 | 461 | 95 | 59 |
| 65 | 483 | 54 | 487 | 101 | 57 | 486 | 101 | 59 | 465 | 96 | 57 |
| 69 ^a | 482 | 43 | 490 | 102 | 46 | 485 | 101 | 49 | 466 | 97 | 46 |
| 73 | 474 | 42 | 478 | 101 | 45 | 472 | 99 | 49 | 458 | 97 | 45 |
| 77 | 475 | 40 | 480 | 101 | 41 | 474 | 100 | 43 | 465 | 98 | 41 |
| 80 | 472 | 34 | 471 | 100 | 39 | 469 | 99 | 41 | 458 | 97 | 39 |
| 85 | 463 | 31 | 463 | 100 | 35 | 469 | 101 | 37 | 459 | 99 | 38 |
| 89 | 462 | 30 | 468 | 101 | 33 | 473 | 102 | 34 | 462 | 100 | 36 |
| 93 | 455 | 27 | 458 | 101 | 30 | 470 | 103 | 31 | 457 | 101 | 34 |
| 97 | 442 | 24 | 447 | 101 | 27 | 451 | 102 | 26 | 447 | 101 | 32 |
| 101 | 449 | 17 | 441 | 98 | 20 | 460 | 102 | 19 | 444 | 99 | 26 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 278 | | 277 | 100 | | 276 | 99 | | 262 | 94 | |
| 14-52 | 443 | | 443 | 100 | | 445 | 100 | | 422 | 95 | |
| 53-101 | 469 | | 473 | 101 | | 474 | 101 | | 459 | 98 | |

^a Interim evaluation occurred during week 66.

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Weeks on Study | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|----------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 105 | 60 | 106 | 101 | 60 | 103 | 98 | 60 | 103 | 98 | 60 |
| 2 | 125 | 60 | 125 | 100 | 60 | 125 | 100 | 60 | 122 | 98 | 60 |
| 3 | 137 | 60 | 137 | 100 | 60 | 134 | 98 | 60 | 131 | 96 | 60 |
| 4 | 149 | 60 | 149 | 100 | 60 | 146 | 99 | 60 | 143 | 96 | 60 |
| 5 | 156 | 60 | 157 | 100 | 60 | 154 | 99 | 60 | 150 | 96 | 60 |
| 6 | 166 | 60 | 168 | 101 | 60 | 164 | 99 | 60 | 160 | 97 | 60 |
| 7 | 173 | 60 | 174 | 101 | 60 | 170 | 98 | 60 | 167 | 97 | 60 |
| 8 | 175 | 60 | 177 | 101 | 60 | 173 | 99 | 60 | 171 | 98 | 60 |
| 9 | 178 | 60 | 179 | 101 | 60 | 176 | 99 | 60 | 172 | 97 | 60 |
| 10 | 184 | 60 | 185 | 101 | 60 | 183 | 100 | 60 | 179 | 98 | 60 |
| 11 | 190 | 60 | 191 | 101 | 60 | 188 | 99 | 60 | 183 | 96 | 60 |
| 12 | 191 | 60 | 193 | 101 | 60 | 191 | 100 | 60 | 187 | 98 | 60 |
| 13 | 192 | 60 | 192 | 100 | 60 | 188 | 98 | 60 | 184 | 96 | 60 |
| 17 | 203 | 60 | 205 | 101 | 60 | 193 | 95 | 60 | 199 | 98 | 60 |
| 21 | 211 | 60 | 211 | 100 | 60 | 207 | 98 | 60 | 203 | 96 | 60 |
| 25 | 218 | 60 | 218 | 100 | 59 | 212 | 97 | 60 | 206 | 95 | 60 |
| 29 | 217 | 60 | 220 | 102 | 59 | 216 | 100 | 60 | 208 | 96 | 60 |
| 33 | 228 | 60 | 226 | 99 | 59 | 221 | 97 | 60 | 212 | 93 | 60 |
| 37 | 236 | 60 | 235 | 100 | 59 | 227 | 97 | 59 | 219 | 93 | 60 |
| 41 | 242 | 60 | 243 | 101 | 59 | 234 | 97 | 59 | 224 | 93 | 60 |
| 45 | 249 | 60 | 249 | 100 | 59 | 242 | 97 | 59 | 230 | 92 | 60 |
| 50 | 262 | 60 | 261 | 100 | 59 | 254 | 97 | 59 | 237 | 91 | 60 |
| 53 | 271 | 60 | 269 | 99 | 59 | 262 | 97 | 59 | 243 | 90 | 60 |
| 57 | 280 | 60 | 276 | 99 | 59 | 267 | 95 | 57 | 245 | 87 | 60 |
| 61 | 290 | 60 | 282 | 97 | 59 | 273 | 94 | 57 | 253 | 87 | 60 |
| 65 | 298 | 60 | 289 | 97 | 58 | 280 | 94 | 56 | 257 | 86 | 59 |
| 69 ^a | 306 | 50 | 297 | 97 | 48 | 286 | 94 | 45 | 267 | 87 | 49 |
| 73 | 308 | 49 | 299 | 97 | 46 | 290 | 94 | 42 | 264 | 86 | 47 |
| 77 | 316 | 49 | 304 | 96 | 44 | 292 | 92 | 41 | 270 | 86 | 44 |
| 81 | 321 | 48 | 308 | 96 | 43 | 298 | 93 | 39 | 274 | 86 | 42 |
| 85 | 324 | 47 | 313 | 97 | 41 | 296 | 92 | 38 | 270 | 84 | 40 |
| 89 | 334 | 46 | 323 | 97 | 41 | 306 | 92 | 37 | 282 | 85 | 38 |
| 93 | 332 | 45 | 324 | 98 | 40 | 305 | 92 | 36 | 283 | 85 | 36 |
| 97 | 337 | 40 | 327 | 97 | 35 | 316 | 94 | 30 | 289 | 86 | 35 |
| 101 | 341 | 31 | 331 | 97 | 25 | 314 | 92 | 24 | 288 | 84 | 28 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 163 | | 164 | 101 | | 161 | 99 | | 158 | 97 | |
| 14-52 | 230 | | 230 | 100 | | 223 | 97 | | 215 | 93 | |
| 53-101 | 312 | | 303 | 97 | | 291 | 93 | | 268 | 86 | |

^a Interim evaluation occurred during week 66.

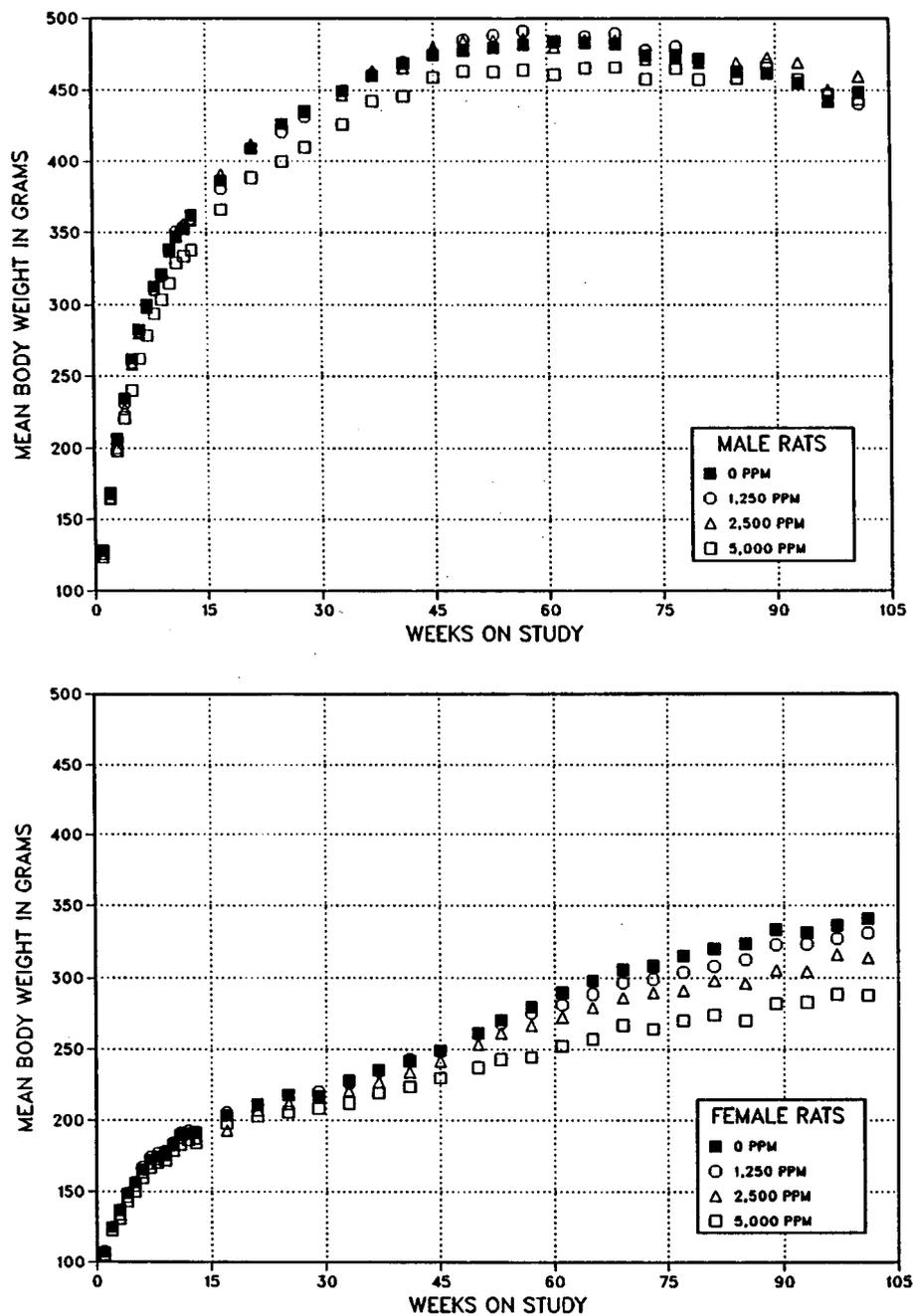


FIGURE 3
Growth Curves for Rats Administered *p*-Nitrobenzoic Acid in Feed for 2 Years

Pathology and Statistical Evaluation

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

Preputial/Clitoral gland: The incidence of preputial gland carcinoma in 5,000 ppm males was significantly greater than that of the controls by the logistic regression test but not by the life table test. However, the incidences of preputial gland adenoma and of preputial gland adenoma or carcinoma (combined) in exposed males were not statistically different from those of the controls (Table 9). Twenty-one of the 25 preputial gland neoplasms occurred in animals sacrificed in a moribund condition prior to the end of the study, suggesting that these were generally lethal neoplasms. Thus, the most appropriate test for these neoplasms is life table analysis rather than logistic regression. These preputial gland neoplasms were observed on gross pathology examination and the average diameter of the preputial gland carcinomas was 31 mm. No preputial gland hyperplasia was observed and few preputial gland adenomas were observed at 15 months.

At the end of the study, all exposed female groups had incidences of clitoral gland adenoma and adenoma or carcinoma (combined) that were significantly greater than those of the controls (Tables 10 and B3). The incidences of clitoral gland adenoma and of clitoral gland adenoma or carcinoma (combined) in groups exposed to *p*-nitrobenzoic acid exceeded the historical control ranges in female F344/N rats in recent 2-year NTP feed studies (Table B4a). Clitoral gland hyperplasia was not observed at the 15-month interim evaluation, and the incidences of hyperplasia in exposed females were marginally lower than that of the controls at 2 years (Tables 10 and B5). Because there was no dose response for clitoral gland neoplasms and the time to neoplasm occurrence was similar (approximately 700 days) in control and exposed groups, and because there was no increase in the incidence of clitoral gland hyperplasia, the increased incidences of clitoral gland neoplasms were considered to be only some evidence of carcinogenic activity in female rats exposed to *p*-nitrobenzoic acid.

Preputial and clitoral gland adenomas were generally circumscribed and sometimes caused compression of the surrounding tissue. The neoplastic cells formed acini and clusters, which were spherical to elongated in shape and varied in size. Many of the neoplastic cells had discrete borders and granular cytoplasm. Foci of cellular debris, necrosis, and cysts were often present. Carcinomas were generally larger masses and less circumscribed than adenomas and often infiltrated the adjacent normal tissue.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Preputial Gland in Male Rats
in the 2-Year Feed Study of p-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|---|----------------------|-----------|------------|------------|
| 15-Month Interim Evaluation | | | | |
| Preputial Gland ^a | 10 | 10 | 10 | 10 |
| Adenoma ^b | 1 | 0 | 1 | 1 |
| 2-Year Study | | | | |
| Preputial Gland | 50 | 50 | 49 | 50 |
| Hyperplasia | 4 (2.0) ^c | 0 | 1 (2.0) | 3 (1.3) |
| Adenoma | | | | |
| Overall rate ^d | 3/50 (6%) | 3/50 (6%) | 4/49 (8%) | 3/50 (6%) |
| Adjusted rate ^e | 21.1% | 13.6% | 16.5% | 10.4% |
| Terminal rate ^f | 2/12 (17%) | 0/13 (0%) | 1/13 (8%) | 1/21 (5%) |
| First incidence (days) | 689 | 588 | 532 | 617 |
| Life table test ^g | P=0.382N | P=0.603N | P=0.555 | P=0.434N |
| Logistic regression test ^g | P=0.522N | P=0.611N | P=0.560 | P=0.554N |
| Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 4/49 (8%) | 6/50 (12%) |
| Adjusted rate | 2.7% | 2.9% | 15.9% | 15.0% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 0/13 (0%) | 0/21 (0%) |
| First incidence (days) | 548 | 602 | 651 | 518 |
| Life table test | P=0.031 | P=0.744N | P=0.234 | P=0.094 |
| Logistic regression test | P=0.002 | P=0.743 | P=0.192 | P=0.009 |
| Adenoma or Carcinoma^h | | | | |
| Overall rate | 4/50 (8%) | 4/50 (8%) | 8/49 (16%) | 9/50 (18%) |
| Adjusted rate | 23.2% | 16.1% | 29.8% | 23.8% |
| Terminal rate | 2/12 (17%) | 0/13 (0%) | 1/13 (8%) | 1/21 (5%) |
| First incidence (days) | 548 | 588 | 532 | 518 |
| Life table test | P=0.176 | P=0.579N | P=0.254 | P=0.278 |
| Logistic regression test | P=0.024 | P=0.607N | P=0.219 | P=0.055 |

^a Number of animals with preputial gland examined microscopically

^b Number of animals with lesion

^c Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

^d Number of animals with neoplasm per number of animals examined microscopically

^e Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^f Observed incidence in animals surviving until the end of the study

^g In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test regards neoplasms in animals dying prior to terminal sacrifice as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by N.

^h Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 139/1,169 (11.9% ± 7.8%), range 2%-30%

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Clitoral Gland in Female Rats
in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|---------------------------------------|-----------------------|-------------|-------------|-------------|
| Clitoral Gland ^a | 50 | 49 | 49 | 50 |
| Hyperplasia ^b | 10 (2.0) ^c | 6 (1.8) | 6 (2.2) | 7 (2.3) |
| Adenoma | | | | |
| Overall rate ^d | 4/50 (8%) | 12/49 (24%) | 10/49 (20%) | 12/50 (24%) |
| Adjusted rate ^e | 11.9% | 42.5% | 33.7% | 42.1% |
| Terminal rate ^f | 2/27 (7%) | 7/22 (32%) | 4/20 (20%) | 7/21 (33%) |
| First incidence (days) | 653 | 665 | 496 | 483 |
| Life table test ^g | P=0.034 | P=0.013 | P=0.030 | P=0.013 |
| Logistic regression test ^g | P=0.046 | P=0.013 | P=0.050 | P=0.023 |
| Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 2/49 (4%) | 5/49 (10%) | 4/50 (8%) |
| Adjusted rate | 3.7% | 6.0% | 19.3% | 11.7% |
| Terminal rate | 1/27 (4%) | 0/22 (0%) | 3/20 (15%) | 0/21 (0%) |
| First incidence (days) | 730 (T) | 694 | 499 | 528 |
| Life table test | P=0.085 | P=0.460 | P=0.056 | P=0.139 |
| Logistic regression test | P=0.117 | P=0.459 | P=0.084 | P=0.224 |
| Adenoma or Carcinoma ^h | | | | |
| Overall rate | 4/50 (8%) | 14/49 (29%) | 15/49 (31%) | 15/50 (30%) |
| Adjusted rate | 11.9% | 45.9% | 48.9% | 47.7% |
| Terminal rate | 2/27 (7%) | 7/22 (32%) | 7/20 (35%) | 7/21 (33%) |
| First incidence (days) | 653 | 665 | 496 | 483 |
| Life table test | P=0.008 | P=0.005 | P=0.001 | P=0.002 |
| Logistic regression test | P=0.011 | P=0.004 | P=0.003 | P=0.004 |

(T)Terminal sacrifice

^a Number of animals with clitoral gland examined microscopically

^b Number of animals with lesion

^c Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

^d Number of animals with neoplasm per number of animals examined microscopically

^e Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^f Observed incidence in animals surviving until the end of the study

^g In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test regards neoplasms in animals dying prior to terminal sacrifice as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal.

^h Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 120/1,096 (10.9% ± 5.3%), range 2%-21%

Kidney: The severity of nephropathy was decreased at 15 months in males exposed to 2,500 and 5,000 ppm *p*-nitrobenzoic acid. At 2 years, the incidences of nephropathy for all groups of rats were 98% to 100% (Tables 11, A5, and B5); however, the severity decreased as exposure level increased in males and females. Nephropathy was less severe in females than in males, consistent with differences normally seen between aging male and female rats. Nephropathy was characterized by glomerulosclerosis, thickening of renal tubule basement membrane, degeneration and atrophy of tubule epithelium, dilatation of tubule lumens by pale pink acellular material (hyaline casts), interstitial fibrosis, and chronic inflammation. Regeneration of the renal tubule epithelium was also observed and the extent and severity of this process paralleled the overall severity of the degenerative changes. Few hyaline droplets were observed in renal tubule epithelial cells of male rats at the end of the 2-year study. It was difficult to further evaluate karyomegaly of renal tubule cells in males and females at 2 years because the karyomegaly could not be differentiated from regenerative changes associated with nephropathy.

There was an increase in the severity of pigmentation of renal tubule epithelial cells at the 15-month interim evaluation and at 2 years (Table 11). The pigment was primarily in the cytoplasm of proximal convoluted renal tubule epithelial cells and was variably brown to light brown or golden. Representative sections of kidney were stained for iron by Perls' method. Positive ferric iron staining was characterized by a distinct medium to dark blue coloration primarily in very small granules, most were 1 micron or less in size. These granules were irregularly distributed, primarily in proximal convoluted renal tubule epithelial cells in the outer cortex. The positive staining indicated the presence of ferric iron, a form compatible with that present in hemosiderin. The severity of the pigment stained by Perls' method was similar in almost all instances to the severity of yellowish brown pigment deposition detected in the hematoxylin and eosin slides.

Proliferative lesions and neoplasms were also present in the kidneys of males. In the 2,500 ppm group, one male had a renal tubule adenoma and one male had a renal tubule carcinoma (Tables 11 and A1). Renal tubule hyperplasia, a possible precursor of adenomas, occurred in three 1,250 ppm males, one 2,500 ppm male, and one 5,000 ppm male. Oncocytic hyper-

plasia of renal tubules was also present in one control male, one 2,500 ppm male, and five 5,000 ppm males. One 1,250 ppm female and five 5,000 ppm females had oncocytic hyperplasia; however, there were no renal tubule adenomas or carcinomas in females.

Initially, a single hematoxylin and eosin-stained section of each kidney was prepared. Primarily because of the increased incidence of renal tubule hyperplasia in exposed males, because of the adenoma and carcinoma observed in 2,500 ppm males, and because of the unusual occurrence of oncocytic hyperplasia in 5,000 ppm males, additional step sections of kidney were prepared from the remaining formalin-fixed tissues. Six to eight additional kidney sections taken at 1 mm intervals were prepared for each male. Additional males with focal hyperplasia or adenoma were identified. The incidences of these proliferative lesions in the step sections and in the single and step sections combined are shown in Table 11. There were no significant increases in the incidence of renal tubule neoplasms.

Renal tubule hyperplasia, as defined in this study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered a preneoplastic lesion. Renal tubule hyperplasia, adenoma, and carcinoma are part of a morphologic continuum. Hyperplasia was generally a focal, minimal to mild lesion consisting of tubules that were dilated to 1.5 to 2 times normal diameter and were lined by increased numbers of tubule epithelial cells, which partially or totally filled the tubule lumen. Cells within hyperplastic lesions varied slightly in size and sometimes stained more basophilic than normal cells but otherwise appeared similar to normal tubule epithelial cells. Renal tubule adenomas were larger discrete lesions, ranging from greater than five tubule diameters to 1 mm or more in size. Cells within adenomas were mildly to moderately pleomorphic, sometimes had vacuolated cytoplasm, and tended to form complex patterns, particularly microtubular structures. A few adenomas contained varying amounts of hyaline basement membrane material that divided the epithelial cells into small irregular clusters.

Oncocytic hyperplasia was characterized by individual tubules or small clusters of tubules, which were somewhat dilated and totally filled by large polygonal

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|--|-----------------------|------------|------------|------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Kidney ^a | 10 | 10 | 10 | 10 |
| Pigmentation ^b | 10 (1.0) ^c | 10 (1.1) | 10 (1.9)** | 10 (2.4)** |
| Nephropathy | 10 (2.0) | 10 (2.0) | 10 (1.8) | 10 (1.7) |
| Renal Tubule Hyperplasia | 0 | 0 | 1 (2.0) | 0 |
| 2-Year Study | | | | |
| Single Sections (Standard Evaluation) | | | | |
| Kidney | 50 | 50 | 50 | 50 |
| Pigmentation | 50 (1.8) | 50 (2.3)** | 50 (2.4)** | 50 (2.6)** |
| Nephropathy | 50 (2.7) | 50 (2.5) | 50 (1.9)** | 50 (1.6)** |
| Oncocytic Hyperplasia | 1 (2.0) | 0 | 1 (1.0) | 5 (1.6) |
| Renal Tubule Hyperplasia | 0 | 3 (1.7) | 1 (1.0) | 1 (2.0) |
| Renal Tubule Adenoma | 0 | 0 | 1 | 0 |
| Renal Tubule Carcinoma | 0 | 0 | 1 | 0 |
| Renal Tubule Adenoma or Carcinoma ^d | 0 | 0 | 2 | 0 |
| Step Sections (Extended Evaluation) | | | | |
| Oncocytic Hyperplasia | 0 | 0 | 0 | 5 |
| Renal Tubule Hyperplasia | 1 | 4 | 4 | 4 |
| Renal Tubule Adenoma | 1 | 1 | 1 | 3 |
| Single and Step Sections Combined | | | | |
| Oncocytic Hyperplasia | 1 | 0 | 1 | 10* |
| Renal Tubule Hyperplasia | 1 | 7* | 5 | 4 |
| Renal Tubule Adenoma | 1 | 1 | 2 | 3 |
| Renal Tubule Carcinoma | 0 | 0 | 1 | 0 |
| Renal Tubule Adenoma or Carcinoma ^e | 1 | 1 | 3 | 3 |
| (continued) | | | | |

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study
of *p*-Nitrobenzoic Acid (continued)

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|------------------------------------|----------|------------|------------|------------|
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Kidney | 10 | 10 | 10 | 10 |
| Pigmentation | 10 (1.1) | 10 (2.2)** | 10 (2.9)** | 10 (2.9)** |
| Nephropathy | 10 (1.4) | 10 (1.0) | 9 (1.1) | 9 (1.3) |
| 2-Year Study | | | | |
| Kidney | 50 | 50 | 50 | 50 |
| Pigmentation | 50 (1.8) | 50 (2.1) | 50 (2.4)** | 50 (2.7)** |
| Nephropathy | 50 (1.9) | 49 (1.6) | 49 (1.3)** | 49 (1.2)** |
| Oncocytic Hyperplasia | 0 | 1 (1.0) | 0 | 5* (1.2) |
| Renal Tubule Hyperplasia | 0 | 0 | 1 (1.0) | 0 |

* Significantly different ($P \leq 0.05$) from the control group by logistic regression test

** Significantly different ($P \leq 0.01$) from the control group by the Mann-Whitney U test

^a Number of animals with kidney examined microscopically

^b Number of animals with lesion

^c Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

^d Historical incidence for 2-year NTP feed studies with untreated control groups (mean \pm standard deviation):

15/1,251 (1.2% \pm 1.7%), range 0%-6%

^e Historical incidence for 2-year NTP feed, gavage, and inhalation studies with untreated control groups: 22/608 (3.6% \pm 2.7%), range 0%-8%

cells with abundant brightly eosinophilic granular cytoplasm and small, often centrally located, basophilic nuclei (oncocytes). These lesions are thought to arise from the distal tubule epithelium.

Spleen: There were significant increases in absolute and relative spleen weights of females, but not of males, at 15 months (Table F3). Associated with the increases in splenic weight were increases in yellow-brown pigment in the red pulp consistent with the accumulation of hemosiderin in splenic macrophages at 15 months (Tables A5 and B5). The severity of pigmentation generally increased as the exposure level increased (severity in males: 1.0, 1.0, 1.5, and 1.9; females: 2.0, 1.9, 2.3, and 3.0). At the end of 2 years, it was difficult to evaluate the severity of splenic pigmentation because rats with mononuclear cell leukemia usually had greatly distended spleens that were packed with leukemia cells. Due to obliteration of the spleen with mononuclear cell leukemia, the Pathology Working Group could not confirm a chemical-related effect for splenic pigmentation at the end of the 2-year study. As with splenic pigmentation, it was difficult to assess extramedullary hematopoiesis because of the mononuclear cell leukemia. The incidences of bone marrow hypercellularity were not supportive of any chemical-related effect on hematopoietic cell proliferation. Furthermore, the majority of animals with increased hematopoietic cell proliferation had complicating neoplasms or inflammatory lesions, which probably accounted for increased hematopoietic cell proliferation.

Mononuclear cell leukemia: There were significant dose-related trends in the incidences of mononuclear cell leukemia in males and females (Table 12). The incidences of mononuclear cell leukemia in 5,000 ppm males (29/50, 35/50, 26/50, 2/50; Table A3) and in 2,500 and 5,000 ppm females (17/50, 11/50,

3/50, 0/50; Table B3) were significantly lower than those of the controls. The incidences in controls (males, 58%; females, 34%), while within the range of historical controls (males, 32% to 62%, Table A4c; females, 14% to 52%, Table B4b), were greater than the mean historical rates. The decrease in the incidences of total malignant neoplasms in 5,000 ppm males and females and increased survival of 5,000 ppm males were attributed to the decreased incidences of mononuclear cell leukemia in these groups.

Liver: The incidences of fatty cellular change (males: 15/49, 13/50, 11/50, 7/50; females: 14/50, 13/50, 7/50, 7/50), multifocal hyperplasia (males: 13/49, 12/50, 13/50, 4/50; females: 9/50, 10/50, 3/50, 2/50), and centrilobular atrophy (males: 22/49, 27/50, 23/50, 5/50; females: 14/50, 11/50, 4/50, 2/50) (Tables A5 and B5) in 5,000 ppm rats were generally lower than those of the controls. The fatty change, focal hyperplasia, and centrilobular atrophy were secondary to the mononuclear cell leukemia, and the lower incidences of these lesions parallel those of mononuclear cell leukemia.

Thyroid gland: The incidence of thyroid gland C-cell adenoma in 5,000 ppm females was marginally lower than that of the controls (9/50, 5/49, 4/50, 2/50; Table B3). No chemical-related differences in the incidences of C-cell carcinoma or C-cell hyperplasia were observed. C-cell adenomas are common lesions of aging F344 rats. C-cell adenomas may be single, multiple, or bilateral. They occur as discrete focal masses of C-cells but may contain widely separated, isolated follicles. The neoplasm is usually well demarcated and causes some compression of the surrounding parenchyma. There were no chemical-related decreased incidences of thyroid gland C-cell neoplasms in male rats.

TABLE 12
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|--|-------------|-------------|-------------|--------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Mononuclear Cell Leukemia ^a | 0/10 | 1/10 | 0/10 | 0/10 |
| 2-Year Study | | | | |
| Mononuclear Cell Leukemia ^b | | | | |
| Overall rate | 29/50 (58%) | 35/50 (70%) | 26/50 (52%) | 2/50 (4%) |
| Adjusted rate ^c | 76.2% | 79.9% | 76.3% | 4.9% |
| Terminal rate ^d | 4/12 (33%) | 5/13 (38%) | 7/13 (54%) | 0/21 (0%) |
| First incidence (days) | 503 | 415 | 506 | 445 |
| Life table test ^e | P<0.001N | P=0.424 | P=0.215N | P<0.001N |
| Logistic regression test ^e | P<0.001N | P=0.177 | P=0.127N | P<0.001N |
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Mononuclear Cell Leukemia | 0/10 | 1/10 | 0/10 | 0/10 |
| 2-Year Study | | | | |
| Mononuclear Cell Leukemia ^f | | | | |
| Overall rate | 17/50 (34%) | 11/50 (22%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted rate | 38.6% | 32.5% | 8.5% | 0.0% |
| Terminal rate | 3/27 (11%) | 4/23 (17%) | 0/21 (0%) | 0/21 (0%) |
| First incidence (days) | 490 | 566 | 492 | ^g |
| Life table test | P<0.001N | P=0.272N | P=0.008N | P<0.001N |
| Logistic regression test | P<0.001N | P=0.159N | P<0.001N | P<0.001N |

^a Number of animals with neoplasm per number of animals necropsied

^b Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 603/1,253 (48.1% ± 8.7%), range 32%-62%

^c Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^d Observed incidence in animals surviving until the end of the study

^e In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test regards neoplasms in animals dying prior to terminal sacrifice as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by N.

^f Historical incidence: 324/1,251 (25.9% ± 8.6%), range 14%-52%

^g Not applicable; no neoplasms in animal group

MICE

14-DAY STUDY

Three males and two females receiving 40,000 ppm died during days 5 through 8 of the study. All other animals survived until the end of the study (Table 13). Males exposed to 20,000 and 40,000 ppm *p*-nitrobenzoic acid and females exposed to 20,000 ppm lost weight. Mean body weight gains of 20,000 and 40,000 ppm males and of 10,000, 20,000, and 40,000 ppm females were significantly lower than those of the controls. There were no clinical findings relating to organ-specific toxicity, although lethargy

and ataxia were observed in 40,000 ppm mice. Feed consumption by males and females was similar to that by the controls, although the feed consumption data varied. Scattering of feed by these mice might have contributed to the variability in the data. Dietary levels of 2,500, 5,000, 10,000, 20,000, or 40,000 ppm *p*-nitrobenzoic acid delivered average daily doses of 1,000, 2,000, 3,500, 8,500, or 14,000 mg/kg body weight to males and 1,000, 2,000, 4,000, 9,500, or 21,500 mg/kg to females.

TABLE 13
Survival, Mean Body Weights, and Feed Consumption of Mice in the 14-Day Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) | Feed Consumption ^c | |
|---------------|-----------------------|-----------------------------------|--------------|--------------|---------------------------------------|-------------------------------|--------|
| | | Initial | Final | Change | | Week 1 | Week 2 |
| Male | | | | | | | |
| 0 | 5/5 | 18.2 ± 1.0 | 19.5 ± 0.9 | 1.3 ± 0.2 | | 5.4 | 5.7 |
| 2,500 | 5/5 | 18.3 ± 0.6 | 19.1 ± 0.7 | 0.8 ± 0.3 | 98 | 8.0 | 6.8 |
| 5,000 | 5/5 | 17.8 ± 0.5 | 18.4 ± 0.5 | 0.5 ± 0.2 | 94 | 6.2 | 8.3 |
| 10,000 | 5/5 | 18.4 ± 0.4 | 19.0 ± 0.4 | 0.7 ± 0.4 | 98 | 5.3 | 7.9 |
| 20,000 | 5/5 | 18.4 ± 0.3 | 17.9 ± 0.5 | -0.5 ± 0.4** | 92 | 6.4 | 8.6 |
| 40,000 | 2/5 ^d | 18.2 ± 0.8 | 18.0 ± 0.4 | -0.7 ± 0.4** | 92 | 4.0 | 8.5 |
| Female | | | | | | | |
| 0 | 5/5 | 15.5 ± 0.7 | 16.8 ± 0.7 | 1.3 ± 0.5 | | 6.3 | 7.1 |
| 2,500 | 5/5 | 15.6 ± 0.3 | 16.5 ± 0.4 | 0.8 ± 0.4 | 98 | 6.8 | 8.3 |
| 5,000 | 5/5 | 15.7 ± 0.6 | 15.9 ± 0.4 | 0.2 ± 0.3 | 95 | 5.8 | 7.6 |
| 10,000 | 5/5 | 15.4 ± 0.2 | 15.3 ± 0.2* | -0.1 ± 0.1* | 91 | 5.0 | 8.0 |
| 20,000 | 5/5 | 15.2 ± 0.6 | 14.6 ± 0.3** | -0.6 ± 0.3* | 87 | 6.9 | 7.3 |
| 40,000 | 3/5 ^e | 15.1 ± 0.7 | 15.3 ± 0.7* | 0.8 ± 0.9* | 91 | 8.8 | 7.5 |

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

** $P \leq 0.01$

^a Number of animals surviving/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.

^c Feed consumption is expressed as grams of feed consumed per animal per day.

^d Day of death: 5, 5, 6

^e Day of death: 7, 8

Absolute and relative thymus weights of 20,000 ppm males and 10,000, 20,000, and 40,000 ppm females were significantly less than those of the controls (Table F4). Relative liver weights of 20,000 and 40,000 ppm males and females and of 10,000 ppm females were significantly greater than those of the controls (Table F4). Differences in the absolute and relative weights of other organs were related to decreased body weights. No biologically significant differences in hematology parameters occurred in exposed males or females (Table G4).

There were no gross lesions observed at necropsy that were considered to be related to chemical administration. Degeneration of the germinal epithelium of

the testis was observed in 20,000 and 40,000 ppm males (Table 14). Microscopically, testicular degeneration was characterized by multinucleated giant cells, pyknosis, and cytoplasmic vacuolization of germinal cells. Bone marrow hemorrhage and bone marrow atrophy occurred in the 40,000 ppm female mice that died early. It was uncertain if these effects were due to the reduced body weight or if they were a chemical effect. Other lesions, including bone marrow atrophy and hemorrhage in males, were considered secondary to stress and inanition.

Based on mortality and reduced mean body weights at 40,000 ppm, the high concentration selected for the 13-week feed study in mice was 20,000 ppm.

TABLE 14
Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Day Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 2,500 | 5,000 | 10,000 | 20,000 | 40,000 |
|-----------------------------------|---|----------------|-------|--------|--------|--------|
| Male | | | | | | |
| Bone Marrow ^a | 5 | — ^b | — | — | 5 | 5 |
| Atrophy ^c | 0 | — | — | — | 0 | 2 |
| Hemorrhage | 0 | — | — | — | 0 | 3 |
| Testis | 5 | — | — | 5 | 5 | 5 |
| Degeneration, Germinal Epithelium | 0 | — | — | 0 | 3 | 2 |
| Female | | | | | | |
| Bone Marrow | 5 | — | — | — | 5 | 5 |
| Atrophy | 0 | — | — | — | 0 | 3 |
| Hemorrhage | 0 | — | — | — | 0 | 2 |

^a Number of animals with organ examined microscopically

^b Organ not examined in this exposure group

^c Number of animals with lesion

13-WEEK STUDY

One female exposed to 1,250 ppm was accidentally killed during week 3 of the study. All other mice survived until the end of the study (Table 15). Final mean body weights and mean body weight gains of all exposed males and of females exposed to 5,000, 10,000, and 20,000 ppm were significantly lower than those of controls. No chemical-related clinical findings were observed. Feed consumption by exposed groups was similar to or greater than that by the controls throughout the study. Dietary levels of 1,250, 2,500, 5,000, 10,000, or 20,000 ppm *p*-nitrobenzoic acid delivered average daily doses of 170, 330, 670, 1,900, or 4,000 mg/kg body weight to males and 240, 460, 970, 2,500, or 4,900 mg/kg to females.

Differences in absolute and relative organ weights in exposed mice were considered to be related to lower body weights (Table F5). Microscopically, minimal degeneration of the germinal epithelium of the seminiferous tubules was observed in the testis of six 20,000 ppm males. Testicular degeneration was considered to be related to the reduced body weight effect of *p*-nitrobenzoic acid exposure.

Dose Selection Rationale: Based on lower final mean body weights, the dietary levels of *p*-nitrobenzoic acid selected for the 2-year feed study in mice were 0, 1,250, 2,500, and 5,000 ppm.

TABLE 15
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) | Feed Consumption ^c | |
|---------------|-----------------------|-----------------------------------|--------------|-------------|---------------------------------------|-------------------------------|---------|
| | | Initial | Final | Change | | Week 1 | Week 13 |
| Male | | | | | | | |
| 0 | 10/10 | 21.5 ± 0.4 | 33.8 ± 0.6 | 12.4 ± 0.6 | | 3.5 | 3.8 |
| 1,250 | 10/10 | 21.6 ± 0.6 | 31.5 ± 0.7** | 9.9 ± 0.7* | 93 | 3.5 | 3.8 |
| 2,500 | 10/10 | 21.9 ± 0.4 | 31.6 ± 0.7** | 9.7 ± 0.6** | 93 | 3.7 | 3.4 |
| 5,000 | 10/10 | 22.0 ± 0.4 | 29.6 ± 0.7** | 7.6 ± 0.7** | 88 | 3.3 | 3.6 |
| 10,000 | 10/10 | 21.8 ± 0.6 | 26.8 ± 0.3** | 4.9 ± 0.3** | 79 | 4.0 | 5.3 |
| 20,000 | 10/10 | 21.9 ± 0.6 | 23.4 ± 0.4** | 1.6 ± 0.9** | 69 | 4.6 | 5.0 |
| Female | | | | | | | |
| 0 | 10/10 | 17.5 ± 0.2 | 26.3 ± 0.5 | 8.8 ± 0.4 | | 3.6 | 4.2 |
| 1,250 | 9/10 ^d | 17.6 ± 0.2 | 25.3 ± 0.5 | 7.7 ± 0.5 | 96 | 3.9 | 4.4 |
| 2,500 | 10/10 | 17.6 ± 0.2 | 26.0 ± 0.3 | 8.4 ± 0.3 | 99 | 3.7 | 4.4 |
| 5,000 | 10/10 | 17.6 ± 0.4 | 24.7 ± 0.3** | 7.1 ± 0.3** | 94 | 3.6 | 4.6 |
| 10,000 | 10/10 | 17.6 ± 0.3 | 22.6 ± 0.3** | 5.0 ± 0.3** | 86 | 4.2 | 5.8 |
| 20,000 | 10/10 | 17.5 ± 0.2 | 20.1 ± 0.4** | 2.6 ± 0.5** | 76 | 4.3 | 4.8 |

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

** $P \leq 0.01$

^a Number of animals surviving/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.

^c Feed consumption is expressed as grams of feed consumed per animal per day.

^d Week of death: 3 (accidental)

2-YEAR STUDY

Survival

Estimates of survival probabilities for male and female mice are presented in Table 16 and in the Kaplan-Meier curves in Figure 4. Two-year survival rates of exposed mice were similar to those of the controls.

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of 5,000 ppm males were 6% to 12% lower than those of the controls after week 17 of the study (Table 17 and Figure 5). Mean body weights of 5,000 ppm females were 12% to 24%

lower than those of the controls after week 16 (Table 18 and Figure 5). The final mean body weight of 5,000 ppm males was 90% that of the controls, and the final mean body weight of 5,000 ppm females was 81% that of the controls. Mean body weights of the other exposure groups were similar to those of the controls.

Feed consumption by exposed groups was similar to that by the control groups (Tables I3 and I4). Dietary levels of 1,250, 2,500, or 5,000 ppm *p*-nitrobenzoic acid delivered 150, 300, or 675 mg/kg body weight per day to males and 170, 365, or 905 mg/kg per day to females. There were no clinical findings of organ-specific toxicity in mice.

TABLE 16
Survival of Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|--|----------|---------|---------|----------|
| Male | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Accidental deaths ^a | | 1 | | 2 |
| Moribund | 9 | 4 | 10 | 2 |
| Natural deaths | 2 | 9 | 1 | 2 |
| Animals surviving to study termination | 39 | 36 | 39 | 44 |
| Percent probability of survival at end of study ^b | 78 | 74 | 78 | 92 |
| Mean survival (days) ^c | 666 | 654 | 664 | 641 |
| Survival analyses ^d | P=0.077N | P=0.764 | P=1.000 | P=0.125N |
| Female | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Accidental deaths ^a | | | 1 | 2 |
| Moribund | 9 | 8 | 12 | 10 |
| Natural deaths | 3 | 5 | 4 | 8 |
| Animals surviving to study termination | 38 | 36 | 33 | 30 |
| Missing ^a | | 1 | | |
| Percent probability of survival at end of study | 77 | 74 | 67 | 63 |
| Mean survival (days) | 660 | 658 | 649 | 625 |
| Survival analyses | P=0.118 | P=0.925 | P=0.465 | P=0.194 |

^a Censored from survival analyses

^b Kaplan-Meier determinations based on the number of animals alive on first day of terminal sacrifice

^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 exposure columns. A negative trend or a lower mortality in an exposure group is indicated by N.

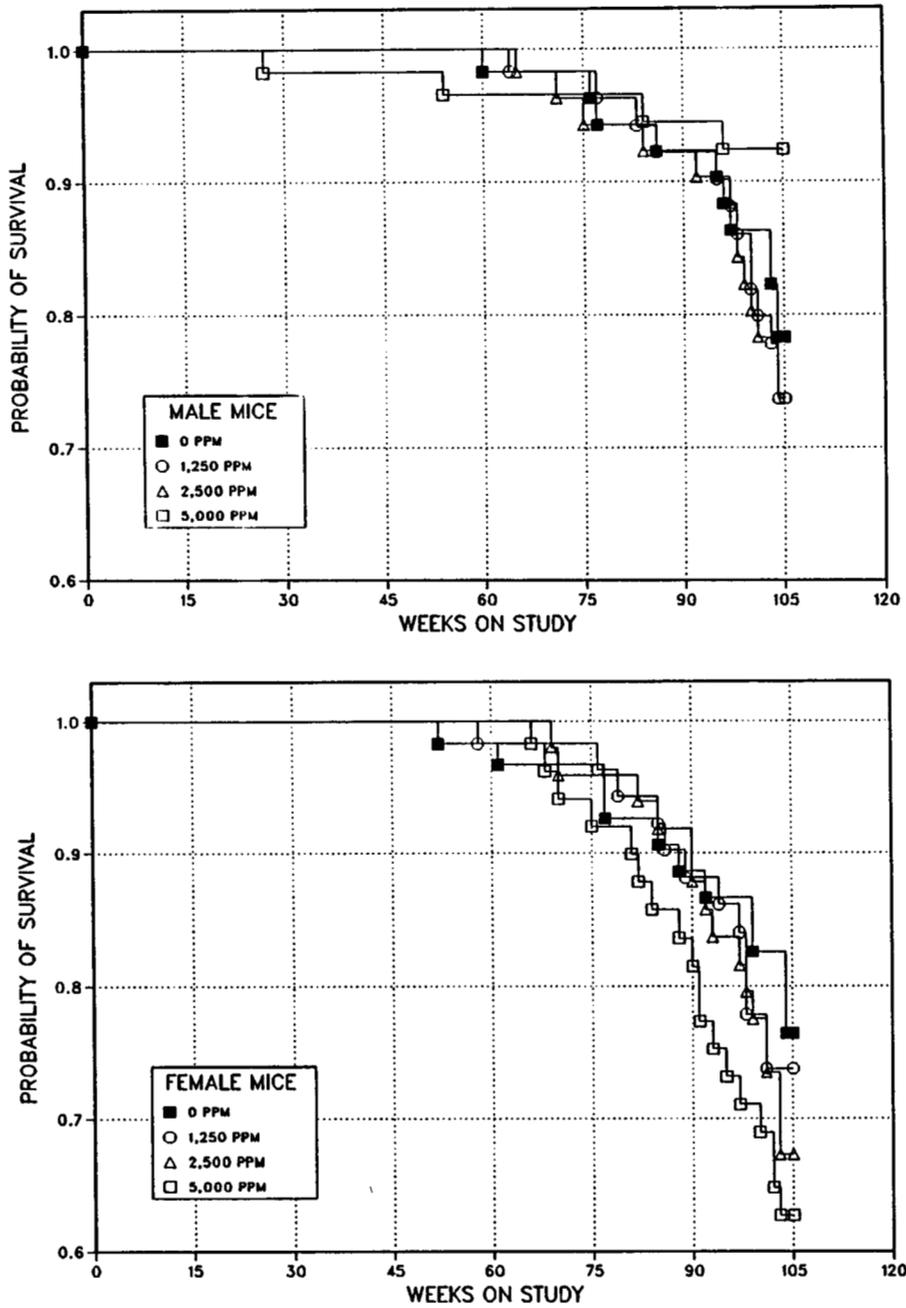


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered
***p*-Nitrobenzoic Acid in Feed for 2 Years**

TABLE 17
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Weeks on Study | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|----------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 23.7 | 60 | 22.7 | 96 | 60 | 23.0 | 97 | 60 | 22.9 | 97 | 60 |
| 2 | 24.8 | 60 | 24.3 | 98 | 59 | 24.4 | 98 | 60 | 23.9 | 96 | 58 |
| 3 | 26.0 | 60 | 25.4 | 98 | 59 | 25.7 | 99 | 60 | 25.1 | 97 | 58 |
| 4 | 26.8 | 60 | 26.5 | 99 | 59 | 26.4 | 99 | 60 | 25.9 | 97 | 58 |
| 5 | 27.5 | 60 | 27.3 | 99 | 59 | 27.4 | 100 | 60 | 26.8 | 98 | 58 |
| 6 | 28.1 | 60 | 27.9 | 99 | 59 | 27.9 | 99 | 60 | 27.3 | 97 | 58 |
| 7 | 28.6 | 60 | 28.0 | 98 | 59 | 28.4 | 99 | 60 | 27.5 | 96 | 58 |
| 8 | 29.3 | 60 | 28.8 | 98 | 59 | 29.3 | 100 | 60 | 28.3 | 97 | 58 |
| 9 | 30.5 | 60 | 29.9 | 98 | 59 | 30.2 | 99 | 60 | 29.3 | 96 | 58 |
| 10 | 31.1 | 60 | 30.1 | 97 | 59 | 30.8 | 99 | 60 | 29.5 | 95 | 58 |
| 11 | 32.0 | 60 | 31.2 | 98 | 59 | 31.7 | 99 | 60 | 30.3 | 95 | 58 |
| 12 | 31.7 | 60 | 31.8 | 100 | 59 | 31.5 | 99 | 60 | 30.7 | 97 | 58 |
| 13 | 33.1 | 60 | 32.8 | 99 | 59 | 33.1 | 100 | 60 | 31.7 | 96 | 58 |
| 17 | 35.4 | 60 | 34.9 | 99 | 59 | 34.9 | 99 | 60 | 33.2 | 94 | 58 |
| 21 | 37.2 | 60 | 36.5 | 98 | 59 | 36.4 | 98 | 60 | 34.5 | 93 | 58 |
| 25 | 38.4 | 60 | 38.0 | 99 | 59 | 37.8 | 98 | 60 | 35.3 | 92 | 58 |
| 29 | 40.8 | 60 | 40.2 | 99 | 59 | 39.8 | 98 | 60 | 37.5 | 92 | 57 |
| 33 | 41.9 | 60 | 41.9 | 100 | 59 | 41.6 | 99 | 60 | 38.7 | 92 | 57 |
| 37 | 43.4 | 60 | 43.5 | 100 | 59 | 43.3 | 100 | 60 | 40.8 | 94 | 57 |
| 41 | 45.5 | 60 | 45.1 | 99 | 59 | 44.8 | 99 | 60 | 41.8 | 92 | 57 |
| 45 | 46.1 | 60 | 45.4 | 99 | 59 | 45.4 | 99 | 60 | 41.9 | 91 | 57 |
| 49 | 47.5 | 60 | 46.9 | 99 | 59 | 46.7 | 98 | 60 | 43.3 | 91 | 57 |
| 53 | 47.4 | 60 | 47.3 | 100 | 59 | 46.7 | 99 | 60 | 43.7 | 92 | 57 |
| 57 | 47.6 | 60 | 46.8 | 98 | 59 | 46.3 | 97 | 60 | 42.9 | 90 | 56 |
| 61 | 47.3 | 59 | 46.5 | 98 | 59 | 46.0 | 97 | 60 | 42.8 | 91 | 56 |
| 65 | 47.9 | 59 | 47.5 | 99 | 58 | 46.2 | 97 | 60 | 43.5 | 91 | 56 |
| 69 ^a | 47.8 | 49 | 48.0 | 100 | 48 | 46.3 | 97 | 49 | 42.8 | 90 | 46 |
| 73 | 49.5 | 49 | 48.9 | 99 | 48 | 48.2 | 97 | 48 | 44.4 | 90 | 46 |
| 77 | 49.2 | 48 | 48.8 | 99 | 48 | 48.2 | 98 | 47 | 44.7 | 91 | 46 |
| 81 | 49.2 | 47 | 48.1 | 98 | 47 | 47.1 | 96 | 47 | 43.5 | 88 | 46 |
| 85 | 48.0 | 47 | 48.4 | 101 | 46 | 47.3 | 99 | 46 | 43.6 | 91 | 45 |
| 89 | 48.9 | 46 | 48.8 | 100 | 45 | 47.4 | 97 | 46 | 43.3 | 89 | 45 |
| 93 | 48.0 | 46 | 49.0 | 102 | 45 | 47.5 | 99 | 45 | 43.1 | 90 | 45 |
| 97 | 46.5 | 43 | 47.5 | 102 | 44 | 46.6 | 100 | 44 | 41.8 | 90 | 44 |
| 101 | 45.9 | 43 | 47.7 | 104 | 39 | 46.4 | 101 | 40 | 41.4 | 90 | 44 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 28.7 | | 28.2 | 98 | | 28.4 | 99 | | 27.6 | 96 | |
| 14-52 | 41.8 | | 41.4 | 99 | | 41.2 | 99 | | 38.6 | 92 | |
| 53-101 | 47.9 | | 47.9 | 100 | | 46.9 | 98 | | 43.2 | 90 | |

^a Interim evaluation occurred during week 66.

TABLE 18
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Weeks on Study | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|----------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 17.9 | 60 | 18.0 | 101 | 60 | 17.7 | 99 | 60 | 17.8 | 99 | 60 |
| 2 | 20.4 | 60 | 20.2 | 99 | 60 | 20.1 | 99 | 59 | 19.8 | 97 | 58 |
| 3 | 22.4 | 60 | 22.3 | 100 | 60 | 22.1 | 99 | 59 | 21.5 | 96 | 58 |
| 4 | 23.1 | 60 | 23.0 | 100 | 60 | 22.7 | 98 | 59 | 21.9 | 95 | 58 |
| 5 | 24.1 | 60 | 24.1 | 100 | 60 | 23.6 | 98 | 59 | 22.7 | 94 | 58 |
| 6 | 24.3 | 60 | 24.5 | 101 | 60 | 24.2 | 100 | 59 | 23.3 | 96 | 58 |
| 7 | 25.2 | 60 | 25.5 | 101 | 60 | 24.9 | 99 | 59 | 23.9 | 95 | 58 |
| 8 | 25.4 | 60 | 25.8 | 102 | 60 | 25.3 | 100 | 59 | 24.5 | 97 | 58 |
| 9 | 25.9 | 60 | 25.8 | 100 | 60 | 25.4 | 98 | 59 | 24.5 | 95 | 58 |
| 10 | 27.1 | 60 | 27.0 | 100 | 60 | 26.1 | 96 | 59 | 25.3 | 93 | 58 |
| 11 | 28.2 | 60 | 28.0 | 99 | 60 | 27.1 | 96 | 59 | 26.0 | 92 | 58 |
| 12 | 28.8 | 60 | 28.5 | 99 | 60 | 27.6 | 96 | 59 | 26.4 | 92 | 58 |
| 16 | 31.2 | 60 | 30.2 | 97 | 60 | 29.7 | 95 | 59 | 27.4 | 88 | 58 |
| 20 | 33.4 | 60 | 32.3 | 97 | 60 | 31.5 | 94 | 59 | 28.9 | 87 | 58 |
| 24 | 34.3 | 60 | 33.7 | 98 | 60 | 32.1 | 94 | 59 | 29.7 | 87 | 58 |
| 28 | 36.8 | 60 | 35.7 | 97 | 60 | 34.8 | 95 | 59 | 31.1 | 85 | 58 |
| 32 | 39.2 | 60 | 38.1 | 97 | 60 | 37.2 | 95 | 59 | 32.9 | 84 | 58 |
| 36 | 40.4 | 60 | 39.7 | 98 | 60 | 38.6 | 96 | 59 | 34.1 | 84 | 58 |
| 40 | 42.6 | 60 | 42.6 | 100 | 60 | 41.0 | 96 | 59 | 35.6 | 84 | 58 |
| 44 | 44.6 | 60 | 44.1 | 99 | 60 | 42.5 | 95 | 59 | 36.4 | 82 | 58 |
| 48 | 46.0 | 60 | 45.7 | 99 | 60 | 44.0 | 96 | 59 | 38.1 | 83 | 58 |
| 52 | 47.0 | 60 | 46.3 | 99 | 60 | 45.0 | 96 | 59 | 38.5 | 82 | 58 |
| 56 | 46.3 | 59 | 45.8 | 99 | 60 | 43.8 | 95 | 59 | 37.0 | 80 | 58 |
| 60 | 47.0 | 59 | 46.9 | 100 | 59 | 44.5 | 95 | 59 | 37.2 | 79 | 58 |
| 64 | 48.8 | 58 | 48.0 | 98 | 59 | 44.7 | 92 | 59 | 37.6 | 77 | 58 |
| 68 ^a | 48.5 | 48 | 48.7 | 100 | 49 | 45.9 | 95 | 49 | 38.0 | 78 | 46 |
| 72 | 49.7 | 48 | 49.5 | 100 | 49 | 47.2 | 95 | 47 | 39.0 | 79 | 45 |
| 76 | 51.2 | 48 | 51.4 | 100 | 47 | 49.0 | 96 | 47 | 40.0 | 78 | 44 |
| 80 | 52.1 | 46 | 51.0 | 98 | 46 | 48.2 | 93 | 47 | 39.9 | 77 | 44 |
| 84 | 51.2 | 46 | 51.0 | 100 | 46 | 48.2 | 94 | 46 | 40.5 | 79 | 41 |
| 88 | 52.0 | 44 | 50.6 | 97 | 44 | 48.1 | 93 | 45 | 39.6 | 76 | 41 |
| 93 | 50.6 | 43 | 49.5 | 98 | 43 | 47.4 | 94 | 42 | 38.5 | 76 | 37 |
| 96 | 49.9 | 43 | 48.5 | 97 | 42 | 46.5 | 93 | 41 | 39.3 | 79 | 35 |
| 100 | 49.8 | 41 | 48.6 | 98 | 38 | 46.2 | 93 | 38 | 38.8 | 78 | 33 |
| 104 | 48.2 | 38 | 47.7 | 99 | 36 | 45.2 | 94 | 33 | 38.8 | 81 | 30 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 24.4 | | 24.4 | 100 | | 23.9 | 98 | | 23.1 | 95 | |
| 14-52 | 39.6 | | 38.8 | 98 | | 37.6 | 95 | | 33.3 | 84 | |
| 53-104 | 49.6 | | 49.0 | 99 | | 46.5 | 94 | | 38.8 | 78 | |

^a Interim evaluation occurred during week 66.

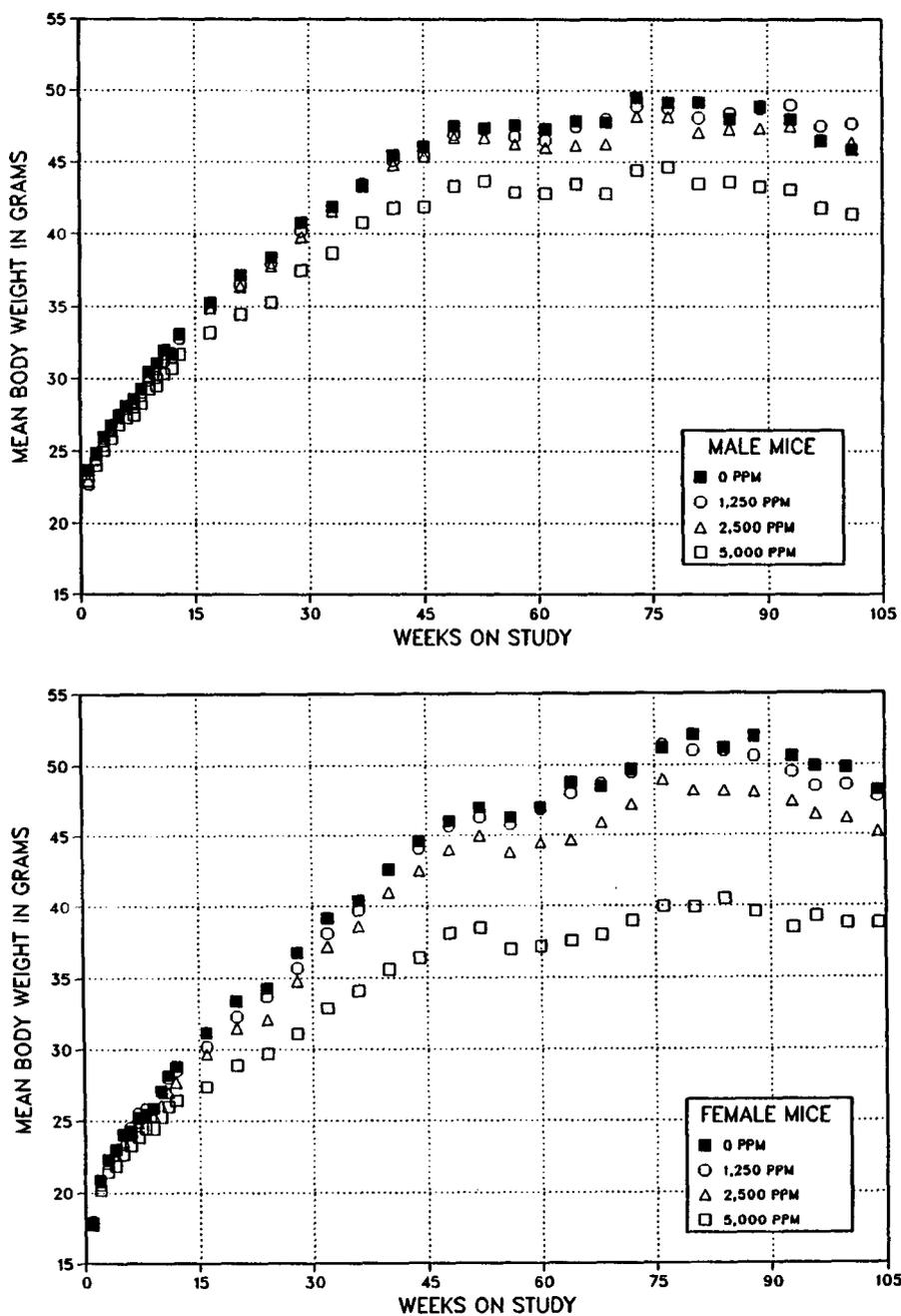


FIGURE 5
Growth Curves for Mice Administered *p*-Nitrobenzoic Acid in Feed for 2 Years

Hematology

The results of hematology evaluations are shown in Table G5. No chemical-related effects on hematology parameters were observed at 15 months.

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the lung and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the biologically significant neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Lung: The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 1,250 and 5,000 ppm females were significantly greater than that of the controls by pairwise comparisons but not by the trend statistics, and the highest incidence in these groups (20%) fell within the historical control range of 2% to 26% (Tables 19, D3, and D4). The historical incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in control female B6C3F₁ mice from recent NTP studies is 106/1,371 (7.7%). At the 15-month interim evaluation, no hyperplasia or neoplasms were observed in females. At 2 years, the incidences of alveolar epithelial hyperplasia in exposed females were similar to that of the controls.

At the 15-month interim evaluation, a few alveolar/bronchiolar adenomas were observed in exposed male mice. In addition, alveolar epithelial hyperplasia was observed in one 1,250 ppm and one 2,500 ppm male but not in controls. At 2 years, incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in exposed groups of male mice were not

significantly different than that of the controls (Tables 19 and C3). The incidence of alveolar epithelial hyperplasia in 5,000 ppm males was greater than that of the controls.

Alveolar epithelial hyperplasia is considered a precursor lesion of alveolar/bronchiolar adenoma and carcinoma. Hyperplasia consists of a focal increase in cellularity of the alveolar epithelium with retention of the alveolar architecture. In contrast, alveolar/bronchiolar adenomas are discrete expansile masses that compress adjacent tissue. Adenomas lack normal architecture and consist of somewhat pleomorphic to columnar cells arranged in regular or papillary patterns. Alveolar/bronchiolar carcinomas are similar but consist of heterogeneous cell populations with varying degrees of cellular pleomorphism and atypia. Adenocarcinomas are larger, highly anaplastic neoplasms, often containing areas of hemorrhage or necrosis.

Kidney: The relative kidney weight of females exposed to 5,000 ppm was significantly greater than that of the controls at 15 months (Table F6); however, this effect was considered to be related to the lower mean body weight in this exposure group. The incidence of mineralization of the kidney was lower than that of the controls in 5,000 ppm females (15/50, 7/49, 7/50, 5/50; Table D5). The mineralization was minimal in severity (1.0, 1.0, 1.0, 1.2) and was not considered to be chemical related. The incidences of mineralization in males exposed to 2,500 and 5,000 ppm were also lower than that of the controls (41/50, 33/49, 23/50, 31/48; Table C5). At 15 months and 2 years, there were marginal decreases in the incidence of renal tubule regeneration in male and female mice. In addition, at 2 years, there were marginal decreases in the incidence of cortical cysts in male mice.

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|--|------------|-------------|-------------|-------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Lung ^a | 10 | 10 | 10 | 10 |
| Alveolar Epithelial Hyperplasia ^b | 0 | 1 | 1 | 0 |
| Alveolar/bronchiolar Adenoma | 0 | 1 | 2 | 1 |
| 2-Year Study | | | | |
| Lung | 50 | 50 | 50 | 50 |
| Alveolar Epithelial Hyperplasia | 2 | 7 | 7 | 8* |
| Alveolar/bronchiolar Adenoma | | | | |
| Overall rate ^c | 6/50 (12%) | 12/50 (24%) | 8/50 (16%) | 9/50 (18%) |
| Adjusted rate ^d | 15.4% | 29.2% | 20.5% | 20.5% |
| Terminal rate ^e | 6/39 (15%) | 8/36 (22%) | 8/39 (21%) | 9/44 (20%) |
| First incidence (days) | 729 (T) | 537 | 729 (T) | 729 (T) |
| Life table test ^f | P=0.545 | P=0.077 | P=0.385 | P=0.378 |
| Logistic regression test ^f | P=0.393 | P=0.091 | P=0.385 | P=0.378 |
| Alveolar/bronchiolar Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 2.6% | 8.0% | 5.1% | 11.4% |
| Terminal rate | 1/39 (3%) | 2/36 (6%) | 2/39 (5%) | 5/44 (11%) |
| First incidence (days) | 729 (T) | 725 | 729 (T) | 729 (T) |
| Life table test | P=0.116 | P=0.279 | P=0.500 | P=0.133 |
| Logistic regression test | P=0.095 | P=0.279 | P=0.500 | P=0.133 |
| Alveolar/bronchiolar Adenoma or Carcinoma ^g | | | | |
| Overall rate | 7/50 (14%) | 14/50 (28%) | 10/50 (20%) | 13/50 (26%) |
| Adjusted rate | 17.9% | 34.2% | 25.6% | 29.5% |
| Terminal rate | 7/39 (18%) | 10/36 (28%) | 10/39 (26%) | 13/44 (30%) |
| First incidence (days) | 729 (T) | 537 | 729 (T) | 729 (T) |
| Life table test | P=0.303 | P=0.053 | P=0.293 | P=0.166 |
| Logistic regression test | P=0.165 | P=0.064 | P=0.293 | P=0.166 |

(continued)

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| Dose (ppm) | 0 | 1,250 | 2,500 | 5,000 |
|--|----------------|-------------|-----------|------------|
| Female | | | | |
| Lung | 50 | 49 | 50 | 50 |
| Alveolar Epithelial Hyperplasia | 3 | 3 | 0 | 1 |
| Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 3/50 (6%) | 5/49 (10%) | 3/50 (6%) | 8/50 (16%) |
| Adjusted rate | 7.5% | 12.9% | 8.7% | 24.3% |
| Terminal rate | 2/38 (5%) | 3/36 (8%) | 2/33 (6%) | 6/30 (20%) |
| First incidence (days) | 689 | 685 | 715 | 570 |
| Life table test | P=0.035 | P=0.324 | P=0.599 | P=0.050 |
| Logistic regression test | P=0.052 | P=0.343 | P=0.643 | P=0.071 |
| Alveolar/bronchiolar Carcinoma | | | | |
| Overall rate | 0/50 (0%) | 5/49 (10%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 13.9% | 2.9% | 3.3% |
| Terminal rate | 0/38 (0%) | 5/36 (14%) | 0/33 (0%) | 1/30 (3%) |
| First incidence (days) | ^h — | 730 (T) | 720 | 730 (T) |
| Life table test | P=0.572N | P=0.029 | P=0.468 | P=0.453 |
| Logistic regression test | P=0.568N | P=0.029 | P=0.491 | P=0.453 |
| Alveolar/bronchiolar Adenoma or Carcinoma ⁱ | | | | |
| Overall rate | 3/50 (6%) | 10/49 (20%) | 4/50 (8%) | 9/50 (18%) |
| Adjusted rate | 7.5% | 26.1% | 11.3% | 27.5% |
| Terminal rate | 2/38 (5%) | 8/36 (22%) | 2/33 (6%) | 7/30 (23%) |
| First incidence (days) | 689 | 685 | 715 | 570 |
| Life table test | P=0.063 | P=0.031 | P=0.428 | P=0.027 |
| Logistic regression test | P=0.088 | P=0.031 | P=0.475 | P=0.039 |

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test

(T) Terminal sacrifice

^a Number of animals with lung examined microscopically

^b Number of animals with lesion

^c Number of animals with neoplasm per number of animals examined microscopically

^d Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^e Observed incidence in animals surviving until the end of the study

^f In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test 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. A negative trend in an exposure group is indicated by N.

^g Historical incidence for 2-year NTP feed studies with untreated control groups (mean \pm standard deviation): 242/1,369 (17.7% \pm 7.3%), range 4%-30%

^h Not applicable; no neoplasms in animal group

ⁱ Historical incidence: 106/1,371 (7.7% \pm 5.0%), range 2%-26%

GENETIC TOXICOLOGY

p-Nitrobenzoic acid, tested in a preincubation protocol at concentrations of 1 to 3,333 $\mu\text{g}/\text{plate}$, with and without induced rat or hamster S9, was mutagenic in strain TA100 (Table E1; Zeiger *et al.*, 1987). No mutagenicity was detected in strains TA98, TA1535, or TA1537, with or without S9.

In cytogenetic tests with cultured Chinese hamster ovary cells, *p*-nitrobenzoic acid induced significant increases in sister chromatid exchanges (Table E2; Zeiger *et al.*, 1987) and chromosomal aberrations (Table E3; Zeiger *et al.*, 1987) at dose levels which induced cell cycle delay in the absence of S9; no increases in either endpoint were observed in the presence of S9. In the sister chromatid exchange test without S9, doses ranging from 498 to 1,000 $\mu\text{g}/\text{mL}$

produced positive responses, induced cell cycle delay, and required use of an extended harvest protocol to allow accumulation of sufficient cells for metaphase analysis. Doses producing positive responses in the chromosomal aberrations assay without S9 ranged from 875 to 1,750 $\mu\text{g}/\text{mL}$ *p*-nitrobenzoic acid. As with the sister chromatid exchange test, cell harvest was delayed to permit a sufficient number of cells to progress to metaphase for analysis.

Despite the positive results obtained in the *in vitro* studies, results of a single NTP *in vivo* genotoxicity study were negative. In this study, the frequencies of micronucleated normochromatic erythrocytes in the peripheral blood of male and female mice were unaffected by exposure to *p*-nitrobenzoic acid in feed for 13 weeks (Table E4).

DISCUSSION AND CONCLUSIONS

p-Nitrobenzoic acid is produced in large volumes for organic synthesis and as an intermediate in the manufacture of pesticides, dyes, and industrial solvents. *p*-Nitrobenzoic acid is also a hydrolysis product or metabolite of other high-volume production chemicals including *p*-nitrobenzoyl chloride and *o*-nitrotoluene. Despite the widespread use and occurrence of *p*-nitrobenzoic acid, there is little information on the toxic and carcinogenic effects of this chemical after long-term exposure. These toxicity and carcinogenicity rodent studies were conducted to provide this information.

Many nitroaromatic compounds are toxic to the hematopoietic system (Beard and Noe, 1981; Beutler, 1985; Bus and Popp, 1987). Generalized toxicity to the hematopoietic system of the rat was observed in studies of the nitroaromatic compounds *o*-, *m*-, and *p*-nitrotoluene (NTP, 1992), *p*-chloroaniline (NCI, 1979a), and *o*-nitroanisole (NTP, 1993). The mechanism of the anemia is thought to involve oxidative damage to hemoglobin leading to Heinz body formation and decreased erythrocyte survival, followed by macrophage ingestion of the injured erythrocytes and removal of macrophages by the spleen resulting in splenic congestion and hemosiderin accumulation. The characteristic hematologic toxicity induced by amine/nitroaromatic compounds is attributed to the formation of a hydroxylamino compound (Facchini and Griffiths, 1981), and it has been observed in a variety of animals, including rodents, dogs, and cats, exposed to nitroaromatic compounds (Kiese, 1966) and in humans exposed to aniline and nitroaromatic compounds (Finch, 1948; Smith, 1991). *p*-Nitrobenzoic acid was toxic to the hematopoietic system of rats in the current studies.

In the 14-day rat study, there were decreases in erythrocyte count and hemoglobin and hematocrit values, and increases in nucleated erythrocytes, reticulocyte counts, and methemoglobin concentration, which were most pronounced in the 20,000 and 40,000 ppm groups. Hypertrophy of the follicular epithelium of the thyroid gland was observed in rats

at doses of 10,000 ppm and greater in the 14-day studies, but this change was not seen in the 13-week or 2-year studies, probably because lower doses were used and rats were able to adapt to this effect. In the 13-week rat study, a mild hemolytic (regenerative) anemia was characterized by decreases in hematocrit and hemoglobin values and increases in methemoglobin concentration, reticulocyte counts, and Heinz body formation in 10,000 ppm rats. Hemosiderin accumulation in the spleen was present in 2,500, 5,000, and 10,000 ppm male and female rats, and congestion was observed in 2,500 and 5,000 ppm males and 10,000 ppm males and females. There were increased incidences of renal tubule pigmentation (hemosiderin) in 5,000 and 10,000 ppm males. Increases in absolute and relative spleen weights were observed in 10,000 ppm rats in the 13-week study. At the 15-month interim evaluation in the 2-year rat study, there were decreases in erythrocyte count, hemoglobin, and hematocrit levels, and increases in nucleated erythrocytes that were most pronounced in 5,000 ppm females. In exposed males and females, increased severity of pigmentation (hemosiderin) of renal tubule epithelial cells was also supportive of this hemolytic anemia.

Hematologic toxicity was not observed in mice in these studies. In studies of other nitroaromatic compounds (e.g., nitrotoluenes, *p*-chloroaniline, and *p*-nitroanisole) the hematologic toxicity was also less severe in mice than in rats. Studies with *p*-chloroaniline hydrochloride (NTP, 1989a) and aniline hydrochloride (NCI, 1978) suggest that these chemicals are cleared from blood more quickly in mice than in rats (McCarthy *et al.*, 1985). Species differences in clearance of *p*-nitrobenzoic acid and its metabolites from blood may also account for the fact that mice are less susceptible to hematologic toxicity than rats. Methemoglobin can be reduced to hemoglobin in mammalian species by NADH-dependent methemoglobin reductase located in erythrocytes. Mice have higher levels of this reductase than rats (Smith, 1991), and species differences in the ability to reduce methemoglobin may be another reason why mice are less susceptible than rats to the hematologic toxicity of *p*-nitrobenzoic acid.

Because of the hematologic toxicity in rats and decreased body weights of 10,000, 20,000, and 40,000 ppm rats and mice in the 14-day studies and 10,000 ppm rats and mice in the 13-week studies, the highest exposure level selected for the 2-year studies was 5,000 ppm. While there were no chemical-related decreases in survival of exposed groups in the 2-year studies, the mean body weights of 5,000 ppm female rats, 5,000 ppm male mice, and 5,000 ppm female mice were consistently lower than those of the respective control groups. The 2-year studies were considered to be adequate assessments of the carcinogenic potential of *p*-nitrobenzoic acid in the F344/N rat and B6C3F₁ mouse.

In the 2-year study, the incidences of mononuclear cell leukemia were decreased in exposed groups of male and female rats. The incidences of mononuclear cell leukemia in 5,000 ppm male rats (4%) and 2,500 (6%) and 5,000 ppm (0%) female rats were below the historical control ranges from recent NTP 2-year feed studies (males: range 32% to 62%, 603/1,253, mean 48%; females: range 14% to 52%, 324/1,251, mean 26%). While the mechanism for this decrease is unknown, a decrease in the incidence of mononuclear cell leukemia has also been observed with other amine/nitro compounds including aniline hydrochloride (NCI, 1978) and *p*-chloroaniline (NCI, 1979a). Injury to splenic cells associated with hematologic toxicity may decrease the chance for the development of mononuclear cell leukemia, which arises from splenic cells in the Fischer rat (Losco and Ward, 1984; Stromberg, 1985). The splenic toxicity appears to be less severe with *p*-nitrobenzoic acid than with aniline hydrochloride or *p*-chloroaniline (Stefanski *et al.*, 1990). In studies of aniline hydrochloride (NCI, 1978), *p*-chloroaniline (NCI, 1979a), *o*-toluidine (NCI, 1979b), and D&C Red No. 9 (NTP, 1982a) splenic damage was more extensive and led to fibrosis and the development of sarcomas of the spleen (Goodman *et al.*, 1984; Weinberger *et al.*, 1985).

While decreased incidences of mononuclear cell leukemia have been observed in rats treated with amine/nitro compounds (Table 17), not all chemicals of this class produced this effect [e.g., *o*-nitroanisole (NTP, 1993)]. Decreased incidences of mononuclear cell leukemia have also been observed with aromatic compounds that do not contain the amine/nitro substitution [e.g., 4-hexylresorcinol (NTP, 1988a) and

α -methylbenzyl alcohol (NTP, 1990)]. Some of the chemicals that caused decreased incidences of mononuclear cell leukemia [nitrobenzoic acid (King and Henschel, 1941; Rosenthal and Bauer, 1941) and 4-hexylresorcinol (NTP, 1988a; Burnens and Vurma-Pupp, 1989; Collins and Levett, 1989)] share an antibacteriostatic activity, but studies to determine if this activity is related to inhibition of nucleic acid synthesis or cell proliferation/growth have not been reported. It has previously been reported (Rao *et al.*, 1987) that decreased incidences of naturally occurring neoplasms (e.g., neoplasms of the liver or mammary gland) may occur when a chemical causes a decrease in body weight. In the present 2-year studies of *p*-nitrobenzoic acid, there were decreases in body weights in exposed groups of rats where there were also decreases in the incidences of mononuclear cell leukemia. Further studies are needed to explain the relationship between body weight and neoplasm occurrence in rodents and to determine if body weight was a factor in the decreased incidences of mononuclear cell leukemia in rats exposed to *p*-nitrobenzoic acid.

In the 13-week rat study, chemical-related effects on the kidney included hyaline droplet accumulation in males and karyomegaly in males and females. In the 2-year rat study, chemical-related effects included tubule epithelial cell hyperplasia in 1,250 ppm males and oncocytic hyperplasia in 5,000 ppm males and females. Also at 2 years, nephropathy severity decreased with increasing dose in both males and females. However, the association between hyaline droplet accumulation ($\alpha_{2\mu}$ -globulin-associated nephrotoxicity) and kidney neoplasms was not observed in the 2-year study. Chemicals that cause protein droplet accumulation have been found to bind to $\alpha_{2\mu}$ -globulin (Dietrich and Swenberg, 1991). Evidence suggests that the chemical binding is responsible for the accumulation of this protein. Chemical-mediated accumulation of $\alpha_{2\mu}$ -globulin is thought to be responsible for cell death, which in turn stimulates cell division as the kidney attempts to repair itself. With prolonged chemical exposure, repeated cycles of cytotoxicity and reparative replication are proposed to be responsible for the observed tumorigenic response (USEPA, 1991). In the present studies, the typical cytotoxicity associated with hyaline droplet ($\alpha_{2\mu}$ -globulin-associated) nephrotoxicity (such as single cell necrosis of the P2 segment epithelium, accumulation of granular casts, linear mineralization

TABLE 17
Results of Carcinogenicity Tests of Selected Chemicals Causing Decreased Incidences
of Mononuclear Cell Leukemia in Male and Female Fisher Rats

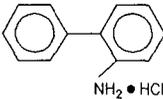
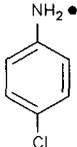
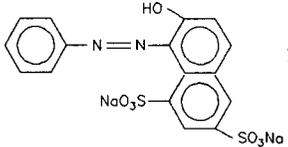
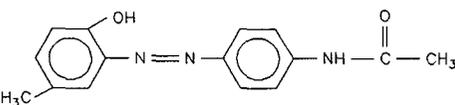
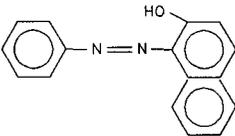
| Chemical | Incidences of Leukemia ^a | | Carcinogenicity ^b | | | | Salmonella Test Result |
|---|-------------------------------------|-------------------------|------------------------------|----------|------------|------------|------------------------------|
| | Male Rats | Female Rats | ♂ Rat | ♀ Rat | ♂ Mouse | ♀ Mouse | |
| 2-Biphenylamine Hydrochloride NTP TR 233  <chem>Nc1ccccc1-c2ccccc2</chem> $\text{NH}_2 \cdot \text{HCl}$ | 15/50, 1/50, 4/50 | 5/50, 1/49, 3/50 | - | - | + | - | |
| p-Chloroaniline Hydrochloride NTP TR 351  <chem>Nc1ccc(Cl)cc1</chem> $\text{NH}_2 \cdot \text{HCl}$ | 21/49, 3/50, 2/50, 3/50 | 10/50, 2/50, 1/50, 1/50 | + | - | + | - | + |
| C.I. Acid Orange 10 NTP TR 211  <chem>Oc1ccc2c(c1)ccc(S(=O)(=O)[Na])cc2=N=Nc3ccccc3</chem> | 22/90, 4/50, 3/50 | 16/88, 2/50, 0/50 | - | - | - | - | - |
| C.I. Disperse Yellow 3 NTP TR 222  <chem>CC(=O)Nc1ccc(N=Nc2cc(O)c(C)cc2)cc1</chem> | 13/50, 2/50, 1/50 | 8/50, 2/50, 1/50 | + | - | - | + | + |
| C.I. Solvent Yellow 14 NTP TR 226  <chem>Oc1ccc2c(c1)ccc3ccccc32=N=Nc4ccccc4</chem> | 25/50, 2/50, 4/50 | 11/50, 2/49, 0/49 | + | + | - | - | + |

TABLE 17
Results of Carcinogenicity Tests of Selected Chemicals Causing Decreased Incidences
of Mononuclear Cell Leukemia in Male and Female Fisher Rats (continued)

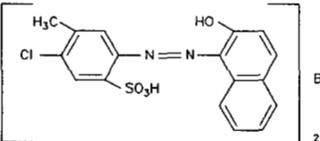
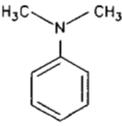
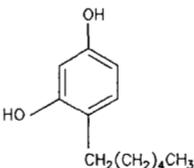
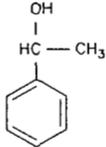
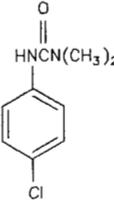
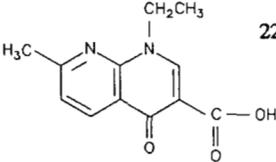
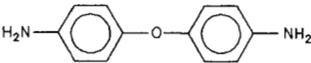
| Chemical | Incidences of Leukemia | | Carcinogenicity | | | | <i>Salmonella</i> Test Result |
|---|------------------------|-------------------|-----------------|-------|---------|---------|----------------------------------|
| | Male Rats | Female Rats | ♂ Rat | ♀ Rat | ♂ Mouse | ♀ Mouse | |
| D & C Red 9 NTP TR 225  | 12/50, 4/50, 3/50 | 11/50, 5/50, 3/50 | + S,L | - | - | - | + |
| <i>N,N</i>-Dimethylaniline NTP TR 360  | 13/50, 4/50, 3/50 | 11/50, 7/50, 0/50 | + S | - | - | - | - |
| 4-Hexylresorcinol NTP TR 330  | 12/49, 7/50, 1/50 | 16/50, 3/50, 2/50 | - | - | - | - | - |
| α-Methylbenzyl Alcohol NTP TR 369  | 15/50, 2/50, 0/50 | 12/50, 2/50, 2/50 | + K | - | - | - | - |
| Monuron NTP TR 266  | 5/50, 0/50, 0/50 | 10/50, 2/50, 2/50 | + K,L | - | - | - | - |

TABLE 17
Results of Carcinogenicity Tests of Selected Chemicals Causing Decreased Incidences
of Mononuclear Cell Leukemia in Male and Female Fisher Rats (continued)

| Chemical | Incidences of Leukemia | | Carcinogenicity | | | | Salmonella Test Result |
|--|---------------------------|--------------------------|-----------------|-------|---------|---------|------------------------|
| | Male Rats | Female Rats | ♂ Rat | ♀ Rat | ♂ Mouse | ♀ Mouse | |
| <p>Nalidixic Acid NTP 368</p>  | 22/50, 13/50, 17/50 | 20/50, 9/50, 7/50 | + | + | - | - | - |
| <p>p-Nitrobenzoic Acid NTP TR 442</p>  | 29/50, 35/50, 26/50, 2/50 | 17/50, 11/50, 3/50, 0/50 | - | + | - | - | + |
| <p>4,4'-Oxydianiline NCI TR 205</p>  | 27/50, 4/50, 4/50, 3/50 | 3/50, 1/50, 2/50, 0/50 | + | + | + | + | + |

^a Incidences given in increasing order of dose level beginning with control.
^b Levels of carcinogenic evidence: + = some or clear evidence, - = no or equivocal evidence; C = clitoral gland, H = harderian gland, He = hemangiosarcoma, K = kidney, L = liver, P = preputial gland, S = spleen, T = thyroid gland.

of tubules within the renal papilla, hyperplasia of the renal pelvis, or significant increases in the incidences of kidney neoplasms) was not observed. In addition, compared to $\alpha_{2\mu}$ -globulin-associated nephrotoxicity where there is an exacerbation of spontaneous chronic progressive nephropathy, there was a decrease in the severity of nephropathy in rats exposed to *p*-nitrobenzoic acid. The lack of cytotoxicity in male rats with hyaline droplet accumulation would suggest that *p*-nitrobenzoic acid might not bind strongly to $\alpha_{2\mu}$ -globulin, thus $\alpha_{2\mu}$ -globulin accumulation might be below the concentration that elicits a cytotoxic response and subsequently a carcinogenic effect.

In NTP studies, oncocytic hyperplasia has not commonly been associated with the spectrum of renal proliferative lesions thought to be important in the development of renal tubule adenomas and carcinomas. Renal tubule neoplasms are thought to originate from proximal tubules, while oncocytic proliferative lesions are thought to originate from distal tubules (Bannasch *et al.*, 1986). In addition, oncocytic proliferative lesions are different from renal tubule proliferative lesions in that cells in oncocytic proliferative lesions are usually much larger and have a more densely packed granular eosinophilic cytoplasm; the characteristic granular appearance of the cytoplasm is caused by populations of atypical

mitochondria. In the present studies, oncocytic hyperplastic lesions did not progress to oncocytomas. Rat renal oncocytomas appear to be benign end stage lesions that do not progress to malignant neoplasms (Bannasch *et al.*, 1986). In the NTP database, kidney proliferative lesions and neoplasms include documented oncocytic hyperplasias and oncocytomas, but no malignant oncocytic neoplasms have been observed.

The step sections revealed additional renal tubule neoplasms and hyperplasia in control and exposed male rats, but, in exposed males, incidences of renal tubule adenoma or carcinoma (combined) from both the single- and step-section evaluations were similar to that of the controls (1/50, 1/50, 3/50, 3/50). The incidences of renal tubule hyperplasia in 1,250 ppm males and of oncocytic hyperplasia in 5,000 ppm males were significantly greater than those of the controls. An increase in nonneoplastic lesions alone is not considered to be evidence of a carcinogenic effect. Further, the incidences of renal tubule adenoma (1/50, 1/50, 1/50, 3/50) from the step-section evaluation fell within the historical range for renal tubule adenoma from step-section evaluations in male control rats from other NTP studies (range 0% to 8%, 18/608, mean 3%; Table A4b). Thus, *p*-nitrobenzoic acid did not cause chemical-related increases in the incidences of kidney neoplasms either by the initial single-section evaluation or by the step-section evaluation. There were no renal tubule neoplasms in female rats.

In the 2-year rat study, the incidences of clitoral gland adenoma in 1,250, 2,500, and 5,000 ppm females, the incidence of clitoral gland carcinoma in 2,500 ppm females, and the incidences of clitoral gland adenoma or carcinoma (combined) in all exposed groups of females (0 ppm, 4/50; 1,250 ppm, 14/49; 2,500 ppm, 15/49; 5,000 ppm, 15/50) were significantly greater than those in the controls by both the life table and logistic regression tests. The incidences of clitoral gland adenoma or carcinoma (combined) in each exposed group (29% to 31%) were greater than that in historical controls (mean incidence, 11%; range, 2% to 21%). Based on these clitoral gland neoplasms, there was some evidence of a carcinogenic effect of *p*-nitrobenzoic acid in the female rat. The neoplasm incidences were not considered to represent a clear carcinogenic response because the incidences of clitoral gland neoplasms were approximately the same in each exposure group,

despite a fourfold increase in dose from the lowest exposure level to the highest. In addition, there was no notable decrease in the time to occurrence of neoplasm (mean time to diagnosis: 0 ppm, 699 days; 1,250 ppm, 712 days; 2,500 ppm, 672 days; or 5,000 ppm, 683 days) nor was there an increased incidence in clitoral gland hyperplasia.

A chemical-related increase in the incidence of clitoral gland neoplasms is often accompanied by an increase in the incidence of preputial gland neoplasms (male counterparts of clitoral gland neoplasms). In this study, a slight increase in the incidence of preputial gland neoplasms was observed in animals receiving 5,000 ppm *p*-nitrobenzoic acid. However, because of improved survival in this group, some increase in neoplasm incidence would be expected to occur by chance, and the life table test indicated that the slight increase was not statistically significant. The life table test was given primary emphasis because 21 of the 25 neoplasms occurred in animals that died early, suggesting that these neoplasms may have contributed to their deaths. Moreover, the neoplasm incidence in 5,000 ppm males (18%) fell well within the historical control range (2%-30%), and there was no chemical-related increase in preputial gland hyperplasia. Thus, the slight increase in preputial gland neoplasms was not considered to be chemical related.

The mechanism for the formation of clitoral gland neoplasms following exposure to *p*-nitrobenzoic acid could not be fully explained by the results of the present studies. Clitoral gland neoplasms have usually been observed with those chemicals that are genotoxic (Ashby and Tennant, 1991). Chemicals shown to induce clitoral gland neoplasms generally are strong mutagens in a variety of *Salmonella* test strains, and also induce neoplasms of the Zymbal's gland, skin, mammary gland, or a combination of these sites (Copeland-Haines and Eustis, 1990). *p*-Nitrobenzoic acid was positive in only one of the *Salmonella* test strains used and was negative in the *in vivo* mouse micronucleus test.

The incidences of thyroid gland C-cell adenoma (9/50, 5/49, 4/50, 2/50) and adenoma or carcinoma (combined) (10/50, 5/49, 6/50, 2/50) were significantly decreased in 5,000 ppm female rats. This response could not be conclusively related to *p*-nitrobenzoic acid exposure because the incidence of C-cell adenoma or carcinoma (combined) is highly variable

in historical controls. There were no chemical-related decreased incidences of thyroid gland C-cell neoplasms in male rats.

At the end of the 2-year mouse study, the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 1,250 and 5,000 ppm females were significantly greater than that of the controls by pairwise comparison (3/50, 10/49, 4/50, 9/50). However, the occurrence of lung neoplasms was not considered to be related to chemical administration because these neoplasms were not increased by the trend statistic; the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were within the historical range for control female B6C3F₁ mice in recent NTP feed studies (range 2% to 26%, 106/1,371, mean 8%); and there was no increase in the incidence of alveolar epithelial hyperplasia, a preneoplastic lesion. In other NTP studies where the lung was a target site for chemical-induced neoplasms in mice, the chemical is usually genotoxic, there are preneoplastic lesions, and males and females are both affected.

Nitroaromatic compounds are an important class of chemicals and it is estimated that 10% of chemicals used in various chemical industries are nitroaromatic chemicals. In a review of 301 chemicals studied by NTP, there were 84 aromatic amino/nitro-type chemicals: 59 were carcinogenic (93% of these chemicals were positive in the *S. typhimurium* assay); 8 gave only equivocal evidence of a carcinogenic response (63% were positive in the *S. typhimurium* assay); and 17 gave no evidence of a carcinogenic response (71% were positive in the *S. typhimurium* assay) (Ashby and Tennant, 1991). *p*-Nitrobenzoic acid fell into the class of aromatic amino/nitro-type chemicals that gave only some evidence for carcinogenic activity in the rodent. The benzoic acid moiety on the aromatic ring allows for *p*-nitrobenzoic acid to be conjugated with glucuronic acid, and may allow for more rapid excretion of the chemical in the urine than the other aromatic/amino chemicals that have been shown to cause some or clear evidence of carcinogenic activity in the rodent.

The regional position of substitutions on the aromatic ring plays an important role in the eventual metabolism and carcinogenic activity of the chemical (Jakoby *et al.*, 1982; Rickert, 1987). In several series of aromatic isomers tested for carcinogenic activity, the ortho-substituted chemical was more carcinogenic than the meta- or para-substituted chemicals. For example, in a 13-week study of *o*-, *m*-, and *p*-nitrotoluene, *o*-nitrotoluene caused mesothelioma and mesothelial cell hyperplasia in male rats, but no preneoplastic lesions or neoplasms were observed with *m*- or *p*-nitrotoluene (NTP, 1992). Weisburger *et al.* (1978) reported that ortho-substituted aromatic compounds are more potent carcinogens than corresponding isomers with meta- or para-substitutions. This was observed with *o*-, *m*-, and *p*-toluidine, where *o*-toluidine was carcinogenic in rats, while carcinogenic activity was not reported in rats treated with *m*- or *p*-toluidine. Information on the carcinogenic potential of *m*- and *o*-nitrobenzoic acid is not available, and the results on the carcinogenic activity of *p*-nitrobenzoic acid reported here may not be predictive of the carcinogenic activity of the other isomers.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of *p*-nitrobenzoic acid in male F344/N rats exposed to 1,250, 2,500, or 5,000 ppm. There was *some evidence of carcinogenic activity* of *p*-nitrobenzoic acid in female F344/N rats based on increases in the incidences of clitoral gland adenoma and of clitoral gland adenoma or carcinoma (combined). There was *no evidence of carcinogenic activity* of *p*-nitrobenzoic acid in male or female B6C3F₁ mice exposed to 1,250, 2,500, or 5,000 ppm.

There were chemical-related decreases in the incidences of mononuclear cell leukemia in exposed male and female rats. *p*-Nitrobenzoic acid caused mild hematologic toxicity in female rats.

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

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS
IN THE 2-YEAR FEED STUDY
OF *p*-NITROBENZOIC ACID

| | | |
|------------------|--|------------|
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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---------------------------------------|---------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Moribund | 32 | 34 | 34 | 25 |
| Natural deaths | 6 | 3 | 3 | 4 |
| Survivors | | | | |
| Terminal sacrifice | 12 | 13 | 13 | 21 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Endocrine System | | | | |
| Adrenal medulla | (10) | (10) | (10) | (10) |
| Pheochromocytoma benign | | | 1 (10%) | |
| Pituitary gland | (10) | (10) | (10) | (10) |
| Pars distalis, adenoma | 1 (10%) | 2 (20%) | 4 (40%) | 1 (10%) |
| Genital System | | | | |
| Preputial gland | (10) | (10) | (10) | (10) |
| Adenoma | 1 (10%) | | 1 (10%) | 1 (10%) |
| Testes | (10) | (10) | (10) | (10) |
| Bilateral, interstitial cell, adenoma | 4 (40%) | 7 (70%) | 7 (70%) | |
| Interstitial cell, adenoma | 5 (50%) | 1 (10%) | 2 (20%) | 1 (10%) |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Lymph node, mandibular | (10) | (10) | (10) | (10) |
| Lymph node, mesenteric | (10) | (10) | (10) | (10) |
| Spleen | (10) | (10) | (10) | (10) |
| Histiocytic sarcoma | | | | 1 (10%) |
| Thymus | (9) | (9) | (10) | (10) |
| Epithelial cell, thymoma benign | | | 1 (10%) | |
| Integumentary System | | | | |
| Mammary gland | (10) | (8) | (9) | (8) |
| Fibroadenoma | 1 (10%) | | | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar/bronchiolar adenoma | | | | 1 (10%) |

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (10) | (10) | (10) | (10) |
| Histiocytic sarcoma | | | | 1 (10%) |
| Leukemia mononuclear | | 1 (10%) | | |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Special Senses System | | | | |
| Urinary System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (49) | (50) | (49) | (50) |
| Polyp adenomatous | | | 1 (2%) | |
| Intestine large, rectum | (49) | (50) | (49) | (50) |
| Intestine large, cecum | (49) | (50) | (49) | (50) |
| Hemangiosarcoma | 1 (2%) | | | |
| Intestine small, duodenum | (48) | (48) | (49) | (50) |
| Intestine small, jejunum | (49) | (49) | (49) | (50) |
| Carcinoma | 2 (4%) | | | |
| Intestine small, ileum | (48) | (49) | (49) | (50) |
| Liver | (49) | (50) | (50) | (50) |
| Hepatocellular carcinoma | 3 (6%) | | | 2 (4%) |
| Hepatocellular adenoma | 2 (4%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Hepatocellular adenoma, multiple | | 2 (4%) | | |
| Osteosarcoma, metastatic, bone | | | 1 (2%) | |
| Mesentery | (17) | (13) | (13) | (17) |
| Carcinoma, metastatic, kidney | | | 1 (8%) | |
| Schwannoma malignant, metastatic, peripheral nerve | | 1 (8%) | | |
| Pancreas | (49) | (50) | (49) | (50) |
| Schwannoma malignant, metastatic, peripheral nerve | | 1 (2%) | | |
| Acinar cell, adenoma | 2 (4%) | 3 (6%) | | |
| Pharynx | (1) | | (3) | |
| Palate, squamous cell papilloma | 1 (100%) | | 2 (67%) | |
| Salivary glands | (49) | (49) | (50) | (50) |
| Schwannoma malignant | 1 (2%) | | | 1 (2%) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Stomach, glandular | (50) | (49) | (49) | (50) |
| Leiomyoma | | | 1 (2%) | |
| Tongue | | | | (2) |
| Squamous cell papilloma | | | | 1 (50%) |

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Adenoma | | | 1 (2%) | |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Pheochromocytoma malignant | 2 (4%) | | 1 (2%) | 2 (4%) |
| Pheochromocytoma benign | 8 (16%) | 7 (14%) | 5 (10%) | 11 (22%) |
| Bilateral, pheochromocytoma benign | 2 (4%) | 4 (8%) | 2 (4%) | 1 (2%) |
| Islets, pancreatic | (49) | (50) | (49) | (50) |
| Adenoma | 2 (4%) | 1 (2%) | 2 (4%) | |
| Carcinoma | 1 (2%) | | | |
| Parathyroid gland | (48) | (49) | (47) | (49) |
| Pituitary gland | (49) | (50) | (49) | (49) |
| Pars distalis, adenoma | 19 (39%) | 12 (24%) | 16 (33%) | 12 (24%) |
| Pars intermedia, adenoma | | 1 (2%) | | |
| Thyroid gland | (49) | (49) | (49) | (50) |
| C-cell, adenoma | 4 (8%) | 2 (4%) | 2 (4%) | 3 (6%) |
| C-cell, carcinoma | | 1 (2%) | 3 (6%) | 1 (2%) |
| Follicular cell, carcinoma | | | | 1 (2%) |
| General Body System | | | | |
| Tissue NOS | (1) | (2) | | (2) |
| Genital System | | | | |
| Epididymis | (50) | (50) | (49) | (50) |
| Preputial gland | (50) | (50) | (49) | (50) |
| Adenoma | 3 (6%) | 2 (4%) | 4 (8%) | 3 (6%) |
| Carcinoma | 1 (2%) | 1 (2%) | 3 (6%) | 5 (10%) |
| Bilateral, adenoma | | 1 (2%) | | |
| Bilateral, carcinoma | | | 1 (2%) | 1 (2%) |
| Duct, squamous cell papilloma | | | 1 (2%) | |
| Prostate | (50) | (50) | (50) | (50) |
| Seminal vesicle | (50) | (50) | (49) | (50) |
| Schwannoma malignant, metastatic, peripheral nerve | | 1 (2%) | | |
| Testes | (50) | (50) | (49) | (50) |
| Bilateral, interstitial cell, adenoma | 34 (68%) | 36 (72%) | 35 (71%) | 21 (42%) |
| Interstitial cell, adenoma | 10 (20%) | 9 (18%) | 9 (18%) | 15 (30%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Lymph node | (24) | (26) | (30) | (14) |
| Mediastinal, carcinoma, metastatic, kidney | | | 1 (3%) | |
| Pancreatic, carcinoma, metastatic, kidney | | | 1 (3%) | |
| Renal, carcinoma, metastatic, kidney | | | 1 (3%) | |

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System (continued) | | | | |
| Lymph node, mandibular | (49) | (49) | (49) | (50) |
| Lymph node, mesenteric | (49) | (50) | (49) | (50) |
| Carcinoma, metastatic, kidney | | | 1 (2%) | |
| Spleen | (50) | (50) | (50) | (50) |
| Fibrosarcoma | | | 1 (2%) | |
| Histiocytic sarcoma | | | | 1 (2%) |
| Thymus | (48) | (50) | (48) | (46) |
| Epithelial cell, thymoma benign | 1 (2%) | 1 (2%) | 1 (2%) | |
| Integumentary System | | | | |
| Mammary gland | (49) | (49) | (49) | (46) |
| Fibroadenoma | 2 (4%) | 1 (2%) | 4 (8%) | 2 (4%) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell adenoma | | | 1 (2%) | 1 (2%) |
| Keratoacanthoma | 3 (6%) | 3 (6%) | 3 (6%) | 2 (4%) |
| Squamous cell papilloma | | | 2 (4%) | |
| Trichoepithelioma | | | | 1 (2%) |
| Subcutaneous tissue, fibroma | 4 (8%) | 6 (12%) | 4 (8%) | 7 (14%) |
| Subcutaneous tissue, fibrosarcoma | 1 (2%) | | | 4 (8%) |
| Subcutaneous tissue, lipoma | | 1 (2%) | | 1 (2%) |
| Subcutaneous tissue, sarcoma | 1 (2%) | | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteosarcoma | | 2 (4%) | 1 (2%) | 1 (2%) |
| Skeletal muscle | (1) | | (1) | (1) |
| Schwannoma malignant, metastatic, peripheral nerve | 1 (100%) | | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Glioma malignant | 1 (2%) | | | |
| Peripheral nerve | (2) | (5) | (1) | |
| Schwannoma malignant | 1 (50%) | 1 (20%) | | |
| Spinal cord | (2) | (4) | (2) | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | | | 1 (2%) | 2 (4%) |
| Alveolar/bronchiolar carcinoma | | 1 (2%) | | |
| Carcinoma, multiple, metastatic, kidney | | | 1 (2%) | |
| Osteosarcoma, multiple, metastatic, bone | | 1 (2%) | 1 (2%) | |
| Squamous cell carcinoma | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |
| Nasolacrimal duct, squamous cell carcinoma | 1 (2%) | | | |
| Trachea | (49) | (49) | (50) | (50) |

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Special Senses System | | | | |
| Ear | | (1) | (1) | |
| Pinna, fibrosarcoma | | 1 (100%) | | |
| Zymbal's gland | (2) | | | |
| Adenoma | 1 (50%) | | | |
| Carcinoma | 1 (50%) | | | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Pelvis, transitional epithelium, papilloma | 1 (2%) | | | |
| Renal tubule, adenoma | | | 1 (2%) | |
| Renal tubule, carcinoma | | | 1 (2%) | |
| Urinary bladder | (50) | (50) | (49) | (50) |
| Leiomyosarcoma | 1 (2%) | | | |
| Schwannoma malignant, metastatic, peripheral nerve | | 1 (2%) | | |
| Systemic Lesions | | | | |
| Multiple organs | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Leukemia mononuclear | 29 (58%) | 35 (70%) | 26 (52%) | 2 (4%) |
| Mesothelioma benign | | | 1 (2%) | |
| Mesothelioma malignant | | 2 (4%) | 1 (2%) | 1 (2%) |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 9 | 9 | 10 | 4 |
| 2-Year study | 48 | 50 | 49 | 46 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 12 | 11 | 16 | 5 |
| 2-Year study | 146 | 138 | 139 | 108 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 9 | 9 | 10 | 4 |
| 2-Year study | 47 | 48 | 48 | 41 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 12 | 10 | 16 | 4 |
| 2-Year study | 99 | 94 | 101 | 85 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | | 1 | | 1 |
| 2-Year study | 39 | 38 | 34 | 19 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | | 1 | | 1 |
| 2-Year study | 47 | 44 | 38 | 23 |
| Total animals with metastatic neoplasms | | | | |
| 2-Year study | 1 | 2 | 2 | |
| Total metastatic neoplasms | | | | |
| 2-Year study | 1 | 5 | 8 | |

^a Number of animals examined microscopically at site and number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid: 0 ppm

| Number of Days on Study | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | |
|------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Carcass ID Number | 4 | 9 | 8 | 9 | 1 | 3 | 5 | 0 | 0 | 1 | 3 | 4 | 4 | 4 | 5 | 5 | 6 | 8 | 8 | 0 | 1 | 1 | 3 | 4 | 4 |
| | 6 | 9 | 1 | 2 | 9 | 8 | 1 | 3 | 5 | 7 | 5 | 1 | 7 | 8 | 6 | 6 | 1 | 4 | 8 | 2 | 7 | 7 | 1 | 5 | 5 |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, cecum | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Intestine small, duodenum | + | + | + | + | + | + | + | + | + | + | A | M | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, jejunum | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Intestine small, ileum | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | M | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Mesentery | + | + | | | | | | | | | + | + | + | + | + | | | | | | + | + | | | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Acinar cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pharynx | | | | | | | | | | | | | | | | | | | | | | | | | |
| Palate, squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Schwannoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | + | + | + | + | I | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Bilateral, pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pars distalis, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tissue NOS | | | | | | | | | | | | | | | | | | | | | | | | | + |

+: 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 Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid: 5,000 ppm (continued)

| | | |
|--------------------------------|--|-----------------------------|
| Number of Days on Study | 7 | |
| | 1 1 1 2 | |
| | 0 0 9 0 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 | |
| Carcass ID Number | 1 1 2 2 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 | Total Tissues/ Tumors |
| | 8 9 1 2 8 8 8 8 9 9 9 9 0 0 0 0 1 1 1 1 1 2 2 2 3 6 6 5 0 2 3 4 9 0 1 4 9 2 3 7 8 0 1 2 7 8 1 6 9 0 | |
| Special Senses System | | |
| Eye | | + |
| | | 1 |
| Urinary System | | |
| Kidney | + | 50 |
| Urinary bladder | + | 50 |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Histiocytic sarcoma | | 1 |
| Leukemia mononuclear | | 2 |
| Mesothelioma malignant | | 1 |

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------------|----------------|------------|-------------|
| Adrenal Medulla: Benign Pheochromocytoma | | | | |
| Overall rate ^a | 10/50 (20%) | 11/50 (22%) | 7/50 (14%) | 12/50 (24%) |
| Adjusted rate ^b | 44.4% | 57.2% | 33.4% | 42.6% |
| Terminal rate ^c | 3/12 (25%) | 6/13 (46%) | 3/13 (23%) | 6/21 (29%) |
| First incidence (days) | 631 | 591 | 541 | 608 |
| Life table test ^d | P=0.253N | P=0.580N | P=0.228N | P=0.339N |
| Logistic regression test ^d | P=0.476N | P=0.587N | P=0.206N | P=0.500 |
| Cochran-Armitage test ^d | P=0.406 | | | |
| Fisher exact test ^d | | P=0.500 | P=0.298N | P=0.405 |
| Adrenal Medulla: Benign or Malignant Pheochromocytoma | | | | |
| Overall rate | 11/50 (22%) | 11/50 (22%) | 8/50 (16%) | 13/50 (26%) |
| Adjusted rate | 50.6% | 57.2% | 35.7% | 44.8% |
| Terminal rate | 4/12 (33%) | 6/13 (46%) | 3/13 (23%) | 6/21 (29%) |
| First incidence (days) | 631 | 591 | 541 | 608 |
| Life table test | P=0.259N | P=0.483N | P=0.229N | P=0.303N |
| Logistic regression test | P=0.494N | P=0.475N | P=0.202N | P=0.531 |
| Cochran-Armitage test | P=0.374 | | | |
| Fisher exact test | | P=0.595N | P=0.306N | P=0.408 |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 2/49 (4%) | 4/50 (8%) | 1/50 (2%) | 2/50 (4%) |
| Adjusted rate | 11.7% | 23.0% | 7.7% | 7.9% |
| Terminal rate | 1/12 (8%) | 2/13 (15%) | 1/13 (8%) | 1/21 (5%) |
| First incidence (days) | 645 | 538 | 729 (T) | 694 |
| Life table test | P=0.242N | P=0.386 | P=0.465N | P=0.513N |
| Logistic regression test | P=0.336N | P=0.385 | P=0.448N | P=0.605N |
| Cochran-Armitage test | P=0.424N | | | |
| Fisher exact test | | P=0.349 | P=0.492N | P=0.684N |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 3/49 (6%) | 0/50 (0%) | 0/50 (0%) | 2/50 (4%) |
| Adjusted rate | 11.8% | 0.0% | 0.0% | 9.5% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 0/13 (0%) | 2/21 (10%) |
| First incidence (days) | 617 | - ^e | - | 729 (T) |
| Life table test | P=0.396N | P=0.104N | P=0.104N | P=0.324N |
| Logistic regression test | P=0.476N | P=0.109N | P=0.107N | P=0.422N |
| Cochran-Armitage test | P=0.525N | | | |
| Fisher exact test | | P=0.117N | P=0.117N | P=0.490N |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 4/49 (8%) | 4/50 (8%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 19.2% | 23.0% | 7.7% | 17.1% |
| Terminal rate | 1/12 (8%) | 2/13 (15%) | 1/13 (8%) | 3/21 (14%) |
| First incidence (days) | 617 | 538 | 729 (T) | 694 |
| Life table test | P=0.277N | P=0.581N | P=0.154N | P=0.381N |
| Logistic regression test | P=0.402N | P=0.592N | P=0.140N | P=0.516N |
| Cochran-Armitage test | P=0.511N | | | |
| Fisher exact test | | P=0.631N | P=0.175N | P=0.631N |

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------------|-------------|-------------|-------------|
| Mammary Gland: Fibroadenoma | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted rate | 16.7% | 5.6% | 24.0% | 8.6% |
| Terminal rate | 2/12 (17%) | 0/13 (0%) | 1/13 (8%) | 1/21 (5%) |
| First incidence (days) | 729 (T) | 710 | 709 | 710 |
| Life table test | P=0.470N | P=0.468N | P=0.389 | P=0.492N |
| Logistic regression test | P=0.552N | P=0.442N | P=0.396 | P=0.526N |
| Cochran-Armitage test | P=0.477 | | | |
| Fisher exact test | | P=0.500N | P=0.339 | P=0.691N |
| Pancreas: Adenoma | | | | |
| Overall rate | 2/49 (4%) | 3/50 (6%) | 0/49 (0%) | 0/50 (0%) |
| Adjusted rate | 16.7% | 18.9% | 0.0% | 0.0% |
| Terminal rate | 2/12 (17%) | 2/13 (15%) | 0/13 (0%) | 0/21 (0%) |
| First incidence (days) | 729 (T) | 674 | — | — |
| Life table test | P=0.032N | P=0.538 | P=0.217N | P=0.124N |
| Logistic regression test | P=0.040N | P=0.571 | P=0.217N | P=0.124N |
| Cochran-Armitage test | P=0.072N | | | |
| Fisher exact test | | P=0.510 | P=0.247N | P=0.242N |
| Pancreatic Islets: Adenoma or Carcinoma | | | | |
| Overall rate | 3/49 (6%) | 1/50 (2%) | 2/49 (4%) | 0/50 (0%) |
| Adjusted rate | 22.2% | 3.0% | 13.1% | 0.0% |
| Terminal rate | 2/12 (17%) | 0/13 (0%) | 1/13 (8%) | 0/21 (0%) |
| First incidence (days) | 714 | 624 | 709 | — |
| Life table test | P=0.052N | P=0.268N | P=0.467N | P=0.048N |
| Logistic regression test | P=0.065N | P=0.259N | P=0.434N | P=0.052N |
| Cochran-Armitage test | P=0.102N | | | |
| Fisher exact test | | P=0.301N | P=0.500N | P=0.117N |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 19/49 (39%) | 12/50 (24%) | 16/49 (33%) | 12/49 (24%) |
| Adjusted rate | 79.7% | 45.5% | 57.0% | 48.1% |
| Terminal rate | 8/12 (67%) | 4/13 (31%) | 4/13 (31%) | 9/21 (43%) |
| First incidence (days) | 547 | 505 | 506 | 608 |
| Life table test | P=0.013N | P=0.070N | P=0.224N | P=0.003N |
| Logistic regression test | P=0.072N | P=0.054N | P=0.188N | P=0.020N |
| Cochran-Armitage test | P=0.145N | | | |
| Fisher exact test | | P=0.085N | P=0.337N | P=0.096N |
| Preputial Gland: Adenoma | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 4/49 (8%) | 3/50 (6%) |
| Adjusted rate | 21.1% | 13.6% | 16.5% | 10.4% |
| Terminal rate | 2/12 (17%) | 0/13 (0%) | 1/13 (8%) | 1/21 (5%) |
| First incidence (days) | 689 | 588 | 532 | 617 |
| Life table test | P=0.382N | P=0.603N | P=0.555 | P=0.434N |
| Logistic regression test | P=0.522N | P=0.611N | P=0.560 | P=0.554N |
| Cochran-Armitage test | P=0.556 | | | |
| Fisher exact test | | P=0.661N | P=0.489 | P=0.661N |

TABLE A3

Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|------------|------------|------------|------------|
| Preputial Gland: Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 4/49 (8%) | 6/50 (12%) |
| Adjusted rate | 2.7% | 2.9% | 15.9% | 15.0% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 0/13 (0%) | 0/21 (0%) |
| First incidence (days) | 548 | 602 | 651 | 518 |
| Life table test | P=0.031 | P=0.744N | P=0.234 | P=0.094 |
| Logistic regression test | P=0.002 | P=0.743 | P=0.192 | P=0.009 |
| Cochran-Armitage test | P=0.013 | | | |
| Fisher exact test | | P=0.753N | P=0.175 | P=0.056 |
| Preputial Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 4/50 (8%) | 8/49 (16%) | 9/50 (18%) |
| Adjusted rate | 23.2% | 16.1% | 29.8% | 23.8% |
| Terminal rate | 2/12 (17%) | 0/13 (0%) | 1/13 (8%) | 1/21 (5%) |
| First incidence (days) | 548 | 588 | 532 | 518 |
| Life table test | P=0.176 | P=0.579N | P=0.254 | P=0.278 |
| Logistic regression test | P=0.024 | P=0.607N | P=0.219 | P=0.055 |
| Cochran-Armitage test | P=0.052 | | | |
| Fisher exact test | | P=0.643N | P=0.168 | P=0.117 |
| Skin: Keratoacanthoma | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 10.5% | 10.5% | 19.4% | 6.9% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 2/13 (15%) | 0/21 (0%) |
| First incidence (days) | 505 | 631 | 696 | 694 |
| Life table test | P=0.242N | P=0.600N | P=0.595N | P=0.350N |
| Logistic regression test | P=0.345N | P=0.655N | P=0.618N | P=0.479N |
| Cochran-Armitage test | P=0.397N | | | |
| Fisher exact test | | P=0.661N | P=0.661N | P=0.500N |
| Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 5/50 (10%) | 3/50 (6%) |
| Adjusted rate | 10.5% | 10.5% | 30.1% | 10.1% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 3/13 (23%) | 0/21 (0%) |
| First incidence (days) | 505 | 631 | 687 | 694 |
| Life table test | P=0.386N | P=0.600N | P=0.433 | P=0.482N |
| Logistic regression test | P=0.535N | P=0.655N | P=0.421 | P=0.633N |
| Cochran-Armitage test | P=0.537 | | | |
| Fisher exact test | | P=0.661N | P=0.357 | P=0.661N |
| Skin (Subcutaneous Tissue): Fibroma | | | | |
| Overall rate | 4/50 (8%) | 6/50 (12%) | 4/50 (8%) | 7/50 (14%) |
| Adjusted rate | 21.7% | 28.5% | 20.6% | 21.2% |
| Terminal rate | 1/12 (8%) | 2/13 (15%) | 2/13 (15%) | 1/21 (5%) |
| First incidence (days) | 602 | 392 | 609 | 483 |
| Life table test | P=0.509 | P=0.441 | P=0.583N | P=0.496 |
| Logistic regression test | P=0.298 | P=0.414 | P=0.572N | P=0.303 |
| Cochran-Armitage test | P=0.254 | | | |
| Fisher exact test | | P=0.370 | P=0.643N | P=0.262 |

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------|--------------|--------------|-------------|
| Skin (Subcutaneous Tissue): Fibrosarcoma | | | | |
| Overall rate | 1/50 (2%) | 0/50 (0%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted rate | 3.7% | 0.0% | 0.0% | 14.1% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 0/13 (0%) | 1/21 (5%) |
| First incidence (days) | 645 | — | — | 615 |
| Life table test | P=0.065 | P=0.479N | P=0.472N | P=0.319 |
| Logistic regression test | P=0.036 | P=0.493N | P=0.492N | P=0.223 |
| Cochran-Armitage test | P=0.028 | | | |
| Fisher exact test | | P=0.500N | P=0.500N | P=0.181 |
| Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma | | | | |
| Overall rate | 2/50 (4%) | 0/50 (0%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted rate | 5.9% | 0.0% | 0.0% | 14.1% |
| Terminal rate | 0/12 (0%) | 0/13 (0%) | 0/13 (0%) | 1/21 (5%) |
| First incidence (days) | 451 | — | — | 615 |
| Life table test | P=0.181 | P=0.221N | P=0.213N | P=0.501 |
| Logistic regression test | P=0.104 | P=0.270N | P=0.307N | P=0.346 |
| Cochran-Armitage test | P=0.104 | | | |
| Fisher exact test | | P=0.247N | P=0.247N | P=0.339 |
| Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma | | | | |
| Overall rate | 6/50 (12%) | 6/50 (12%) | 4/50 (8%) | 11/50 (22%) |
| Adjusted rate | 26.3% | 28.5% | 20.6% | 32.5% |
| Terminal rate | 1/12 (8%) | 2/13 (15%) | 2/13 (15%) | 2/21 (10%) |
| First incidence (days) | 451 | 392 | 609 | 483 |
| Life table test | P=0.294 | P=0.544N | P=0.311N | P=0.399 |
| Logistic regression test | P=0.106 | P=0.595N | P=0.322N | P=0.170 |
| Cochran-Armitage test | P=0.086 | | | |
| Fisher exact test | | P=0.620N | P=0.370N | P=0.143 |
| Testes: Adenoma | | | | |
| Overall rate | 44/50 (88%) | 45/50 (90%) | 44/49 (90%) | 36/50 (72%) |
| Adjusted rate | 100.0% | 100.0% | 100.0% | 91.9% |
| Terminal rate | 12/12 (100%) | 13/13 (100%) | 13/13 (100%) | 18/21 (86%) |
| First incidence (days) | 381 | 392 | 505 | 483 |
| Life table test | P<0.001N | P=0.374N | P=0.293N | P=0.001N |
| Logistic regression test | P<0.001N | P=0.580N | P=0.437N | P=0.007N |
| Cochran-Armitage test | P=0.010N | | | |
| Fisher exact test | | P=0.500 | P=0.514 | P=0.039N |
| Thyroid Gland (C-cell): Adenoma | | | | |
| Overall rate | 4/49 (8%) | 2/49 (4%) | 2/49 (4%) | 3/50 (6%) |
| Adjusted rate | 19.0% | 9.9% | 15.4% | 11.0% |
| Terminal rate | 1/12 (8%) | 1/13 (8%) | 2/13 (15%) | 1/21 (5%) |
| First incidence (days) | 547 | 538 | 729 (T) | 608 |
| Life table test | P=0.290N | P=0.303N | P=0.297N | P=0.308N |
| Logistic regression test | P=0.414N | P=0.315N | P=0.280N | P=0.431N |
| Cochran-Armitage test | P=0.469N | | | |
| Fisher exact test | | P=0.339N | P=0.339N | P=0.489N |

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|--------------|--------------|--------------|-------------|
| Thyroid Gland (C-cell): Carcinoma | | | | |
| Overall rate | 0/49 (0%) | 1/49 (2%) | 3/49 (6%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 7.7% | 11.2% | 2.9% |
| Terminal rate | 0/12 (0%) | 1/13 (8%) | 0/13 (0%) | 0/21 (0%) |
| First incidence (days) | – | 729 (T) | 604 | 618 |
| Life table test | P=0.478 | P=0.516 | P=0.159 | P=0.545 |
| Logistic regression test | P=0.382 | P=0.516 | P=0.133 | P=0.497 |
| Cochran-Armitage test | P=0.358 | | | |
| Fisher exact test | | P=0.500 | P=0.121 | P=0.505 |
| Thyroid Gland (C-cell): Adenoma or Carcinoma | | | | |
| Overall rate | 4/49 (8%) | 3/49 (6%) | 5/49 (10%) | 4/50 (8%) |
| Adjusted rate | 19.0% | 17.4% | 24.9% | 13.5% |
| Terminal rate | 1/12 (8%) | 2/13 (15%) | 2/13 (15%) | 1/21 (5%) |
| First incidence (days) | 547 | 538 | 604 | 608 |
| Life table test | P=0.396N | P=0.456N | P=0.575 | P=0.442N |
| Logistic regression test | P=0.567N | P=0.464N | P=0.569 | P=0.587N |
| Cochran-Armitage test | P=0.513 | | | |
| Fisher exact test | | P=0.500N | P=0.500 | P=0.631N |
| All Organs: Mononuclear Cell Leukemia | | | | |
| Overall rate | 29/50 (58%) | 35/50 (70%) | 26/50 (52%) | 2/50 (4%) |
| Adjusted rate | 76.2% | 79.9% | 76.3% | 4.9% |
| Terminal rate | 4/12 (33%) | 5/13 (38%) | 7/13 (54%) | 0/21 (0%) |
| First incidence (days) | 503 | 415 | 506 | 445 |
| Life table test | P<0.001N | P=0.424 | P=0.215N | P<0.001N |
| Logistic regression test | P<0.001N | P=0.177 | P=0.127N | P<0.001N |
| Cochran-Armitage test | P<0.001N | | | |
| Fisher exact test | | P=0.149 | P=0.344N | P<0.001N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 48/50 (96%) | 48/50 (96%) | 48/50 (96%) | 42/50 (84%) |
| Adjusted rate | 100.0% | 100.0% | 100.0% | 97.7% |
| Terminal rate | 12/12 (100%) | 13/13 (100%) | 13/13 (100%) | 20/21 (95%) |
| First incidence (days) | 246 | 392 | 505 | 483 |
| Life table test | P=0.002N | P=0.322N | P=0.283N | P=0.003N |
| Logistic regression test | P<0.001N | P=0.457N | P=0.374N | P=0.005N |
| Cochran-Armitage test | P=0.010N | | | |
| Fisher exact test | | P=0.691N | P=0.691N | P=0.046N |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 39/50 (78%) | 38/50 (76%) | 35/50 (70%) | 19/50 (38%) |
| Adjusted rate | 84.6% | 83.3% | 86.0% | 50.3% |
| Terminal rate | 5/12 (42%) | 6/13 (46%) | 8/13 (62%) | 5/21 (24%) |
| First incidence (days) | 381 | 310 | 506 | 445 |
| Life table test | P<0.001N | P=0.317N | P=0.172N | P<0.001N |
| Logistic regression test | P<0.001N | P=0.478N | P=0.092N | P<0.001N |
| Cochran-Armitage test | P<0.001N | | | |
| Fisher exact test | | P=0.500N | P=0.247N | P<0.001N |

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------|--------------|--------------|--------------|
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 49/50 (98%) | 50/50 (100%) | 49/50 (98%) | 46/50 (92%) |
| Adjusted rate | 100.0% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 12/12 (100%) | 13/13 (100%) | 13/13 (100%) | 21/21 (100%) |
| First incidence (days) | 246 | 310 | 505 | 445 |
| Life table test | P=0.005N | P=0.366N | P=0.279N | P=0.009N |
| Logistic regression test | P=0.002N | P=0.773 | P=0.297N | P=0.026N |
| Cochran-Armitage test | P=0.034N | | | |
| Fisher exact test | | P=0.500 | P=0.753N | P=0.181N |

(T)Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, pancreas, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; 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 exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test 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 an exposure group is indicated by N.

^e Not applicable; no neoplasms in animal group

TABLE A4a
Historical Incidence of Renal Tubule Neoplasms in Untreated Male F344/N Rats^a

| Study | Incidence in Controls | | |
|--|-----------------------|----------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Southern Research Institute | | | |
| Benzyl Acetate | 0/50 | 0/50 | 0/50 |
| C.I. Pigment Red 23 | 0/50 | 0/50 | 0/50 |
| C.I. Pigment Red 3 | 0/50 | 1/50 | 1/50 |
| Nitrofurantoin | 0/50 | 0/50 | 0/50 |
| o-Nitroanisole | 0/49 | 0/49 | 0/49 |
| Polysorbate 80 | 0/50 | 1/50 | 1/50 |
| Rhodamine 6G | 0/50 | 0/50 | 0/50 |
| Roxarsone | 1/50 | 1/50 | 2/50 |
| Total | 1/399 (0.3%) | 3/399 (0.8%) | 4/399 (1.0%) |
| Standard deviation | 0.7% | 1.0% | 1.5% |
| Range | 0%-2% | 0%-2% | 0%-4% |
| Overall Historical Incidence | | | |
| Total | 9/1,251 (0.7%) | 6/1,251 (0.5%) | 15/1,251 (1.2%) |
| Standard deviation | 1.5% | 1.1% | 1.7% |
| Range | 0%-6% | 0%-4% | 0%-6% |

^a Data as of 20 August 1992

TABLE A4b
Historical Incidence of Renal Tubule Lesions from Single and Step Sections in Male F344/N Rats

| Study | Incidence in Controls | | | |
|---|-----------------------|---------------|--------------|----------------------|
| | Hyperplasia | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Single Sections (Standard Evaluation) | | | | |
| Nitrofurantoin ^a | 2/50 | 0/50 | 0/50 | 0/50 |
| Furosemide ^a | 4/50 | 1/50 | 0/50 | 1/50 |
| Phenylbutazone ^b | 3/50 | 0/50 | 0/50 | 0/50 |
| α -Methylbenzyl Alcohol ^b | 0/50 | 0/50 | 0/50 | 0/50 |
| Toluene ^c | 4/60 | 0/60 | 0/60 | 0/60 |
| 2,4-Diaminophenol Dihydrochloride ^b | 0/50 | 0/50 | 1/50 | 1/50 |
| Mercuric Chloride ^b | 1/50 | 0/50 | 0/50 | 0/50 |
| Quercetin ^a | 1/50 | 0/50 | 0/50 | 0/50 |
| Coumarin ^b | 1/49 | 1/49 | 0/49 | 1/49 |
| 3,4-Dihydrocoumarin ^b | 0/50 | 0/50 | 0/50 | 0/50 |
| <i>o</i> -Benzyl- <i>p</i> -Chlorophenol ^b | 0/50 | 1/50 | 0/50 | 1/50 |
| C.I. Pigment Red 23 ^a | 3/50 | 0/50 | 0/50 | 0/50 |
| Overall Historical Incidence | | | | |
| Total | 19/609 (3.1%) | 3/609 (0.5%) | 1/609 (0.2%) | 4/609 (0.7%) |
| Standard deviation | 3.0% | 0.9% | 0.6% | 1.0% |
| Range | 0%-8% | 0%-2% | 0%-2% | 0%-2% |
| Step Sections (Extended Evaluations) | | | | |
| Nitrofurantoin | 9/50 | 3/50 | 0/50 | 3/50 |
| Furosemide | 2/50 | 2/50 | 0/50 | 2/50 |
| Phenylbutazone | 2/50 | 0/50 | 0/50 | 0/50 |
| α -Methylbenzyl Alcohol | 1/49 | 1/49 | 0/49 | 1/49 |
| Toluene | 0/60 | 5/60 | 0/60 | 5/60 |
| 2,4-Diaminophenol Dihydrochloride | 3/50 | 0/50 | 0/50 | 0/50 |
| Mercuric Chloride | 2/50 | 4/50 | 0/50 | 4/50 |
| Quercetin | 2/50 | 1/50 | 0/50 | 1/50 |
| Coumarin | 2/49 | 0/49 | 0/49 | 0/49 |
| 3,4-Dihydrocoumarin | 0/50 | 1/50 | 0/50 | 1/50 |
| <i>o</i> -Benzyl- <i>p</i> -Chlorophenol | 3/50 | 0/50 | 0/50 | 0/50 |
| C.I. Pigment Red 23 | 3/50 | 1/50 | 0/50 | 1/50 |
| Overall Historical Incidence | | | | |
| Total | 29/608 (4.8%) | 18/608 (3.0%) | 0/608 (0%) | 18/608 (3.0%) |
| Standard deviation | 4.6% | 3.0% | | 3.0% |
| Range | 0%-18% | 0%-8% | | 0%-8% |

TABLE A4b
Historical Incidence of Renal Tubule Lesions from Single and Step Sections in Male F344/N Rats (continued)

| Study | Incidence in Controls | | | |
|--|-----------------------|---------------|--------------|----------------------|
| | Hyperplasia | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Single and Step Sections Combined | | | | |
| Nitrofurantoin | 10/50 | 3/50 | 0/50 | 3/50 |
| Furosemide | 6/50 | 3/50 | 0/50 | 3/50 |
| Phenylbutazone | 5/50 | 0/50 | 0/50 | 0/50 |
| α -Methylbenzyle Alcohol | 1/49 | 1/49 | 0/49 | 1/49 |
| Toluene | 4/60 | 5/60 | 0/60 | 5/60 |
| 2,4-Diaminophenol Dihydrochloride | 3/50 | 0/50 | 1/50 | 1/50 |
| Mercuric Chloride | 3/50 | 4/50 | 0/50 | 4/50 |
| Quercetin | 3/50 | 1/50 | 0/50 | 1/50 |
| Coumarin | 3/49 | 1/49 | 0/49 | 1/49 |
| 3,4-Dihydrocoumarin | 0/50 | 1/50 | 0/50 | 1/50 |
| <i>o</i> -Benzyl- <i>p</i> -Chlorophenol | 3/50 | 1/50 | 0/50 | 1/50 |
| C.I. Pigment Red 23 | 6/50 | 1/50 | 0/50 | 1/50 |
| Overall Historical Incidence | | | | |
| Total | 47/608 (7.7%) | 21/608 (3.5%) | 1/608 (0.2%) | 22/608 (3.6%) |
| Standard deviation | 5.2% | 2.9% | 0.8% | 2.7% |
| Range | 0%-20% | 0%-8% | 0%-2% | 0%-8% |

- ^a Feed study
^b Gavage study
^c Inhalation study

TABLE A4c
Historical Incidence of Preputial Gland Neoplasms in Untreated Male F344/N Rats^a

| Study | Incidence in Controls | | |
|--|-----------------------|-----------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Southern Research Institute | | | |
| Benzyl Acetate | 2/50 | 0/50 | 2/50 |
| C.I. Pigment Red 23 | 3/49 | 2/49 | 5/49 |
| C.I. Pigment Red 3 | 6/49 | 1/49 | 7/49 |
| Nitrofurantoin | 6/48 | 6/48 | 12/48 |
| <i>o</i> -Nitroanisole | 4/50 | 7/50 | 11/50 |
| Polysorbate 80 | 5/48 | 5/48 | 10/48 |
| Rhodamine 6G | 2/49 | 2/49 | 4/49 |
| Roxarsone | 7/49 | 0/49 | 7/49 |
| Total | 35/392 (8.9%) | 23/392 (5.9%) | 58/392 (14.8%) |
| Standard deviation | 4.0% | 5.7% | 7.4% |
| Range | 4%-14% | 0%-14% | 4%-25% |
| Overall Historical Incidence | | | |
| Total ^b | 94/1,169 (8.0%) | 46/1,169 (3.9%) | 139/1,169 (11.9%) |
| Standard deviation | 5.6% | 4.0% | 7.8% |
| Range | 2%-24% | 0%-14% | 2%-30% |

^a Data as of 20 August 1992

^b Data from Quercetin, TR 409, censored due to low denominator (adenoma, 2/13; carcinoma, 1/13; adenoma or carcinoma, 3/13)

TABLE A4d
Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats^a

| Study | Incidence in Controls |
|--|--|
| | Mononuclear Cell Leukemia ^b |
| Historical Incidence at Southern Research Institute | |
| Benzyl Acetate | 16/50 |
| C.I. Pigment Red 23 | 28/50 |
| C.I. Pigment Red 3 | 22/50 |
| Nitrofurantoin | 23/50 |
| <i>o</i> -Nitroanisole | 26/50 |
| Polysorbate 80 | 23/50 |
| Rhodamine 6G | 27/50 |
| Roxarsone | 27/50 |
| Total | 192/400 (48.0%) |
| Standard deviation | 7.9% |
| Range | 32%-56% |
| Overall Historical Incidence | |
| Total | 603/1,253 (48.1%) |
| Standard deviation | 8.7% |
| Range | 32%-62% |

^a Data as of 20 August 1992

^b Includes incidences of lymphocytic, monocytic, or undifferentiated leukemia.

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|-----------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Moribund | 32 | 34 | 34 | 25 |
| Natural deaths | 6 | 3 | 3 | 4 |
| Survivors | | | | |
| Terminal sacrifice | 12 | 13 | 13 | 21 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (10) | (10) | (10) | (9) |
| Parasite metazoan | | | | 2 (22%) |
| Intestine large, rectum | (10) | (10) | (10) | (10) |
| Parasite metazoan | 2 (20%) | 1 (10%) | | |
| Liver | (10) | (10) | (10) | (10) |
| Basophilic focus | 5 (50%) | 1 (10%) | 5 (50%) | 2 (20%) |
| Clear cell focus | 2 (20%) | | | |
| Degeneration, cystic | | 1 (10%) | 1 (10%) | |
| Fatty change | 10 (100%) | 8 (80%) | 10 (100%) | 9 (90%) |
| Hepatodiaphragmatic nodule | | | 5 (50%) | |
| Inflammation, focal | 8 (80%) | 6 (60%) | 7 (70%) | 6 (60%) |
| Mixed cell focus | | 1 (10%) | | |
| Bile duct, hyperplasia | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Mesentery | (1) | (1) | (2) | (3) |
| Accessory spleen | 1 (100%) | | 1 (50%) | |
| Fat, necrosis | | 1 (100%) | 1 (50%) | 3 (100%) |
| Pancreas | (10) | (10) | (10) | (10) |
| Accessory spleen | | | | 1 (10%) |
| Atrophy, focal | 7 (70%) | 4 (40%) | 4 (40%) | 6 (60%) |
| Stomach, forestomach | (10) | (10) | (10) | (10) |
| Mineralization, focal | 1 (10%) | | | |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Accessory adrenal cortical nodule | | | | 1 (10%) |
| Focal cellular change | 1 (10%) | | 2 (20%) | 1 (10%) |
| Pituitary gland | (10) | (10) | (10) | (10) |
| Angiectasis | | | 1 (10%) | 1 (10%) |
| Cyst | | 1 (10%) | | 1 (10%) |
| Pars distalis, focal cellular change | 6 (60%) | 4 (40%) | | 3 (30%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Degeneration, cystic | | | 1 (10%) | |
| Ultimobranchial cyst | | | | 1 (10%) |
| C-cell, hyperplasia | 1 (10%) | 3 (30%) | | |
| Follicular cell, hyperplasia | | | | 1 (10%) |

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Genital System | | | | |
| Epididymis | (10) | (10) | (10) | (10) |
| Inflammation, chronic | | 1 (10%) | | |
| Preputial gland | (10) | (10) | (10) | (10) |
| Degeneration, cystic | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Inflammation, chronic | 1 (10%) | | 1 (10%) | |
| Prostate | (10) | (10) | (10) | (10) |
| Inflammation, suppurative | 8 (80%) | 6 (60%) | 7 (70%) | 5 (50%) |
| Testes | (10) | (10) | (10) | (10) |
| Bilateral, interstitial cell, hyperplasia | 1 (10%) | | 1 (10%) | 3 (30%) |
| Germinal epithelium, degeneration | 1 (10%) | 1 (10%) | | |
| Interstitial cell, hyperplasia | 5 (50%) | 2 (20%) | 2 (20%) | 3 (30%) |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Myelofibrosis | | 1 (10%) | | |
| Lymph node | (2) | | (2) | (1) |
| Mediastinal, congestion | 2 (100%) | | 2 (100%) | 1 (100%) |
| Mediastinal, hyperplasia | | | 1 (50%) | |
| Lymph node, mandibular | (10) | (10) | (10) | (10) |
| Congestion | | | 1 (10%) | |
| Hyperplasia | | 1 (10%) | 1 (10%) | |
| Lymph node, mesenteric | (10) | (10) | (10) | (10) |
| Edema | | 1 (10%) | | |
| Hyperplasia, lymphoid | | 1 (10%) | | 1 (10%) |
| Spleen | (10) | (10) | (10) | (10) |
| Pigmentation | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Thymus | (9) | (9) | (10) | (10) |
| Cyst | | | | 1 (10%) |
| Integumentary System | | | | |
| Mammary gland | (10) | (8) | (9) | (8) |
| Dilatation | 1 (10%) | | | |
| Skin | (10) | (10) | (10) | (10) |
| Exudate | | | | 1 (10%) |
| Hemorrhage, focal | | | 1 (10%) | |
| Inflammation, chronic, focal | | | | 1 (10%) |
| Ulcer | | | | 1 (10%) |
| Nervous System | | | | |
| Brain | (10) | (10) | (10) | (10) |
| Compression | | 1 (10%) | | |

TABLE A5

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar epithelium, hyperplasia | | | 1 (10%) | 1 (10%) |
| Nose | (10) | (10) | (10) | (10) |
| Fungus | | 1 (10%) | | 1 (10%) |
| Inflammation, suppurative | | 1 (10%) | 1 (10%) | 1 (10%) |
| Respiratory epithelium, hyperplasia, focal | | | | 1 (10%) |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Nephropathy | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Pelvis, dilatation | | 1 (10%) | | |
| Renal tubule, hyperplasia | | | 1 (10%) | |
| Renal tubule, pigmentation | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Systems Examined With No Lesions Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Special Senses System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (49) | (50) | (49) | (50) |
| Parasite metazoan | 2 (4%) | | 1 (2%) | |
| Intestine large, rectum | (49) | (50) | (49) | (50) |
| Parasite metazoan | 1 (2%) | 3 (6%) | 5 (10%) | 1 (2%) |
| Intestine large, cecum | (49) | (50) | (49) | (50) |
| Inflammation, chronic | | 1 (2%) | | |
| Parasite metazoan | | 1 (2%) | | |
| Intestine small, duodenum | (48) | (48) | (49) | (50) |
| Mucosa, hyperplasia | 1 (2%) | | | |
| Intestine small, jejunum | (49) | (49) | (49) | (50) |
| Necrosis | | 1 (2%) | | |
| Ulcer | | 1 (2%) | | |
| Intestine small, ileum | (48) | (49) | (49) | (50) |
| Diverticulum | | 1 (2%) | | |
| Fibrosis | | 1 (2%) | | |
| Inflammation, chronic, focal | | 1 (2%) | | |
| Ulcer | | 1 (2%) | | |

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Liver | (49) | (50) | (50) | (50) |
| Angiectasis | 4 (8%) | 6 (12%) | 6 (12%) | 1 (2%) |
| Atrophy, focal | 1 (2%) | | | |
| Autolysis | | | 1 (2%) | |
| Basophilic focus | 13 (27%) | 18 (36%) | 25 (50%) | 33 (66%) |
| Clear cell focus | 6 (12%) | 8 (16%) | 9 (18%) | 12 (24%) |
| Congestion, focal | 3 (6%) | 1 (2%) | | |
| Degeneration, cystic | 7 (14%) | 10 (20%) | 10 (20%) | 12 (24%) |
| Developmental malformation | | | | 1 (2%) |
| Eosinophilic focus | 5 (10%) | 5 (10%) | 7 (14%) | 5 (10%) |
| Fatty change | 15 (31%) | 13 (26%) | 11 (22%) | 7 (14%) |
| Fibrosis, focal | | | 1 (2%) | |
| Focal cellular change | | 1 (2%) | 1 (2%) | |
| Hematopoietic cell proliferation | | | 1 (2%) | 2 (4%) |
| Hemorrhage, focal | | | | 1 (2%) |
| Hepatodiaphragmatic nodule | 7 (14%) | 2 (4%) | 7 (14%) | 9 (18%) |
| Hepatodiaphragmatic nodule, multiple | 2 (4%) | 1 (2%) | 1 (2%) | |
| Hyperplasia, histiocytic | 1 (2%) | | | |
| Hyperplasia, lymphoid | 1 (2%) | | | |
| Hyperplasia, multifocal | 13 (27%) | 12 (24%) | 13 (26%) | 4 (8%) |
| Infiltration cellular, mixed cell | 7 (14%) | 5 (10%) | 4 (8%) | 3 (6%) |
| Inflammation, focal | 10 (20%) | 10 (20%) | 15 (30%) | 26 (52%) |
| Mixed cell focus | 2 (4%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Necrosis, focal | | 6 (12%) | 3 (6%) | 1 (2%) |
| Pigmentation | 1 (2%) | | | |
| Thrombosis | | | 2 (4%) | |
| Bile duct, hyperplasia | 48 (98%) | 48 (96%) | 49 (98%) | 48 (96%) |
| Centrilobular, atrophy | 22 (45%) | 27 (54%) | 23 (46%) | 5 (10%) |
| Centrilobular, congestion | 1 (2%) | | | |
| Centrilobular, necrosis | 2 (4%) | | 1 (2%) | 1 (2%) |
| Mesentery | (17) | (13) | (13) | (17) |
| Accessory spleen | | 2 (15%) | | 1 (6%) |
| Angiectasis | | 1 (8%) | | |
| Fibrosis | 1 (6%) | | 1 (8%) | 1 (6%) |
| Hemorrhage | 1 (6%) | | | |
| Inflammation, chronic | | | 1 (8%) | 1 (6%) |
| Fat, necrosis | 12 (71%) | 4 (31%) | 3 (23%) | 13 (76%) |
| Pancreas | (49) | (50) | (49) | (50) |
| Accessory spleen | 1 (2%) | | | |
| Atrophy, diffuse | | 1 (2%) | | |
| Atrophy, focal | 23 (47%) | 28 (56%) | 25 (51%) | 21 (42%) |
| Edema | 1 (2%) | 1 (2%) | | |
| Inflammation, chronic | | | 1 (2%) | 1 (2%) |
| Necrosis | 1 (2%) | | | |
| Acinar cell, focal cellular change | | 1 (2%) | | |
| Acinar cell, hyperplasia | | 1 (2%) | 2 (4%) | |
| Artery, inflammation, chronic | | | 1 (2%) | 1 (2%) |
| Duct, dilatation | | | 2 (4%) | |
| Salivary glands | (49) | (49) | (50) | (50) |
| Atrophy, focal | | | | 1 (2%) |

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Edema | 1 (2%) | 1 (2%) | 1 (2%) | |
| Erosion | 1 (2%) | 1 (2%) | | |
| Inflammation, chronic | 2 (4%) | 2 (4%) | 3 (6%) | |
| Ulcer | 1 (2%) | 2 (4%) | 4 (8%) | |
| Mucosa, hyperplasia | 4 (8%) | 3 (6%) | 5 (10%) | |
| Stomach, glandular | (50) | (49) | (49) | (50) |
| Erosion | 4 (8%) | 2 (4%) | 1 (2%) | |
| Hyperplasia, focal, lymphoid | | | 1 (2%) | |
| Pigmentation, focal | 2 (4%) | 1 (2%) | 1 (2%) | |
| Mucosa, hyperplasia | | 1 (2%) | | |
| Tongue | | | | (2) |
| Hyperplasia, squamous | | | | 1 (50%) |
| Cardiovascular System | | | | |
| Blood vessel | (49) | (50) | (50) | (50) |
| Mesenteric artery, inflammation, chronic | 1 (2%) | 1 (2%) | | 2 (4%) |
| Heart | (50) | (50) | (50) | (50) |
| Inflammation, chronic, focal | 1 (2%) | 1 (2%) | | 1 (2%) |
| Thrombosis | | | 2 (4%) | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Accessory adrenal cortical nodule | 2 (4%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Angiectasis | | | 1 (2%) | 1 (2%) |
| Atrophy | | | 1 (2%) | |
| Congestion | 2 (4%) | | | |
| Focal cellular change | 3 (6%) | 7 (14%) | 9 (18%) | 8 (16%) |
| Hematopoietic cell proliferation | 1 (2%) | 1 (2%) | | |
| Hyperplasia, focal | | | | 1 (2%) |
| Infiltration cellular, lymphocyte | 1 (2%) | | | |
| Vacuolization cytoplasmic | | 1 (2%) | | |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Angiectasis | | 1 (2%) | | |
| Hyperplasia | 11 (22%) | 8 (16%) | 15 (30%) | 9 (18%) |
| Pituitary gland | (49) | (50) | (49) | (49) |
| Angiectasis | 1 (2%) | 3 (6%) | 2 (4%) | 3 (6%) |
| Cyst | 3 (6%) | 3 (6%) | 3 (6%) | 3 (6%) |
| Hemorrhage | 1 (2%) | | 1 (2%) | |
| Pars distalis, focal cellular change | 5 (10%) | 2 (4%) | 6 (12%) | 9 (18%) |
| Pars distalis, hyperplasia, focal | 3 (6%) | 7 (14%) | 4 (8%) | 1 (2%) |
| Pars nervosa, focal cellular change | | | 1 (2%) | |
| Thyroid gland | (49) | (49) | (49) | (50) |
| Degeneration, cystic | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Ultimobranchial cyst | | | 2 (4%) | 1 (2%) |
| C-cell, hyperplasia | 5 (10%) | 10 (20%) | 5 (10%) | 9 (18%) |
| Follicle, dilatation | 1 (2%) | 1 (2%) | | |
| Follicular cell, hyperplasia | 2 (4%) | 1 (2%) | 1 (2%) | |

TABLE A5

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| General Body System | | | | |
| Tissue NOS | (1) | (2) | | (2) |
| Anterior, fibrosis | 1 (100%) | | | |
| Anterior, inflammation, chronic | 1 (100%) | | | |
| Oral, inflammation, chronic | | | | 1 (50%) |
| Genital System | | | | |
| Epididymis | (50) | (50) | (49) | (50) |
| Inflammation, chronic | | | | 2 (4%) |
| Epithelium, degeneration | | 1 (2%) | | |
| Preputial gland | (50) | (50) | (49) | (50) |
| Degeneration, cystic | 47 (94%) | 47 (94%) | 48 (98%) | 48 (96%) |
| Hyperplasia | 4 (8%) | | 1 (2%) | 3 (6%) |
| Inflammation, chronic | 1 (2%) | | 2 (4%) | |
| Prostate | (50) | (50) | (50) | (50) |
| Hemorrhage | | 1 (2%) | | |
| Inflammation, suppurative | 37 (74%) | 31 (62%) | 36 (72%) | 39 (78%) |
| Epithelium, hyperplasia, focal | 1 (2%) | 7 (14%) | 2 (4%) | |
| Seminal vesicle | (50) | (50) | (49) | (50) |
| Dilatation | 2 (4%) | 1 (2%) | | |
| Inflammation, chronic | | 1 (2%) | | 1 (2%) |
| Testes | (50) | (50) | (49) | (50) |
| Congestion | | | | 1 (2%) |
| Mineralization, focal | | | | 1 (2%) |
| Artery, inflammation, chronic | | | | 1 (2%) |
| Bilateral, interstitial cell, hyperplasia | 1 (2%) | | 2 (4%) | 7 (14%) |
| Germinal epithelium, degeneration | 6 (12%) | 11 (22%) | 13 (27%) | 14 (28%) |
| Interstitial cell, hyperplasia | 10 (20%) | 13 (26%) | 7 (14%) | 14 (28%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hemorrhage | | | | 2 (4%) |
| Hypercellularity | 5 (10%) | 3 (6%) | 6 (12%) | 5 (10%) |
| Hyperplasia, focal, histiocytic | 1 (2%) | 1 (2%) | | 3 (6%) |
| Metaplasia, osseous | | 1 (2%) | | |
| Myelofibrosis | | 1 (2%) | 1 (2%) | 1 (2%) |
| Lymph node | (24) | (26) | (30) | (14) |
| Inguinal, hyperplasia | | | 1 (3%) | 4 (29%) |
| Lumbar, hyperplasia | | | | 1 (7%) |
| Mediastinal, angiectasis | | 3 (12%) | | 1 (7%) |
| Mediastinal, congestion | 1 (4%) | 2 (8%) | 4 (13%) | 4 (29%) |
| Mediastinal, hyperplasia | 1 (4%) | 1 (4%) | | 4 (29%) |
| Mediastinal, pigmentation | | | 3 (10%) | |
| Pancreatic, angiectasis | 1 (4%) | | 1 (3%) | 1 (7%) |
| Pancreatic, edema | 2 (8%) | | 1 (3%) | 1 (7%) |
| Pancreatic, hyperplasia | 2 (8%) | | | 1 (7%) |
| Pancreatic, hyperplasia, lymphoid | | | | 1 (7%) |
| Pancreatic, hyperplasia, macrophage | | | | 1 (7%) |

TABLE A5

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System (continued) | | | | |
| Lymph node, mandibular | (49) | (49) | (49) | (50) |
| Angiectasis | | | 1 (2%) | |
| Congestion | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Ectasia | | 1 (2%) | | |
| Edema | | | 1 (2%) | |
| Hemorrhage | | | | 1 (2%) |
| Hyperplasia | 7 (14%) | 7 (14%) | 6 (12%) | 16 (32%) |
| Lymph node, mesenteric | (49) | (50) | (49) | (50) |
| Congestion | | | | 1 (2%) |
| Edema | 1 (2%) | | 2 (4%) | 1 (2%) |
| Hyperplasia | 5 (10%) | 3 (6%) | 1 (2%) | 7 (14%) |
| Hyperplasia, lymphoid | 1 (2%) | | | 2 (4%) |
| Spleen | (50) | (50) | (50) | (50) |
| Angiectasis | 1 (2%) | | | |
| Congestion | 1 (2%) | | | |
| Cyst | 1 (2%) | | | |
| Degeneration, fatty | | | 1 (2%) | |
| Fibrosis | 12 (24%) | 14 (28%) | 12 (24%) | 7 (14%) |
| Hematopoietic cell proliferation | 1 (2%) | 2 (4%) | 7 (14%) | 9 (18%) |
| Necrosis, focal | 1 (2%) | 4 (8%) | 1 (2%) | |
| Pigmentation | 50 (100%) | 50 (100%) | 50 (100%) | 50 (100%) |
| Red pulp, hyperplasia, focal, histiocytic | | 1 (2%) | | |
| Thymus | (48) | (50) | (48) | (46) |
| Congestion | 1 (2%) | | | |
| Cyst | | | | 1 (2%) |
| Hemorrhage | | | 1 (2%) | |
| Epithelial cell, hyperplasia | | 1 (2%) | | |
| Integumentary System | | | | |
| Mammary gland | (49) | (49) | (49) | (46) |
| Dilatation | 18 (37%) | 11 (22%) | 18 (37%) | 9 (20%) |
| Hemorrhage | | 2 (4%) | | |
| Hyperplasia | 6 (12%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 1 (2%) | |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | 1 (2%) | | | |
| Hemorrhage, focal | 1 (2%) | 2 (4%) | | |
| Hyperkeratosis, focal | | 2 (4%) | 4 (8%) | 2 (4%) |
| Hyperplasia, focal | | | 1 (2%) | |
| Inflammation, chronic, focal | 1 (2%) | | 3 (6%) | 1 (2%) |
| Ulcer | 1 (2%) | | 1 (2%) | |
| Epidermis, hyperplasia, focal | | 2 (4%) | 4 (8%) | 2 (4%) |
| Subcutaneous tissue, angiectasis | 1 (2%) | | | |
| Subcutaneous tissue, congestion | | | | 1 (2%) |
| Subcutaneous tissue, inflammation, chronic, focal | | | | 1 (2%) |

TABLE A5

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|-----------------------------------|---------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Fracture | 1 (2%) | | | 2 (4%) |
| Hyperostosis | | | 1 (2%) | 1 (2%) |
| Trabecula, proliferation | | | 1 (2%) | |
| Skeletal muscle | (1) | | (1) | (1) |
| Inflammation, chronic | | | | 1 (100%) |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 8 (16%) | 4 (8%) | 5 (10%) | 2 (4%) |
| Hemorrhage | 2 (4%) | 4 (8%) | | |
| Meninges, fibrosis, focal | | 1 (2%) | | |
| Spinal cord | (2) | (4) | (2) | |
| Demyelination | | 1 (25%) | | |
| Hemorrhage, focal | 1 (50%) | 1 (25%) | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | 2 (4%) | | 2 (4%) | 2 (4%) |
| Edema | 1 (2%) | | | |
| Fibrosis, focal | 1 (2%) | | 1 (2%) | |
| Hemorrhage, focal | 1 (2%) | 3 (6%) | 3 (6%) | |
| Hyperplasia, diffuse, macrophage | | 1 (2%) | | |
| Hyperplasia, focal, macrophage | 2 (4%) | 2 (4%) | 2 (4%) | |
| Infiltration cellular, mixed cell | | | 1 (2%) | 2 (4%) |
| Necrosis, focal | 1 (2%) | | | |
| Alveolar epithelium, hyperplasia | 6 (12%) | 6 (12%) | 6 (12%) | 3 (6%) |
| Nose | (50) | (50) | (50) | (50) |
| Fungus | 6 (12%) | 8 (16%) | 8 (16%) | 7 (14%) |
| Inflammation, suppurative | 8 (16%) | 10 (20%) | 11 (22%) | 8 (16%) |
| Respiratory epithelium, ulcer | | 1 (2%) | | |
| Special Senses System | | | | |
| Eye | | (1) | (2) | (1) |
| Atrophy | | | 1 (50%) | 1 (100%) |
| Cataract | | 1 (100%) | 2 (100%) | 1 (100%) |
| Retina, degeneration | | 1 (100%) | 2 (100%) | 1 (100%) |

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Autolysis | | | 1 (2%) | |
| Congestion | | 1 (2%) | | |
| Cyst | | 1 (2%) | | 1 (2%) |
| Infarct | | | 1 (2%) | |
| Nephropathy | 50 (100%) | 50 (100%) | 50 (100%) | 50 (100%) |
| Thrombosis | | | 1 (2%) | |
| Pelvis, dilatation | 1 (2%) | | | |
| Pelvis, transitional epithelium, hyperplasia | 1 (2%) | 2 (4%) | | |
| Renal tubule, hyperplasia | | 3 (6%) | 1 (2%) | 1 (2%) |
| Renal tubule, hyperplasia, oncocytic | 1 (2%) | | 1 (2%) | 5 (10%) |
| Renal tubule, pigmentation | 50 (100%) | 50 (100%) | 50 (100%) | 50 (100%) |
| Renal tubule, vacuolization cytoplasmic | | | | 1 (2%) |
| Urinary bladder | (50) | (50) | (49) | (50) |
| Dilatation | 1 (2%) | | | |
| Hemorrhage | 2 (4%) | 1 (2%) | | |
| Inflammation, chronic | | | | 1 (2%) |
| Transitional epithelium, hyperplasia | 1 (2%) | | | |

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR FEED STUDY
OF *p*-NITROBENZOIC ACID

| | | |
|------------------|--|------------|
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TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------------------------|---------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Moribund | 21 | 23 | 27 | 26 |
| Natural deaths | 2 | 4 | 2 | 3 |
| Survivors | | | | |
| Terminal sacrifice | 27 | 23 | 21 | 21 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Endocrine System | | | | |
| Pituitary gland | (10) | (10) | (9) | (10) |
| Pars distalis, adenoma | 2 (20%) | 2 (20%) | 1 (11%) | 3 (30%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| C-cell, adenoma | | | 1 (10%) | |
| Genital System | | | | |
| Uterus | (10) | (10) | (10) | (10) |
| Endometrium, polyp stromal | | 3 (30%) | 3 (30%) | 1 (10%) |
| Endometrium, sarcoma stromal | | | 1 (10%) | |
| Hematopoietic System | | | | |
| Lymph node | (1) | (4) | (2) | (2) |
| Lymph node, mesenteric | (10) | (10) | (10) | (10) |
| Spleen | (10) | (10) | (10) | (10) |
| Integumentary System | | | | |
| Mammary gland | (10) | (10) | (10) | (10) |
| Fibroadenoma | | 1 (10%) | | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (10) | (10) | (10) | (10) |
| Leukemia mononuclear | | 1 (10%) | | |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Respiratory System | | | | |
| Special Senses System | | | | |
| Urinary System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, thyroid gland | | | | 1 (2%) |
| Intestine large, colon | (50) | (48) | (50) | (50) |
| Leiomyosarcoma, metastatic, uterus | | 1 (2%) | | |
| Intestine large, cecum | (50) | (47) | (50) | (49) |
| Intestine small, duodenum | (50) | (50) | (50) | (49) |
| Intestine small, ileum | (50) | (47) | (50) | (48) |
| Liver | (50) | (50) | (50) | (50) |
| Hepatocellular adenoma | 2 (4%) | | | |
| Sarcoma, metastatic, mesentery | | | 1 (2%) | |
| Mesentery | (10) | (8) | (9) | (6) |
| Sarcoma | | | 1 (11%) | |
| Schwannoma malignant, metastatic, uterus | | | 1 (11%) | |
| Pancreas | (50) | (50) | (49) | (49) |
| Acinar cell, adenoma | | | 1 (2%) | |
| Pharynx | | | (1) | |
| Palate, squamous cell papilloma | | | 1 (100%) | |
| Salivary glands | (50) | (50) | (50) | (50) |
| Stomach, forestomach | (50) | (50) | (49) | (49) |
| Stomach, glandular | (50) | (50) | (49) | (49) |
| Tongue | | | (1) | (1) |
| Squamous cell papilloma | | | 1 (100%) | |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (50) | (50) |
| Osteosarcoma, metastatic, bone | | 1 (2%) | | |
| Adrenal medulla | (49) | (50) | (50) | (50) |
| Osteosarcoma, metastatic, bone | | 1 (2%) | | |
| Pheochromocytoma benign | 2 (4%) | 1 (2%) | 3 (6%) | |
| Islets, pancreatic | (50) | (50) | (49) | (49) |
| Adenoma | 1 (2%) | | | |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Endocrine System (continued) | | | | |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Osteosarcoma, metastatic, bone | | 1 (2%) | | |
| Pars distalis, adenoma | 18 (36%) | 27 (54%) | 25 (50%) | 23 (47%) |
| Pars distalis, carcinoma | 1 (2%) | | | |
| Pars nervosa, ganglioneuroma | | | | 1 (2%) |
| Thyroid gland | (50) | (49) | (50) | (50) |
| C-cell, adenoma | 9 (18%) | 4 (8%) | 4 (8%) | 2 (4%) |
| C-cell, adenoma, multiple | | 1 (2%) | | |
| C-cell, carcinoma | 1 (2%) | | 2 (4%) | |
| Follicular cell, carcinoma | | 1 (2%) | | 1 (2%) |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Clitoral gland | (50) | (49) | (49) | (50) |
| Adenoma | 4 (8%) | 12 (24%) | 9 (18%) | 11 (22%) |
| Carcinoma | 1 (2%) | 2 (4%) | 4 (8%) | 3 (6%) |
| Bilateral, adenoma | | | 1 (2%) | 1 (2%) |
| Bilateral, carcinoma | | | 1 (2%) | 1 (2%) |
| Ovary | (50) | (50) | (50) | (49) |
| Granulosa cell tumor benign | 1 (2%) | | | |
| Neoplasm NOS | | 1 (2%) | | |
| Uterus | (50) | (50) | (50) | (49) |
| Histiocytic sarcoma | | | 1 (2%) | |
| Leiomyosarcoma | | 1 (2%) | | |
| Endometrium, polyp stromal | 5 (10%) | 10 (20%) | 11 (22%) | 5 (10%) |
| Endometrium, polyp stromal, multiple | | 1 (2%) | 1 (2%) | |
| Endometrium, sarcoma stromal | | 1 (2%) | 1 (2%) | |
| Endometrium, schwannoma malignant | 1 (2%) | | 2 (4%) | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Lymph node | (13) | (10) | (9) | (8) |
| Renal, sarcoma, metastatic, mesentery | | | 1 (11%) | |
| Lymph node, mandibular | (50) | (50) | (50) | (50) |
| Lymph node, mesenteric | (49) | (50) | (50) | (49) |
| Spleen | (50) | (50) | (50) | (49) |
| Fibrosarcoma | | | 1 (2%) | |
| Sarcoma, metastatic, mesentery | | | 1 (2%) | |
| Thymus | (49) | (48) | (50) | (50) |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | | | |
| Carcinoma | 2 (4%) | 2 (4%) | 3 (6%) | 2 (4%) |
| Fibroadenoma | 17 (34%) | 15 (30%) | 19 (38%) | 19 (38%) |
| Fibroadenoma, multiple | 5 (10%) | 7 (14%) | 7 (14%) | 5 (10%) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell carcinoma | | | | 1 (2%) |
| Basosquamous tumor malignant | 1 (2%) | | | |
| Squamous cell papilloma | | | | 1 (2%) |
| Subcutaneous tissue, fibroma | 2 (4%) | 1 (2%) | | 3 (6%) |
| Subcutaneous tissue, lipoma | 1 (2%) | | | |
| Subcutaneous tissue, schwannoma malignant | | 1 (2%) | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteosarcoma | 1 (2%) | 1 (2%) | 1 (2%) | |
| Skeletal muscle | | | (1) | (1) |
| Rhabdomyosarcoma | | | 1 (100%) | |
| Sarcoma | | | | 1 (100%) |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Astrocytoma NOS | | 1 (2%) | | |
| Glioma malignant | 1 (2%) | | | |
| Peripheral nerve | (2) | (4) | (2) | |
| Schwannoma malignant | | 1 (25%) | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | | | 1 (2%) | 1 (2%) |
| Alveolar/bronchiolar carcinoma | 1 (2%) | | | |
| Carcinoma, metastatic, thyroid gland | | | | 1 (2%) |
| Osteosarcoma, multiple, metastatic, bone | | 1 (2%) | | |
| Squamous cell carcinoma | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (49) |
| Trachea | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, thyroid gland | | | | 1 (2%) |
| Special Senses System | | | | |
| Zymbal's gland | | | (1) | |
| Carcinoma | | | 1 (100%) | |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Pelvis, transitional epithelium, mesenchymal tumor | | | 1 (2%) | |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Systemic Lesions | | | | |
| Multiple organs | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | | 1 (2%) | |
| Leukemia mononuclear | 17 (34%) | 11 (22%) | 3 (6%) | |
| Mesothelioma malignant | | | | 1 (2%) |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 2 | 5 | 5 | 4 |
| 2-Year study | 44 | 48 | 50 | 45 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 2 | 7 | 6 | 4 |
| 2-Year study | 95 | 102 | 107 | 83 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 2 | 5 | 4 | 4 |
| 2-Year study | 40 | 41 | 44 | 41 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 2 | 6 | 5 | 4 |
| 2-Year study | 68 | 79 | 84 | 72 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | | 1 | 1 | |
| 2-Year study | 21 | 19 | 21 | 10 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | | 1 | 1 | |
| 2-Year study | 27 | 21 | 22 | 11 |
| Total animals with metastatic neoplasms | | | | |
| 2-Year study | | 2 | 2 | 1 |
| Total metastatic neoplasms | | | | |
| 2-Year study | | 5 | 4 | 3 |
| Total animals with uncertain neoplasms benign or malignant | | | | |
| 2-Year study | | 2 | 1 | |
| Total uncertain neoplasms | | | | |
| 2-Year study | | 2 | 1 | |

^a Number of animals examined microscopically at site and number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid: 0 ppm

| Number of Days on Study | 4 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
|-----------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9 | 5 | 6 | 0 | 3 | 4 | 5 | 5 | 6 | 6 | 7 | 7 | 8 | 8 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 3 | 3 | | |
| | 0 | 6 | 6 | 5 | 1 | 7 | 3 | 7 | 4 | 7 | 9 | 9 | 0 | 7 | 4 | 0 | 0 | 2 | 2 | 9 | 4 | 2 | 4 | 1 | 1 | | | |
| 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 | 2 | 2 | 2 |
| | 8 | 8 | 5 | 6 | 4 | 4 | 6 | 5 | 8 | 7 | 8 | 8 | 6 | 5 | 5 | 6 | 6 | 4 | 6 | 4 | 7 | 7 | 4 | 4 | 4 | | | |
| | 2 | 0 | 9 | 4 | 3 | 4 | 3 | 8 | 8 | 9 | 5 | 7 | 9 | 6 | 2 | 2 | 5 | 7 | 7 | 2 | 6 | 8 | 5 | 1 | 6 | | | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, cecum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, duodenum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, jejunum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, ileum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Mesentery | + | | | | | | | | | + | + | | | | | | | | + | + | + | | | | | | | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tooth | | | | | | | | | | | | | | | | | | | | | | | | | | | | + |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma benign | | | | | | | X | | | | | | | | | | | | | | | | | | | | | X |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pars distalis, adenoma | | | | | | | | | | X | X | X | X | | X | X | X | X | | X | | | | | | X | X | X |
| Pars distalis, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell, adenoma | | | X | | | | | | | | | X | | | | | | | | | | | | | | | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Granulosa cell tumor benign | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endometrium, polyp stromal | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Endometrium, schwannoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Vagina | | | | | | | | | | | | | | | | | | | | | | | | | | | | + |

+: 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 Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid: 5,000 ppm

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--|
| | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | | |
| Number of Days on Study | 4 | 8 | 9 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 0 | 1 | 1 | 2 | 7 | 8 | 8 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | | |
| | 1 | 3 | 3 | 8 | 8 | 2 | 1 | 6 | 5 | 4 | 2 | 1 | 8 | 2 | 4 | 7 | 9 | 5 | 5 | 0 | 2 | 2 | 7 | 8 | 8 | |
| 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 | | |
| | 3 | 4 | 5 | 2 | 5 | 6 | 2 | 3 | 4 | 2 | 5 | 4 | 5 | 5 | 2 | 6 | 6 | 5 | 6 | 4 | 2 | 6 | 6 | 2 | 3 | |
| | 9 | 6 | 6 | 9 | 1 | 6 | 8 | 0 | 8 | 4 | 0 | 1 | 8 | 3 | 5 | 7 | 9 | 4 | 2 | 7 | 6 | 3 | 8 | 1 | 3 | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Carcinoma, metastatic, thyroid gland | | | | | | | | | | | X | | | | | | | | | | | | | | | |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, rectum | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, cecum | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, duodenum | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, jejunum | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, ileum | M | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Mesentery | | | | + | | + | | | | | | | | | | | | | | | | | | | + | |
| Pancreas | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Stomach, forestomach | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Stomach, glandular | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Tongue | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Islets, pancreatic | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pars distalis, adenoma | | | | X | X | X | X | X | | | | X | X | X | X | X | X | X | X | X | X | X | X | X | | |
| Pars nervosa, ganglioneuroma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | |
| C-cell, adenoma | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| Follicular cell, carcinoma | | | | | | | | | | | X | | | | | | | | | | | | | | | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | X | | | X | | | | | | | | | | X | | | | X | | | | X | | | |
| Carcinoma | | | | | X | | | | | | | | | | | | | | X | | | | | | | |
| Bilateral, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bilateral, carcinoma | | | | | | | | | | | | | | | X | | | | | | | | | | | |
| Ovary | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | |
| Uterus | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | |
| Endometrium, polyp stromal | | | | | | | | | X | | | | X | X | X | | | | | | X | | | | | |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid: 5,000 ppm (continued)

| | |
|--------------------------------------|---|
| Number of Days on Study | 4 4 4 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 |
| | 4 8 9 1 2 3 4 5 6 7 0 1 1 2 7 8 8 9 9 0 0 0 0 0 0 |
| | 1 3 3 8 8 2 1 6 5 4 2 1 8 2 4 7 9 5 5 0 2 2 7 8 8 |
| Carcass ID Number | 4 |
| | 3 4 5 2 5 6 2 3 4 2 5 4 5 5 2 6 6 5 6 4 2 6 6 2 3 |
| | 9 6 6 9 1 6 8 0 8 4 0 1 8 3 5 7 9 4 2 7 6 3 8 1 3 |
| Hematopoietic System | |
| Bone marrow | + |
| Lymph node | + |
| Lymph node, mandibular | + |
| Lymph node, mesenteric | + + + M + + + + + + + + + + + + + + + + + + + |
| Spleen | + + + M + + + + + + + + + + + + + + + + + + + |
| Thymus | + |
| Integumentary System | |
| Mammary gland | + |
| Carcinoma | + |
| Fibroadenoma | X |
| Fibroadenoma, multiple | + |
| Skin | + |
| Basal cell carcinoma | X |
| Squamous cell papilloma | |
| Subcutaneous tissue, fibroma | X X |
| Musculoskeletal System | |
| Bone | + |
| Skeletal muscle | + |
| Sarcoma | X |
| Nervous System | |
| Brain | + |
| Respiratory System | |
| Lung | + |
| Alveolar/bronchiolar adenoma | |
| Carcinoma, metastatic, thyroid gland | X |
| Squamous cell carcinoma | |
| Nose | + |
| Trachea | + |
| Carcinoma, metastatic, thyroid gland | X |
| Special Senses System | |
| Eye | + |
| Urinary System | |
| Kidney | + |
| Urinary bladder | + |
| Systemic Lesions | |
| Multiple organs | + |
| Mesothelioma malignant | |

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------------|-------------|-------------|----------------|
| Adrenal Medulla: Benign Pheochromocytoma | | | | |
| Overall rate ^a | 2/49 (4%) | 1/50 (2%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted rate ^b | 5.4% | 4.3% | 11.7% | 0.0% |
| Terminal rate ^c | 0/26 (0%) | 1/23 (4%) | 2/21 (10%) | 0/21 (0%) |
| First incidence (days) | 647 | 730 (T) | 518 | - ^e |
| Life table test ^d | P=0.298N | P=0.557N | P=0.402 | P=0.289N |
| Logistic regression test ^d | P=0.242N | P=0.510N | P=0.526 | P=0.220N |
| Cochran-Armitage test ^d | P=0.237N | | | |
| Fisher exact test ^d | | P=0.492N | P=0.510 | P=0.242N |
| Clitoral Gland: Adenoma | | | | |
| Overall rate | 4/50 (8%) | 12/49 (24%) | 10/49 (20%) | 12/50 (24%) |
| Adjusted rate | 11.9% | 42.5% | 33.7% | 42.1% |
| Terminal rate | 2/27 (7%) | 7/22 (32%) | 4/20 (20%) | 7/21 (33%) |
| First incidence (days) | 653 | 665 | 496 | 483 |
| Life table test | P=0.034 | P=0.013 | P=0.030 | P=0.013 |
| Logistic regression test | P=0.046 | P=0.013 | P=0.050 | P=0.023 |
| Cochran-Armitage test | P=0.066 | | | |
| Fisher exact test | | P=0.024 | P=0.068 | P=0.027 |
| Clitoral Gland: Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 2/49 (4%) | 5/49 (10%) | 4/50 (8%) |
| Adjusted rate | 3.7% | 6.0% | 19.3% | 11.7% |
| Terminal rate | 1/27 (4%) | 0/22 (0%) | 3/20 (15%) | 0/21 (0%) |
| First incidence (days) | 730 (T) | 694 | 499 | 528 |
| Life table test | P=0.085 | P=0.460 | P=0.056 | P=0.139 |
| Logistic regression test | P=0.117 | P=0.459 | P=0.084 | P=0.224 |
| Cochran-Armitage test | P=0.116 | | | |
| Fisher exact test | | P=0.492 | P=0.098 | P=0.181 |
| Clitoral Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 14/49 (29%) | 15/49 (31%) | 15/50 (30%) |
| Adjusted rate | 11.9% | 45.9% | 48.9% | 47.7% |
| Terminal rate | 2/27 (7%) | 7/22 (32%) | 7/20 (35%) | 7/21 (33%) |
| First incidence (days) | 653 | 665 | 496 | 483 |
| Life table test | P=0.008 | P=0.005 | P=0.001 | P=0.002 |
| Logistic regression test | P=0.011 | P=0.004 | P=0.003 | P=0.004 |
| Cochran-Armitage test | P=0.018 | | | |
| Fisher exact test | | P=0.008 | P=0.004 | P=0.005 |
| Mammary Gland: Fibroadenoma | | | | |
| Overall rate | 22/50 (44%) | 22/50 (44%) | 26/50 (52%) | 24/50 (48%) |
| Adjusted rate | 59.6% | 57.1% | 70.4% | 61.4% |
| Terminal rate | 13/27 (48%) | 8/23 (35%) | 11/21 (52%) | 7/21 (33%) |
| First incidence (days) | 566 | 483 | 379 | 483 |
| Life table test | P=0.161 | P=0.363 | P=0.073 | P=0.198 |
| Logistic regression test | P=0.280 | P=0.502 | P=0.187 | P=0.327 |
| Cochran-Armitage test | P=0.333 | | | |
| Fisher exact test | | P=0.580N | P=0.274 | P=0.421 |

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------------|-------------|-------------|-------------|
| Mammary Gland: Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 7.0% | 6.6% | 11.3% | 7.0% |
| Terminal rate | 1/27 (4%) | 1/23 (4%) | 2/21 (10%) | 1/21 (5%) |
| First incidence (days) | 722 | 570 | 241 | 556 |
| Life table test | P=0.481 | P=0.636 | P=0.404 | P=0.605 |
| Logistic regression test | P=0.583N | P=0.691 | P=0.623 | P=0.699N |
| Cochran-Armitage test | P=0.569 | | | |
| Fisher exact test | | P=0.691N | P=0.500 | P=0.691N |
| Mammary Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 9.3% | 6.6% | 11.3% | 7.0% |
| Terminal rate | 1/27 (4%) | 1/23 (4%) | 2/21 (10%) | 1/21 (5%) |
| First incidence (days) | 667 | 570 | 241 | 556 |
| Life table test | P=0.546N | P=0.562N | P=0.554 | P=0.596N |
| Logistic regression test | P=0.422N | P=0.500N | P=0.564N | P=0.490N |
| Cochran-Armitage test | P=0.456N | | | |
| Fisher exact test | | P=0.500N | P=0.661N | P=0.500N |
| Mammary Gland: Adenoma or Fibroadenoma | | | | |
| Overall rate | 23/50 (46%) | 22/50 (44%) | 26/50 (52%) | 24/50 (48%) |
| Adjusted rate | 60.6% | 57.1% | 70.4% | 61.4% |
| Terminal rate | 13/27 (48%) | 8/23 (35%) | 11/21 (52%) | 7/21 (33%) |
| First incidence (days) | 566 | 483 | 379 | 483 |
| Life table test | P=0.196 | P=0.427 | P=0.098 | P=0.247 |
| Logistic regression test | P=0.340 | P=0.576N | P=0.249 | P=0.408 |
| Cochran-Armitage test | P=0.396 | | | |
| Fisher exact test | | P=0.500N | P=0.345 | P=0.500 |
| Mammary Gland: Adenoma, Fibroadenoma, or Carcinoma | | | | |
| Overall rate | 25/50 (50%) | 24/50 (48%) | 28/50 (56%) | 26/50 (52%) |
| Adjusted rate | 64.7% | 60.9% | 73.9% | 65.0% |
| Terminal rate | 14/27 (52%) | 9/23 (39%) | 12/21 (57%) | 8/21 (38%) |
| First incidence (days) | 566 | 483 | 241 | 483 |
| Life table test | P=0.186 | P=0.409 | P=0.093 | P=0.229 |
| Logistic regression test | P=0.362 | P=0.579N | P=0.304 | P=0.408 |
| Cochran-Armitage test | P=0.396 | | | |
| Fisher exact test | | P=0.500N | P=0.344 | P=0.500 |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 18/50 (36%) | 27/50 (54%) | 25/50 (50%) | 23/49 (47%) |
| Adjusted rate | 46.6% | 74.8% | 76.4% | 59.8% |
| Terminal rate | 8/27 (30%) | 15/23 (65%) | 14/21 (67%) | 7/20 (35%) |
| First incidence (days) | 657 | 510 | 538 | 518 |
| Life table test | P=0.112 | P=0.030 | P=0.023 | P=0.096 |
| Logistic regression test | P=0.200 | P=0.034 | P=0.025 | P=0.184 |
| Cochran-Armitage test | P=0.272 | | | |
| Fisher exact test | | P=0.054 | P=0.113 | P=0.184 |

TABLE B3

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------------|-------------|-------------|-------------|
| Pituitary Gland (Pars Distalis): Adenoma or Carcinoma | | | | |
| Overall rate | 19/50 (38%) | 27/50 (54%) | 25/50 (50%) | 23/49 (47%) |
| Adjusted rate | 48.4% | 74.8% | 76.4% | 59.8% |
| Terminal rate | 8/27 (30%) | 15/23 (65%) | 14/21 (67%) | 7/20 (35%) |
| First incidence (days) | 657 | 510 | 538 | 518 |
| Life table test | P=0.139 | P=0.045 | P=0.035 | P=0.125 |
| Logistic regression test | P=0.248 | P=0.051 | P=0.038 | P=0.237 |
| Cochran-Armitage test | P=0.330 | | | |
| Fisher exact test | | P=0.080 | P=0.157 | P=0.243 |
| Skin (Subcutaneous Tissue): Fibroma | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate | 6.6% | 3.1% | 0.0% | 9.9% |
| Terminal rate | 1/27 (4%) | 0/23 (0%) | 0/21 (0%) | 1/21 (5%) |
| First incidence (days) | 702 | 696 | — | 611 |
| Life table test | P=0.294 | P=0.552N | P=0.295N | P=0.427 |
| Logistic regression test | P=0.315 | P=0.535N | P=0.290N | P=0.480 |
| Cochran-Armitage test | P=0.337 | | | |
| Fisher exact test | | P=0.500N | P=0.247N | P=0.500 |
| Thyroid Gland (C-cell): Adenoma | | | | |
| Overall rate | 9/50 (18%) | 5/49 (10%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted rate | 29.3% | 17.4% | 13.6% | 6.7% |
| Terminal rate | 7/27 (26%) | 3/23 (13%) | 1/21 (5%) | 1/21 (5%) |
| First incidence (days) | 556 | 632 | 588 | 483 |
| Life table test | P=0.045N | P=0.284N | P=0.241N | P=0.059N |
| Logistic regression test | P=0.023N | P=0.249N | P=0.165N | P=0.027N |
| Cochran-Armitage test | P=0.019N | | | |
| Fisher exact test | | P=0.205N | P=0.117N | P=0.026N |
| Thyroid Gland (C-cell): Adenoma or Carcinoma | | | | |
| Overall rate | 10/50 (20%) | 5/49 (10%) | 6/50 (12%) | 2/50 (4%) |
| Adjusted rate | 31.4% | 17.4% | 22.2% | 6.7% |
| Terminal rate | 7/27 (26%) | 3/23 (13%) | 3/21 (14%) | 1/21 (5%) |
| First incidence (days) | 556 | 632 | 588 | 483 |
| Life table test | P=0.041N | P=0.213N | P=0.391N | P=0.038N |
| Logistic regression test | P=0.021N | P=0.176N | P=0.308N | P=0.016N |
| Cochran-Armitage test | P=0.016N | | | |
| Fisher exact test | | P=0.140N | P=0.207N | P=0.014N |
| Uterus: Stromal Polyp | | | | |
| Overall rate | 5/50 (10%) | 11/50 (22%) | 12/50 (24%) | 5/50 (10%) |
| Adjusted rate | 15.9% | 38.9% | 37.9% | 13.2% |
| Terminal rate | 3/27 (11%) | 7/23 (30%) | 4/21 (19%) | 0/21 (0%) |
| First incidence (days) | 490 | 521 | 434 | 565 |
| Life table test | P=0.523 | P=0.050 | P=0.023 | P=0.513 |
| Logistic regression test | P=0.406N | P=0.075 | P=0.063 | P=0.534N |
| Cochran-Armitage test | P=0.411N | | | |
| Fisher exact test | | P=0.086 | P=0.054 | P=0.630N |

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------------|-------------|--------------|-------------|
| Uterus: Stromal Polyp or Stromal Sarcoma | | | | |
| Overall rate | 5/50 (10%) | 12/50 (24%) | 13/50 (26%) | 5/50 (10%) |
| Adjusted rate | 15.9% | 40.7% | 39.4% | 13.2% |
| Terminal rate | 3/27 (11%) | 7/23 (30%) | 4/21 (19%) | 0/21 (0%) |
| First incidence (days) | 490 | 521 | 434 | 565 |
| Life table test | P=0.540 | P=0.032 | P=0.014 | P=0.513 |
| Logistic regression test | P=0.379N | P=0.047 | P=0.044 | P=0.534N |
| Cochran-Armitage test | P=0.389N | | | |
| Fisher exact test | | P=0.054 | P=0.033 | P=0.630N |
| All Organs: Mononuclear Cell Leukemia | | | | |
| Overall rate | 17/50 (34%) | 11/50 (22%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted rate | 38.6% | 32.5% | 8.5% | 0.0% |
| Terminal rate | 3/27 (11%) | 4/23 (17%) | 0/21 (0%) | 0/21 (0%) |
| First incidence (days) | 490 | 566 | 492 | — |
| Life table test | P<0.001N | P=0.272N | P=0.008N | P<0.001N |
| Logistic regression test | P<0.001N | P=0.159N | P<0.001N | P<0.001N |
| Cochran-Armitage test | P<0.001N | | | |
| Fisher exact test | | P=0.133N | P<0.001N | P<0.001N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 40/50 (80%) | 41/50 (82%) | 44/50 (88%) | 41/50 (82%) |
| Adjusted rate | 84.9% | 93.0% | 95.6% | 88.8% |
| Terminal rate | 20/27 (74%) | 20/23 (87%) | 19/21 (90%) | 16/21 (76%) |
| First incidence (days) | 490 | 483 | 379 | 483 |
| Life table test | P=0.128 | P=0.206 | P=0.035 | P=0.150 |
| Logistic regression test | P=0.311 | P=0.339 | P=0.072 | P=0.473 |
| Cochran-Armitage test | P=0.424 | | | |
| Fisher exact test | | P=0.500 | P=0.207 | P=0.500 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 22/50 (44%) | 19/50 (38%) | 22/50 (44%) | 11/50 (22%) |
| Adjusted rate | 49.0% | 48.3% | 62.3% | 31.4% |
| Terminal rate | 6/27 (22%) | 6/23 (26%) | 10/21 (48%) | 2/21 (10%) |
| First incidence (days) | 490 | 150 | 241 | 441 |
| Life table test | P=0.113N | P=0.541N | P=0.259 | P=0.104N |
| Logistic regression test | P=0.007N | P=0.188N | P=0.383N | P=0.004N |
| Cochran-Armitage test | P=0.017N | | | |
| Fisher exact test | | P=0.342N | P=0.580N | P=0.016N |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 44/50 (88%) | 48/50 (96%) | 50/50 (100%) | 45/50 (90%) |
| Adjusted rate | 88.0% | 98.0% | 100.0% | 91.8% |
| Terminal rate | 21/27 (78%) | 22/23 (96%) | 21/21 (100%) | 17/21 (81%) |
| First incidence (days) | 490 | 150 | 241 | 441 |
| Life table test | P=0.143 | P=0.103 | P=0.017 | P=0.138 |
| Logistic regression test | P=0.559 | P=0.156 | P=0.032 | P=0.564 |
| Cochran-Armitage test | P=0.519 | | | |
| Fisher exact test | | P=0.134 | P=0.013 | P=0.500 |

TABLE B3

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

(T) Terminal sacrifice

- ^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
- ^b 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 exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test 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 an exposure group is indicated by N.
- ^e Not applicable; no neoplasms in animal group

TABLE B4a
Historical Incidence of Clitoral Gland Neoplasms in Untreated Female F344/N Rats^a

| Study | Incidence in Controls | | |
|--|-----------------------|-----------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Southern Research Institute | | | |
| Benzyl Acetate | 0/50 | 1/50 | 1/50 |
| C.I. Pigment Red 23 | 5/47 | 3/47 | 7/47 |
| C.I. Pigment Red 3 | 9/47 | 0/47 | 9/47 |
| Nitrofurantoin | 1/44 | 4/44 | 5/44 |
| <i>o</i> -Nitroanisole | 3/45 | 4/45 | 7/45 |
| Polysorbate 80 | 3/48 | 7/48 | 10/48 |
| Rhodamine 6G | 5/42 | 1/42 | 6/42 |
| Roxarsone | 1/44 | 1/44 | 2/44 |
| Total | 27/367 (7.4%) | 21/367 (5.7%) | 47/367 (12.8%) |
| Standard deviation | 6.4% | 5.1% | 6.6% |
| Range | 0%-19% | 0%-15% | 2%-21% |
| Overall Historical Incidence | | | |
| Total ^b | 90/1,096 (8.2%) | 31/1,096 (2.8%) | 120/1,096 (10.9%) |
| Standard deviation | 4.6% | 4.0% | 5.3% |
| Range | 0%-19% | 0%-15% | 2%-21% |

^a Data as of 20 August 1992

^b Data from Quercetin, TR 409, censored due to low denominator (adenoma, 4/14; carcinoma, 1/14; adenoma or carcinoma, 5/14)

TABLE B4b
Historical Incidence of Mononuclear Cell Leukemia in Untreated Female F344/N Rats^a

| Study | Incidence in Controls |
|--|--|
| | Mononuclear Cell Leukemia ^b |
| Historical Incidence at Southern Research Institute | |
| Benzyl Acetate | 9/50 |
| C.I. Pigment Red 23 | 14/50 |
| C.I. Pigment Red 3 | 10/50 |
| Nitrofurantoin | 13/50 |
| <i>o</i> -Nitroanisole | 14/50 |
| Polysorbate 80 | 26/50 |
| Rhodamine 6G | 11/50 |
| Roxarsone | 14/50 |
| Total | 111/400 (27.8%) |
| Standard deviation | 10.6% |
| Range | 18%-52% |
| Overall Historical Incidence | |
| Total | 324/1,251 (25.9%) |
| Standard deviation | 8.6% |
| Range | 14%-52% |

^a Data as of 20 August 1992

^b Includes incidences of lymphocytic, monocytic, or undifferentiated leukemia.

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|----------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| <i>15-Month interim evaluation</i> | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Moribund | 21 | 23 | 27 | 26 |
| Natural deaths | 2 | 4 | 2 | 3 |
| Survivors | | | | |
| Terminal sacrifice | 27 | 23 | 21 | 21 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (10) | (10) | (10) | (10) |
| Parasite metazoan | 1 (10%) | | 1 (10%) | |
| Intestine large, rectum | (10) | (10) | (10) | (9) |
| Parasite metazoan | | 1 (10%) | | 1 (11%) |
| Liver | (10) | (10) | (10) | (10) |
| Basophilic focus | 8 (80%) | 5 (50%) | 6 (60%) | 8 (80%) |
| Clear cell focus | | 1 (10%) | | |
| Fatty change | | 1 (10%) | | |
| Hepatodiaphragmatic nodule | 3 (30%) | 1 (10%) | 1 (10%) | 4 (40%) |
| Inflammation, focal | 7 (70%) | 9 (90%) | 4 (40%) | 4 (40%) |
| Mixed cell focus | 1 (10%) | | 1 (10%) | |
| Bile duct, hyperplasia | 4 (40%) | 6 (60%) | 6 (60%) | 6 (60%) |
| Mesentery | (1) | (1) | (1) | |
| Fat, necrosis | 1 (100%) | 1 (100%) | 1 (100%) | |
| Pancreas | (10) | (10) | (10) | (10) |
| Accessory spleen | 1 (10%) | 1 (10%) | | |
| Atrophy, focal | 5 (50%) | 4 (40%) | 2 (20%) | 2 (20%) |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Accessory adrenal cortical nodule | 1 (10%) | | | |
| Focal cellular change | | | 2 (20%) | |
| Pituitary gland | (10) | (10) | (9) | (10) |
| Angiectasis | 7 (70%) | 2 (20%) | 5 (56%) | 3 (30%) |
| Cyst | 1 (10%) | 2 (20%) | | 2 (20%) |
| Pars distalis, focal cellular change | | | | 1 (10%) |
| Pars distalis, hyperplasia, focal | 5 (50%) | 2 (20%) | 5 (56%) | 1 (10%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Ultimobranchial cyst | | 2 (20%) | 1 (10%) | |
| C-cell, hyperplasia | | | | 3 (30%) |

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

TABLE B5

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Genital System | | | | |
| Clitoral gland | (10) | (10) | (10) | (10) |
| Degeneration, cystic | 8 (80%) | 5 (50%) | 8 (80%) | 4 (40%) |
| Ovary | (10) | (10) | (10) | (10) |
| Cyst | | 4 (40%) | 4 (40%) | 1 (10%) |
| Uterus | (10) | (10) | (10) | (10) |
| Hydrometra | | 2 (20%) | 2 (20%) | 1 (10%) |
| Endometrium, hyperplasia, cystic | | 1 (10%) | | 2 (20%) |
| Endometrium, infarct | 1 (10%) | | | |
| Vagina | | (1) | | |
| Cyst | | 1 (100%) | | |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Hyperplasia, focal, histiocytic | 1 (10%) | 2 (20%) | | |
| Lymph node | (1) | (4) | (2) | (2) |
| Mediastinal, congestion | 1 (100%) | 2 (50%) | 2 (100%) | |
| Mediastinal, pigmentation | | | | 1 (50%) |
| Pancreatic, congestion | | 1 (25%) | | |
| Pancreatic, pigmentation | | | | 1 (50%) |
| Lymph node, mandibular | (10) | (10) | (10) | (10) |
| Congestion | | 1 (10%) | | |
| Hyperplasia, lymphoid | | 2 (20%) | | |
| Spleen | (10) | (10) | (10) | (10) |
| Cyst | | | | 1 (10%) |
| Pigmentation | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Integumentary System | | | | |
| Mammary gland | (10) | (10) | (10) | (10) |
| Dilatation | 1 (10%) | 2 (20%) | 3 (30%) | 2 (20%) |
| Hyperplasia | | 1 (10%) | 3 (30%) | 1 (10%) |
| Musculoskeletal System | | | | |
| Bone | (10) | (10) | (10) | (10) |
| Hyperostosis | 1 (10%) | 2 (20%) | | |
| Nervous System | | | | |
| Brain | (10) | (10) | (10) | (10) |
| Compression | | | 1 (10%) | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar epithelium, hyperplasia | | | | 1 (10%) |

TABLE B5

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Special Senses System | | | | |
| Eye | (2) | | | |
| Cataract | 1 (50%) | | | |
| Fibrosis | 1 (50%) | | | |
| Retina, degeneration | 1 (50%) | | | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Nephropathy | 10 (100%) | 10 (100%) | 9 (90%) | 9 (90%) |
| Renal tubule, pigmentation | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Systems Examined With No Lesions Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, rectum | (50) | (48) | (50) | (49) |
| Parasite metazoan | | 6 (13%) | 2 (4%) | 3 (6%) |
| Intestine large, cecum | (50) | (47) | (50) | (49) |
| Parasite metazoan | | 1 (2%) | | |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | 1 (2%) | 2 (4%) | | 2 (4%) |
| Basophilic focus | 32 (64%) | 38 (76%) | 44 (88%) | 47 (94%) |
| Clear cell focus | 4 (8%) | 6 (12%) | 7 (14%) | 12 (24%) |
| Eosinophilic focus | 4 (8%) | 4 (8%) | | 1 (2%) |
| Fatty change | 14 (28%) | 13 (26%) | 7 (14%) | 7 (14%) |
| Fibrosis, focal | 1 (2%) | | 2 (4%) | |
| Focal cellular change | 1 (2%) | 1 (2%) | 2 (4%) | |
| Hematopoietic cell proliferation | | 2 (4%) | 2 (4%) | 1 (2%) |
| Hepatodiaphragmatic nodule | 5 (10%) | 5 (10%) | 11 (22%) | 12 (24%) |
| Hepatodiaphragmatic nodule, multiple | | 2 (4%) | 2 (4%) | 3 (6%) |
| Hyperplasia, histiocytic | | | | 1 (2%) |
| Hyperplasia, lymphoid | | | | 1 (2%) |
| Hyperplasia, multifocal | 9 (18%) | 10 (20%) | 3 (6%) | 2 (4%) |
| Infiltration cellular, mixed cell | 1 (2%) | 4 (8%) | 3 (6%) | 1 (2%) |
| Inflammation, focal | 26 (52%) | 24 (48%) | 37 (74%) | 41 (82%) |
| Mixed cell focus | 4 (8%) | 8 (16%) | 3 (6%) | 7 (14%) |
| Necrosis, focal | 2 (4%) | 1 (2%) | | 1 (2%) |
| Pigmentation | 1 (2%) | | | |
| Thrombosis | 1 (2%) | | | |
| Bile duct, dilatation | | 1 (2%) | | |
| Bile duct, hyperplasia | 23 (46%) | 19 (38%) | 18 (36%) | 27 (54%) |
| Centrilobular, atrophy | 14 (28%) | 11 (22%) | 4 (8%) | 2 (4%) |
| Centrilobular, congestion | | | 1 (2%) | |
| Centrilobular, hemorrhage | 1 (2%) | | | |
| Centrilobular, necrosis | 1 (2%) | | 1 (2%) | |

TABLE B5

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Mesentery | (10) | (8) | (9) | (6) |
| Accessory spleen | | 1 (13%) | | 1 (17%) |
| Cyst | | | 1 (11%) | |
| Inflammation, chronic | | 1 (13%) | 1 (11%) | 1 (17%) |
| Fat, necrosis | 6 (60%) | 3 (38%) | 6 (67%) | 4 (67%) |
| Pancreas | (50) | (50) | (49) | (49) |
| Atrophy, diffuse | 2 (4%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Atrophy, focal | 16 (32%) | 14 (28%) | 19 (39%) | 11 (22%) |
| Cyst | | | | 1 (2%) |
| Inflammation, chronic | | 1 (2%) | 1 (2%) | |
| Acinar cell, depletion secretory | | | 1 (2%) | |
| Duct, dilatation | | 1 (2%) | | |
| Stomach, forestomach | (50) | (50) | (49) | (49) |
| Edema | | 1 (2%) | | |
| Hemorrhage, focal | 1 (2%) | | | |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Ulcer | 1 (2%) | | 2 (4%) | |
| Mucosa, hyperplasia | 2 (4%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Stomach, glandular | (50) | (50) | (49) | (49) |
| Bacterium | 1 (2%) | | | |
| Erosion | 2 (4%) | 1 (2%) | | |
| Inflammation, chronic | 1 (2%) | | | |
| Ulcer | 1 (2%) | | | |
| Tongue | | | (1) | (1) |
| Hypertrophy, squamous | | | | 1 (100%) |
| Tooth | (1) | | | |
| Gingiva, hyperplasia | 1 (100%) | | | |
| Cardiovascular System | | | | |
| Blood vessel | (50) | (50) | (50) | (50) |
| Mesenteric artery, inflammation, chronic | 1 (2%) | | | 2 (4%) |
| Mesenteric artery, thrombosis | 1 (2%) | | | |
| Heart | (50) | (50) | (50) | (50) |
| Bacterium | 1 (2%) | | | |
| Embolus | 1 (2%) | | | |
| Inflammation, chronic, focal | 2 (4%) | | | 1 (2%) |
| Artery, inflammation, chronic | | | 2 (4%) | |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (50) | (50) |
| Accessory adrenal cortical nodule | 1 (2%) | | 1 (2%) | 3 (6%) |
| Angiectasis | 1 (2%) | 2 (4%) | | |
| Congestion | 4 (8%) | 1 (2%) | | 2 (4%) |
| Degeneration, cystic | | | 2 (4%) | |
| Depletion cellular | 1 (2%) | | | |
| Focal cellular change | 13 (27%) | 9 (18%) | 8 (16%) | 13 (26%) |
| Hyperplasia, focal | 1 (2%) | | | |
| Infiltration cellular, lymphocyte | 1 (2%) | | | |
| Vacuolization cytoplasmic | | 1 (2%) | | |
| Capsule, extra adrenal tissue, fibrosis | | | | 1 (2%) |

TABLE B5

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Endocrine System (continued) | | | | |
| Adrenal medulla | (49) | (50) | (50) | (50) |
| Hyperplasia | 2 (4%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Angiectasis | 12 (24%) | 11 (22%) | 7 (14%) | 8 (16%) |
| Cyst | 3 (6%) | 4 (8%) | 2 (4%) | 2 (4%) |
| Granuloma | 1 (2%) | | | |
| Hemorrhage | 1 (2%) | | | |
| Pars distalis, cyst | | 1 (2%) | | 1 (2%) |
| Pars distalis, focal cellular change | 6 (12%) | 4 (8%) | 2 (4%) | 2 (4%) |
| Pars distalis, hyperplasia, focal | 11 (22%) | 9 (18%) | 9 (18%) | 8 (16%) |
| Rathke's cleft, hyperplasia, cystic | | 1 (2%) | | |
| Thyroid gland | (50) | (49) | (50) | (50) |
| Degeneration, cystic | 1 (2%) | | | |
| Inflammation, focal | | 1 (2%) | | |
| Ultimobranchial cyst | | | 2 (4%) | |
| C-cell, hyperplasia | 10 (20%) | 8 (16%) | 11 (22%) | 11 (22%) |
| Follicle, dilatation | | 1 (2%) | | |
| Follicular cell, hyperplasia | | 2 (4%) | | |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Clitoral gland | (50) | (49) | (49) | (50) |
| Atrophy | | 1 (2%) | | |
| Cyst | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Degeneration, cystic | 34 (68%) | 38 (78%) | 39 (80%) | 35 (70%) |
| Hyperplasia | 10 (20%) | 6 (12%) | 6 (12%) | 7 (14%) |
| Inflammation, chronic | | 1 (2%) | 1 (2%) | |
| Duct, hyperplasia, squamous | | 1 (2%) | | |
| Ovary | (50) | (50) | (50) | (49) |
| Angiectasis | | | 1 (2%) | 1 (2%) |
| Atrophy | | | 1 (2%) | |
| Cyst | 5 (10%) | 6 (12%) | 6 (12%) | 8 (16%) |
| Hemorrhage | 1 (2%) | | | |
| Bilateral, cyst | | | 1 (2%) | |
| Corpus luteum, hyperplasia, lymphoid | 2 (4%) | 1 (2%) | | |
| Corpus luteum, thecal cell, hyperplasia | 2 (4%) | 1 (2%) | | |
| Uterus | (50) | (50) | (50) | (49) |
| Hydrometra | 1 (2%) | 4 (8%) | 3 (6%) | 2 (4%) |
| Endometrium, cyst | | 1 (2%) | 1 (2%) | 1 (2%) |
| Endometrium, hyperplasia, cystic | 3 (6%) | 9 (18%) | 4 (8%) | 4 (8%) |
| Endometrium, infarct | 1 (2%) | | | |
| Vagina | (3) | (1) | (3) | |
| Cyst | 2 (67%) | 1 (100%) | 1 (33%) | |
| Cyst, multiple | | | 1 (33%) | |
| Inflammation, chronic | | | 1 (33%) | |
| Mucosa, hyperplasia | 1 (33%) | | | |

TABLE B5

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|-----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hypercellularity | 2 (4%) | 4 (8%) | 2 (4%) | 3 (6%) |
| Hyperplasia, focal, histiocytic | 2 (4%) | 2 (4%) | 4 (8%) | 4 (8%) |
| Metaplasia, osseous | 1 (2%) | | | |
| Myelofibrosis | 1 (2%) | | | |
| Lymph node | (13) | (10) | (9) | (8) |
| Deep cervical, angiectasis | | | 1 (11%) | |
| Inguinal, depletion lymphoid | | 1 (10%) | | |
| Inguinal, hyperplasia | | | | 2 (25%) |
| Mediastinal, angiectasis | | | 1 (11%) | |
| Mediastinal, congestion | | 1 (10%) | 5 (56%) | 1 (13%) |
| Mediastinal, depletion lymphoid | | 1 (10%) | | |
| Mediastinal, hyperplasia | | | | 3 (38%) |
| Mediastinal, hyperplasia, lymphoid | | 1 (10%) | | |
| Mediastinal, hyperplasia, macrophage | | | 1 (11%) | |
| Mediastinal, pigmentation | | | 2 (22%) | 1 (13%) |
| Pancreatic, congestion | | 1 (10%) | | |
| Pancreatic, depletion lymphoid | | 1 (10%) | | |
| Pancreatic, edema | | | | 1 (13%) |
| Pancreatic, hyperplasia, lymphoid | | | | 1 (13%) |
| Pancreatic, inflammation, chronic | | | | 1 (13%) |
| Renal, hyperplasia, macrophage | | | | 1 (13%) |
| Renal, pigmentation | | | | 1 (13%) |
| Lymph node, mandibular | (50) | (50) | (50) | (50) |
| Congestion | 1 (2%) | | 2 (4%) | 4 (8%) |
| Depletion lymphoid | | 1 (2%) | | |
| Hyperplasia | | 2 (4%) | 2 (4%) | 1 (2%) |
| Pigmentation | | | | 2 (4%) |
| Lymph node, mesenteric | (49) | (50) | (50) | (49) |
| Congestion | | 1 (2%) | | |
| Depletion lymphoid | | 1 (2%) | | |
| Hyperplasia | 1 (2%) | | | |
| Hyperplasia, lymphoid | | 1 (2%) | 1 (2%) | |
| Spleen | (50) | (50) | (50) | (49) |
| Fibrosis | 1 (2%) | 6 (12%) | | 1 (2%) |
| Hematopoietic cell proliferation | 2 (4%) | 14 (28%) | 9 (18%) | 8 (16%) |
| Necrosis, focal | 2 (4%) | | 1 (2%) | |
| Pigmentation | 50 (100%) | 50 (100%) | 50 (100%) | 49 (100%) |
| Thymus | (49) | (48) | (50) | (50) |
| Congestion | | | | 1 (2%) |
| Cyst | 1 (2%) | | 1 (2%) | |
| Fibrosis | 1 (2%) | | | |
| Hyperplasia, lymphoid | | 1 (2%) | | |

TABLE B5

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Dilatation | 43 (86%) | 38 (76%) | 39 (78%) | 35 (70%) |
| Hyperplasia | 9 (18%) | 14 (28%) | 10 (20%) | 15 (30%) |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | | | 1 (2%) | |
| Hemorrhage, focal | | | 1 (2%) | |
| Inflammation, chronic, focal | | 1 (2%) | | |
| Ulcer | | 1 (2%) | | |
| Subcutaneous tissue, inflammation, chronic, focal | | | 1 (2%) | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Fracture healed | | | 1 (2%) | |
| Hyperostosis | 17 (34%) | 9 (18%) | 9 (18%) | 9 (18%) |
| Inflammation, chronic, focal | 1 (2%) | | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 8 (16%) | 16 (32%) | 10 (20%) | 14 (28%) |
| Demyelination, focal | | | 1 (2%) | |
| Hemorrhage | 4 (8%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Spinal cord | (1) | (2) | (2) | |
| Hemorrhage, focal | | | 1 (50%) | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Bacterium | 1 (2%) | | | |
| Congestion | | 1 (2%) | 2 (4%) | |
| Fibrosis, focal | 1 (2%) | | | 1 (2%) |
| Hemorrhage, focal | | | 2 (4%) | |
| Hyperplasia, focal, macrophage | | | 1 (2%) | 2 (4%) |
| Infiltration cellular, mixed cell | 1 (2%) | 1 (2%) | 1 (2%) | |
| Metaplasia, focal, osseous | 1 (2%) | | | |
| Thrombosis | 1 (2%) | | | |
| Alveolar epithelium, hyperplasia | 3 (6%) | 3 (6%) | 4 (8%) | 3 (6%) |
| Mediastinum, infiltration cellular, lymphocyte | | 1 (2%) | | |
| Nose | (50) | (50) | (50) | (49) |
| Fungus | | | | 1 (2%) |
| Inflammation, suppurative | 2 (4%) | 4 (8%) | 5 (10%) | 2 (4%) |
| Nasolacrimal duct, cyst | | 1 (2%) | | |

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of p-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Special Senses System | | | | |
| Eye | (2) | (2) | (2) | (1) |
| Atrophy | | | 1 (50%) | |
| Cataract | 2 (100%) | 1 (50%) | 2 (100%) | 1 (100%) |
| Hemorrhage | 1 (50%) | 1 (50%) | 1 (50%) | |
| Inflammation, chronic | 1 (50%) | 1 (50%) | 1 (50%) | |
| Necrosis, focal | | 1 (50%) | | |
| Retina, degeneration | 2 (100%) | 1 (50%) | 2 (100%) | 1 (100%) |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Atrophy, focal | | | 1 (2%) | |
| Bacterium | 1 (2%) | | | |
| Congestion | | 1 (2%) | | |
| Cyst | | | 1 (2%) | 1 (2%) |
| Fibrosis, focal | | | 1 (2%) | |
| Nephropathy | 50 (100%) | 49 (98%) | 49 (98%) | 49 (98%) |
| Cortex, renal tubule, autolysis | 1 (2%) | | | |
| Glomerulus, autolysis | 1 (2%) | | | |
| Interstitial, infarct | | | | 1 (2%) |
| Papilla, epithelium, hyperplasia, focal | | | | 1 (2%) |
| Renal tubule, hyperplasia | | | 1 (2%) | |
| Renal tubule, hyperplasia, oncocytic | | 1 (2%) | | 5 (10%) |
| Renal tubule, pigmentation | 50 (100%) | 50 (100%) | 50 (100%) | 50 (100%) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Transitional epithelium, hyperplasia | | | 1 (2%) | |

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE
IN THE 2-YEAR FEED STUDY
OF *p*-NITROBENZOIC ACID

| | | |
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TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|---------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | | | | |
| Early deaths | | | | |
| Accidental deaths | | 1 | | 2 |
| Moribund | 9 | 4 | 10 | 2 |
| Natural deaths | 2 | 9 | 1 | 2 |
| Survivors | | | | |
| Terminal sacrifice | 39 | 36 | 39 | 44 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Hepatocellular carcinoma | | 2 (20%) | | |
| Hepatocellular adenoma | 1 (10%) | 1 (10%) | 1 (10%) | |
| Hepatocellular adenoma, multiple | 1 (10%) | 1 (10%) | 1 (10%) | |
| Endocrine System | | | | |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Follicular cell, adenoma | 1 (10%) | | | |
| Integumentary System | | | | |
| Skin | (10) | (10) | (10) | (10) |
| Subcutaneous tissue, lipoma | | | 1 (10%) | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar/bronchiolar adenoma | | 1 (10%) | 2 (20%) | 1 (10%) |
| Special Senses System | | | | |
| Ear | | | (1) | |
| Schwannoma benign | | | 1 (100%) | |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Genital System | | | | |
| Hematopoietic System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Urinary System | | | | |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Gallbladder | (48) | (45) | (50) | (48) |
| Intestine small, jejunum | (50) | (43) | (49) | (46) |
| Adenocarcinoma | 3 (6%) | 1 (2%) | | |
| Intestine small, ileum | (50) | (42) | (49) | (46) |
| Liver | (50) | (50) | (50) | (48) |
| Hemangiosarcoma | | 1 (2%) | | |
| Hepatocellular carcinoma | 6 (12%) | 9 (18%) | 12 (24%) | 4 (8%) |
| Hepatocellular carcinoma, multiple | 2 (4%) | 4 (8%) | 4 (8%) | 4 (8%) |
| Hepatocellular adenoma | 14 (28%) | 10 (20%) | 5 (10%) | 11 (23%) |
| Hepatocellular adenoma, multiple | 3 (6%) | 7 (14%) | 7 (14%) | 3 (6%) |
| Hepatocholangiocarcinoma | | | 1 (2%) | |
| Mesentery | (2) | (1) | (2) | (2) |
| Sarcoma | 1 (50%) | | | |
| Pancreas | (50) | (48) | (50) | (47) |
| Sarcoma, metastatic, mesentery | 1 (2%) | | | |
| Stomach, forestomach | (50) | (47) | (50) | (47) |
| Squamous cell papilloma | | | 1 (2%) | |
| Stomach, glandular | (50) | (47) | (50) | (47) |
| Carcinoid tumor NOS | | 1 (2%) | | |
| Sarcoma, metastatic, mesentery | 1 (2%) | | | |
| Cardiovascular System | | | | |
| None | | | | |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (49) | (50) | (49) |
| Sarcoma, metastatic, mesentery | 1 (2%) | | | |
| Adrenal medulla | (50) | (49) | (50) | (49) |
| Pheochromocytoma benign | | | 1 (2%) | |
| Islets, pancreatic | (50) | (49) | (50) | (48) |
| Adenoma | | 1 (2%) | | |
| Pituitary gland | (46) | (46) | (50) | (46) |
| Pars distalis, adenoma | | | | 1 (2%) |
| Pars intermedia, adenoma | | 1 (2%) | | |
| Thyroid gland | (50) | (49) | (50) | (49) |
| C-cell, carcinoma | 1 (2%) | | | |
| Follicular cell, adenoma | 1 (2%) | 1 (2%) | | |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Epididymis | (50) | (49) | (50) | (50) |
| Prostate | (50) | (49) | (50) | (50) |
| Seminal vesicle | (50) | (49) | (50) | (50) |
| Testes | (50) | (49) | (50) | (50) |
| Interstitial cell, adenoma | 1 (2%) | | | |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (49) | (50) | (50) |
| Hemangioma | | 1 (2%) | | |
| Lymph node | (4) | (1) | (4) | (4) |
| Lymph node, mandibular | (49) | (47) | (50) | (50) |
| Mast cell tumor NOS | | | 1 (2%) | |
| Lymph node, mesenteric | (50) | (49) | (50) | (47) |
| Spleen | (50) | (49) | (50) | (49) |
| Hemangiosarcoma | 4 (8%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Integumentary System | | | | |
| Skin | (50) | (49) | (50) | (50) |
| Hemangioma | | 1 (2%) | | |
| Hemangiosarcoma | 1 (2%) | | | |
| Subcutaneous tissue, mast cell tumor NOS | | | 1 (2%) | |
| Musculoskeletal System | | | | |
| Skeletal muscle | (1) | | (1) | |
| Hemangiosarcoma | 1 (100%) | | 1 (100%) | |
| Nervous System | | | | |
| None | | | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 3 (6%) | 10 (20%) | 6 (12%) | 6 (12%) |
| Alveolar/bronchiolar adenoma, multiple | 3 (6%) | 2 (4%) | 2 (4%) | 3 (6%) |
| Alveolar/bronchiolar carcinoma | 1 (2%) | 3 (6%) | 2 (4%) | 5 (10%) |
| Hepatocellular carcinoma, metastatic, liver | 4 (8%) | 2 (4%) | 6 (12%) | 4 (8%) |
| Nose | (50) | (49) | (49) | (50) |
| Nasolacrimal duct, mast cell tumor NOS | | | 1 (2%) | |
| Special Senses System | | | | |
| Harderian gland | (1) | (3) | (3) | (1) |
| Adenoma | 1 (100%) | 3 (100%) | 2 (67%) | 1 (100%) |
| Adenoma, multiple | | | 1 (33%) | |
| Urinary System | | | | |
| Kidney | (50) | (49) | (50) | (48) |
| Transitional epithelium, carcinoma | | 1 (2%) | | |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|--------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (50) | (50) | (50) | (50) |
| Lymphoma malignant lymphocytic | 2 (4%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Lymphoma malignant mixed | | | 1 (2%) | |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 2 | 5 | 6 | 1 |
| 2-Year study | 33 | 36 | 35 | 30 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 3 | 5 | 6 | 1 |
| 2-Year study | 48 | 59 | 52 | 41 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 2 | 3 | 6 | 1 |
| 2-Year study | 20 | 29 | 20 | 22 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 3 | 3 | 6 | 1 |
| 2-Year study | 26 | 37 | 25 | 25 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | | 2 | | |
| 2-Year study | 18 | 19 | 21 | 14 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | | 2 | | |
| 2-Year study | 22 | 21 | 24 | 16 |
| Total animals with metastatic neoplasms | | | | |
| 2-Year study | 5 | 2 | 6 | 4 |
| Total metastatic neoplasms | | | | |
| 2-Year study | 7 | 2 | 6 | 4 |
| Total animals with uncertain neoplasms | | | | |
| benign or malignant | | | | |
| 2-Year study | | 1 | 1 | |
| Total uncertain neoplasms | | | | |
| 2-Year study | | 1 | 3 | |

^a Number of animals examined microscopically at site and number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------------|-------------|-------------|-------------|
| Harderian Gland: Adenoma | | | | |
| Overall rate ^a | 1/50 (2%) | 3/50 (6%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted rate ^b | 2.3% | 7.6% | 6.6% | 2.3% |
| Terminal rate ^c | 0/39 (0%) | 1/36 (3%) | 0/39 (0%) | 1/44 (2%) |
| First incidence (days) | 720 | 681 | 638 | 729 (T) |
| Life table test ^d | P=0.473N | P=0.284 | P=0.303 | P=0.744N |
| Logistic regression test ^d | P=0.538 | P=0.298 | P=0.394 | P=0.761 |
| Cochran-Armitage test ^d | P=0.500N | | | |
| Fisher exact test ^a | | P=0.309 | P=0.309 | P=0.753N |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 17/50 (34%) | 17/50 (34%) | 12/50 (24%) | 14/48 (29%) |
| Adjusted rate | 36.4% | 41.4% | 29.1% | 31.1% |
| Terminal rate | 10/39 (26%) | 13/36 (36%) | 10/39 (26%) | 13/44 (30%) |
| First incidence (days) | 532 | 442 | 681 | 667 |
| Life table test | P=0.145N | P=0.496 | P=0.215N | P=0.250N |
| Logistic regression test | P=0.277N | P=0.557N | P=0.187N | P=0.384N |
| Cochran-Armitage test | P=0.276N | | | |
| Fisher exact test | | P=0.583N | P=0.189N | P=0.384N |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 8/50 (16%) | 13/50 (26%) | 16/50 (32%) | 8/48 (17%) |
| Adjusted rate | 19.1% | 29.6% | 35.7% | 17.4% |
| Terminal rate | 6/39 (15%) | 6/36 (17%) | 11/39 (28%) | 6/44 (14%) |
| First incidence (days) | 597 | 575 | 496 | 582 |
| Life table test | P=0.366N | P=0.145 | P=0.060 | P=0.531N |
| Logistic regression test | P=0.388 | P=0.203 | P=0.052 | P=0.421 |
| Cochran-Armitage test | P=0.513N | | | |
| Fisher exact test | | P=0.163 | P=0.050 | P=0.572 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 22/50 (44%) | 26/50 (52%) | 23/50 (46%) | 19/48 (40%) |
| Adjusted rate | 47.4% | 56.0% | 50.6% | 41.3% |
| Terminal rate | 15/39 (38%) | 16/36 (44%) | 17/39 (44%) | 17/44 (39%) |
| First incidence (days) | 532 | 442 | 496 | 582 |
| Life table test | P=0.121N | P=0.223 | P=0.491 | P=0.234N |
| Logistic regression test | P=0.453N | P=0.295 | P=0.566 | P=0.548 |
| Cochran-Armitage test | P=0.265N | | | |
| Fisher exact test | | P=0.274 | P=0.500 | P=0.406N |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 6/50 (12%) | 12/50 (24%) | 8/50 (16%) | 9/50 (18%) |
| Adjusted rate | 15.4% | 29.2% | 20.5% | 20.5% |
| Terminal rate | 6/39 (15%) | 8/36 (22%) | 8/39 (21%) | 9/44 (20%) |
| First incidence (days) | 729 (T) | 537 | 729 (T) | 729 (T) |
| Life table test | P=0.545 | P=0.077 | P=0.385 | P=0.378 |
| Logistic regression test | P=0.393 | P=0.091 | P=0.385 | P=0.378 |
| Cochran-Armitage test | P=0.413 | | | |
| Fisher exact test | | P=0.096 | P=0.387 | P=0.288 |

TABLE C3

Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|------------|-------------|----------------|-------------|
| Lung: Alveolar/bronchiolar Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 2.6% | 8.0% | 5.1% | 11.4% |
| Terminal rate | 1/39 (3%) | 2/36 (6%) | 2/39 (5%) | 5/44 (11%) |
| First incidence (days) | 729 (T) | 725 | 729 (T) | 729 (T) |
| Life table test | P=0.116 | P=0.279 | P=0.500 | P=0.133 |
| Logistic regression test | P=0.095 | P=0.279 | P=0.500 | P=0.133 |
| Cochran-Armitage test | P=0.078 | | | |
| Fisher exact test | | P=0.309 | P=0.500 | P=0.102 |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 7/50 (14%) | 14/50 (28%) | 10/50 (20%) | 13/50 (26%) |
| Adjusted rate | 17.9% | 34.2% | 25.6% | 29.5% |
| Terminal rate | 7/39 (18%) | 10/36 (28%) | 10/39 (26%) | 13/44 (30%) |
| First incidence (days) | 729 (T) | 537 | 729 (T) | 729 (T) |
| Life table test | P=0.303 | P=0.053 | P=0.293 | P=0.166 |
| Logistic regression test | P=0.165 | P=0.064 | P=0.293 | P=0.166 |
| Cochran-Armitage test | P=0.178 | | | |
| Fisher exact test | | P=0.070 | P=0.298 | P=0.105 |
| Small Intestine (Jejunum): Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 7.4% | 2.8% | 0.0% | 0.0% |
| Terminal rate | 2/39 (5%) | 1/36 (3%) | 0/39 (0%) | 0/44 (0%) |
| First incidence (days) | 728 | 729 (T) | - ^e | - |
| Life table test | P=0.039N | P=0.338N | P=0.126N | P=0.105N |
| Logistic regression test | P=0.042N | P=0.336N | P=0.131N | P=0.113N |
| Cochran-Armitage test | P=0.044N | | | |
| Fisher exact test | | P=0.309N | P=0.121N | P=0.121N |
| Spleen: Hemangiosarcoma | | | | |
| Overall rate | 4/50 (8%) | 1/49 (2%) | 1/50 (2%) | 1/49 (2%) |
| Adjusted rate | 10.3% | 2.8% | 2.4% | 2.3% |
| Terminal rate | 4/39 (10%) | 1/36 (3%) | 0/39 (0%) | 1/44 (2%) |
| First incidence (days) | 729 (T) | 729 (T) | 695 | 729 (T) |
| Life table test | P=0.113N | P=0.204N | P=0.185N | P=0.145N |
| Logistic regression test | P=0.134N | P=0.204N | P=0.185N | P=0.145N |
| Cochran-Armitage test | P=0.137N | | | |
| Fisher exact test | | P=0.187N | P=0.181N | P=0.187N |
| All Organs: Hemangiosarcoma | | | | |
| Overall rate | 6/50 (12%) | 2/50 (4%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 14.9% | 5.6% | 4.9% | 2.3% |
| Terminal rate | 5/39 (13%) | 2/36 (6%) | 1/39 (3%) | 1/44 (2%) |
| First incidence (days) | 728 | 729 (T) | 695 | 729 (T) |
| Life table test | P=0.032N | P=0.164N | P=0.142N | P=0.044N |
| Logistic regression test | P=0.040N | P=0.162N | P=0.143N | P=0.049N |
| Cochran-Armitage test | P=0.042N | | | |
| Fisher exact test | | P=0.134N | P=0.134N | P=0.056N |

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------------|-------------|-------------|-------------|
| All Organs: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 6/50 (12%) | 3/50 (6%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 14.9% | 7.8% | 4.9% | 2.3% |
| Terminal rate | 5/39 (13%) | 2/36 (6%) | 1/39 (3%) | 1/44 (2%) |
| First incidence (days) | 728 | 695 | 695 | 729 (T) |
| Life table test | P=0.028N | P=0.284N | P=0.142N | P=0.044N |
| Logistic regression test | P=0.035N | P=0.261N | P=0.143N | P=0.049N |
| Cochran-Armitage test | P=0.035N | | | |
| Fisher exact test | | P=0.243N | P=0.134N | P=0.056N |
| All Organs: Malignant Lymphoma (Lymphocytic or Mixed) | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 5.1% | 2.8% | 7.0% | 4.5% |
| Terminal rate | 2/39 (5%) | 1/36 (3%) | 2/39 (5%) | 2/44 (5%) |
| First incidence (days) | 729 (T) | 729 (T) | 451 | 729 (T) |
| Life table test | P=0.554 | P=0.528N | P=0.502 | P=0.651N |
| Logistic regression test | P=0.502 | P=0.528N | P=0.508 | P=0.651N |
| Cochran-Armitage test | P=0.500 | | | |
| Fisher exact test | | P=0.500N | P=0.500 | P=0.691N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 20/50 (40%) | 29/50 (58%) | 22/50 (44%) | 23/50 (46%) |
| Adjusted rate | 43.0% | 62.8% | 49.6% | 50.0% |
| Terminal rate | 13/39 (33%) | 19/36 (53%) | 17/39 (44%) | 21/44 (48%) |
| First incidence (days) | 532 | 442 | 451 | 188 |
| Life table test | P=0.351N | P=0.054 | P=0.421 | P=0.500 |
| Logistic regression test | P=0.496 | P=0.056 | P=0.427 | P=0.322 |
| Cochran-Armitage test | P=0.519 | | | |
| Fisher exact test | | P=0.055 | P=0.420 | P=0.343 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 18/50 (36%) | 20/50 (40%) | 22/50 (44%) | 14/50 (28%) |
| Adjusted rate | 40.6% | 44.0% | 48.2% | 30.4% |
| Terminal rate | 13/39 (33%) | 11/36 (31%) | 16/39 (41%) | 12/44 (27%) |
| First incidence (days) | 419 | 442 | 451 | 582 |
| Life table test | P=0.126N | P=0.340 | P=0.286 | P=0.181N |
| Logistic regression test | P=0.201N | P=0.429 | P=0.284 | P=0.286N |
| Cochran-Armitage test | P=0.200N | | | |
| Fisher exact test | | P=0.418 | P=0.270 | P=0.260N |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 33/50 (66%) | 36/50 (72%) | 36/50 (72%) | 31/50 (62%) |
| Adjusted rate | 68.6% | 73.5% | 74.8% | 65.9% |
| Terminal rate | 24/39 (62%) | 23/36 (64%) | 27/39 (69%) | 28/44 (64%) |
| First incidence (days) | 419 | 442 | 451 | 188 |
| Life table test | P=0.137N | P=0.248 | P=0.361 | P=0.235N |
| Logistic regression test | P=0.496N | P=0.337 | P=0.364 | P=0.464N |
| Cochran-Armitage test | P=0.304N | | | |
| Fisher exact test | | P=0.333 | P=0.333 | P=0.418N |

TABLE C3

Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

(T) Terminal sacrifice

- ^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and spleen; 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 exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test 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 an exposure group is indicated by N.
- ^e Not applicable; no neoplasms in animal group

TABLE C4
Historical Incidence of Lung Neoplasms in Untreated Male B6C3F₁ Mice^a

| Study | Incidence in Controls | | |
|--|---------------------------------|-----------------------------------|--|
| | Alveolar/bronchiolar Adenoma | Alveolar/bronchiolar Carcinoma | Alveolar/bronchiolar Adenoma or Carcinoma |
| Historical Incidence at Southern Research Institute | | | |
| Benzyl Acetate | 9/50 | 5/50 | 14/50 |
| C.I. Pigment Red 23 | 4/49 | 2/49 | 5/49 |
| C.I. Pigment Red 3 | 2/50 | 0/50 | 2/50 |
| Ethylene Glycol | 7/54 | 1/54 | 7/54 |
| Nitrofurantoin | 5/50 | 1/50 | 6/50 |
| <i>o</i> -Nitroanisole | 5/50 | 1/50 | 6/50 |
| Polysorbate 80 | 5/49 | 1/49 | 6/49 |
| Rhodamine 6G | 6/50 | 3/50 | 9/50 |
| Roxarsone | 5/50 | 6/50 | 11/50 |
| Total | 48/452 (10.6%) | 20/452 (4.4%) | 66/452 (14.6%) |
| Standard deviation | 3.8% | 4.1% | 7.1% |
| Range | 4%-18% | 2%-12% | 4%-28% |
| Overall Historical Incidence | | | |
| Total | 181/1,369 (13.2%) | 68/1,369 (5.0%) | 242/1,369 (17.7%) |
| Standard deviation | 5.8% | 4.0% | 7.3% |
| Range | 4%-26% | 0%-14% | 4%-30% |

^a Data as of 20 August 1992

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------------------------|----------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| <i>15-Month interim evaluation</i> | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Accidental deaths | | 1 | | 2 |
| Moribund | 9 | 4 | 10 | 2 |
| Natural deaths | 2 | 9 | 1 | 2 |
| Survivors | | | | |
| Terminal sacrifice | 39 | 36 | 39 | 44 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Basophilic focus | | 1 (10%) | | 1 (10%) |
| Inflammation, subacute | | | 1 (10%) | |
| Karyomegaly | | | | 1 (10%) |
| Mixed cell focus | 1 (10%) | | | 1 (10%) |
| Necrosis | | 1 (10%) | | |
| Vacuolization cytoplasmic | 2 (20%) | 2 (20%) | 2 (20%) | 1 (10%) |
| Mesentery | (1) | | | (2) |
| Fat, necrosis | 1 (100%) | | | 2 (100%) |
| Pancreas | (10) | (10) | (10) | (10) |
| Acinus, atrophy | | 1 (10%) | 1 (10%) | |
| Stomach, glandular | (10) | (10) | (10) | (10) |
| Mineralization | | | 1 (10%) | |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Hypertrophy | 1 (10%) | 1 (10%) | 1 (10%) | 2 (20%) |
| Pituitary gland | (10) | (9) | (10) | (10) |
| Pars distalis, hyperplasia | | 1 (11%) | | |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Follicle, degeneration | | | | 1 (10%) |
| Genital System | | | | |
| Preputial gland | (10) | (10) | (10) | (10) |
| Atrophy | | 1 (10%) | | |
| Inflammation, subacute | | | | 1 (10%) |
| Duct, cyst | 2 (20%) | 1 (10%) | 3 (30%) | 3 (30%) |

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

TABLE C5

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Hematopoietic System | | | | |
| Lymph node, mesenteric | (10) | (10) | (10) | (10) |
| Angiectasis | 1 (10%) | 1 (10%) | | |
| Spleen | (10) | (10) | (10) | (10) |
| Hematopoietic cell proliferation | 1 (10%) | 1 (10%) | 1 (10%) | |
| Hyperplasia, lymphoid | | 2 (20%) | | |
| Thymus | (10) | (9) | (10) | (10) |
| Cyst | | | | 1 (10%) |
| Integumentary System | | | | |
| Skin | (10) | (10) | (10) | (10) |
| Pinna, inflammation, subacute | | | 1 (10%) | |
| Subcutaneous tissue, hemorrhage | | | | 1 (10%) |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar epithelium, hyperplasia | | 1 (10%) | 1 (10%) | |
| Nose | (10) | (10) | (10) | (10) |
| Lumen, hemorrhage | | | | 1 (10%) |
| Special Senses System | | | | |
| Eye | (1) | | (2) | (1) |
| Retrolubar, hemorrhage | 1 (100%) | | 2 (100%) | 1 (100%) |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Fibrosis | | | 1 (10%) | |
| Hydronephrosis | | | 1 (10%) | |
| Inflammation, subacute | | | 1 (10%) | |
| Mineralization | 9 (90%) | 4 (40%) | 5 (50%) | 4 (40%) |
| Renal tubule, casts | | 1 (10%) | | |
| Renal tubule, pigmentation | | 1 (10%) | | |
| Renal tubule, regeneration | 7 (70%) | 6 (60%) | 7 (70%) | 3 (30%) |
| Systems Examined With No Lesions Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |

TABLE C5

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Gallbladder | (48) | (45) | (50) | (48) |
| Epithelium, hyperplasia | | | | 1 (2%) |
| Intestine small, jejunum | (50) | (43) | (49) | (46) |
| Peyer's patch, hyperplasia, lymphoid | | 1 (2%) | | |
| Liver | (50) | (50) | (50) | (48) |
| Basophilic focus | 1 (2%) | 3 (6%) | | 4 (8%) |
| Clear cell focus | 4 (8%) | 6 (12%) | 5 (10%) | 2 (4%) |
| Cytologic alterations | | | 1 (2%) | |
| Eosinophilic focus | 4 (8%) | 17 (34%) | 5 (10%) | 4 (8%) |
| Hematopoietic cell proliferation | 2 (4%) | | | |
| Inflammation, subacute | 1 (2%) | | 1 (2%) | |
| Mixed cell focus | 6 (12%) | 5 (10%) | 5 (10%) | 2 (4%) |
| Necrosis | 4 (8%) | | 1 (2%) | |
| Regeneration | 1 (2%) | | | |
| Vacuolization cytoplasmic | 2 (4%) | 1 (2%) | 4 (8%) | 3 (6%) |
| Hepatocyte, hypertrophy, focal | | | | 1 (2%) |
| Mesentery | (2) | (1) | (2) | (2) |
| Fat, inflammation, granulomatous | | | 1 (50%) | |
| Fat, necrosis | 1 (50%) | 1 (100%) | 1 (50%) | 2 (100%) |
| Pancreas | (50) | (48) | (50) | (47) |
| Edema | | | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | | | |
| Acinus, atrophy | 1 (2%) | | 1 (2%) | |
| Acinus, depletion secretory | | 1 (2%) | 1 (2%) | |
| Duct, cyst | 1 (2%) | 1 (2%) | 1 (2%) | |
| Salivary glands | (50) | (49) | (50) | (50) |
| Acinus, atrophy | | 1 (2%) | | |
| Duct, cyst | | 1 (2%) | | |
| Stomach, forestomach | (50) | (47) | (50) | (47) |
| Cyst | | | | 1 (2%) |
| Hyperplasia | 6 (12%) | 4 (9%) | | |
| Inflammation, subacute | 1 (2%) | 3 (6%) | | |
| Ulcer | 1 (2%) | | | |
| Stomach, glandular | (50) | (47) | (50) | (47) |
| Cyst | | 1 (2%) | 2 (4%) | 1 (2%) |
| Ulcer | 2 (4%) | | | |
| Tooth | (2) | | (2) | (2) |
| Dysplasia | | | 1 (50%) | 1 (50%) |
| Inflammation, chronic | | | 1 (50%) | 1 (50%) |
| Inflammation, subacute | 2 (100%) | | | 1 (50%) |
| Cardiovascular System | | | | |
| Heart | (50) | (49) | (50) | (50) |
| Cardiomyopathy | | 1 (2%) | | |
| Fibrosis | | | 1 (2%) | 1 (2%) |
| Inflammation, chronic | | | 1 (2%) | |
| Mineralization | | | | 1 (2%) |
| Artery, inflammation, subacute | | 1 (2%) | | |

TABLE C5

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|-----------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (49) | (50) | (49) |
| Accessory adrenal cortical nodule | 1 (2%) | 2 (4%) | 1 (2%) | |
| Hyperplasia | | 1 (2%) | | 1 (2%) |
| Hypertrophy | 14 (28%) | 17 (35%) | 16 (32%) | 15 (31%) |
| Spindle cell, hyperplasia | | | 1 (2%) | 1 (2%) |
| Islets, pancreatic | (50) | (49) | (50) | (48) |
| Hyperplasia | 1 (2%) | 3 (6%) | | |
| Pituitary gland | (46) | (46) | (50) | (46) |
| Pars distalis, cyst | 2 (4%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Thyroid gland | (50) | (49) | (50) | (49) |
| Follicle, cyst | 2 (4%) | 1 (2%) | | 3 (6%) |
| Follicular cell, hyperplasia | 15 (30%) | 12 (24%) | 8 (16%) | 9 (18%) |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Coagulating gland | (1) | (2) | | |
| Dilatation | 1 (100%) | 1 (50%) | | |
| Epididymis | (50) | (49) | (50) | (50) |
| Cyst, multiple | | | 1 (2%) | |
| Inflammation, granulomatous | | | 1 (2%) | 1 (2%) |
| Preputial gland | (50) | (49) | (50) | (50) |
| Abscess | | 2 (4%) | 1 (2%) | 2 (4%) |
| Atrophy | | | 1 (2%) | |
| Cyst | | | 1 (2%) | 1 (2%) |
| Infiltration cellular, subacute | | | | 1 (2%) |
| Inflammation, chronic | | | 1 (2%) | |
| Inflammation, subacute | 11 (22%) | 2 (4%) | 10 (20%) | 4 (8%) |
| Duct, cyst | 31 (62%) | 36 (73%) | 36 (72%) | 27 (54%) |
| Prostate | (50) | (49) | (50) | (50) |
| Atrophy | 1 (2%) | | | |
| Inflammation, subacute | | | 1 (2%) | |
| Polyarteritis | | | | 1 (2%) |
| Seminal vesicle | (50) | (49) | (50) | (50) |
| Atrophy | 1 (2%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Dilatation | 1 (2%) | 6 (12%) | 1 (2%) | |
| Inflammation, chronic | | | 1 (2%) | |
| Inflammation, subacute | | | 1 (2%) | |

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (49) | (50) | (50) |
| Congestion | | | | 1 (2%) |
| Hypercellularity | 2 (4%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Lymph node | (4) | (1) | (4) | (4) |
| Axillary, hyperplasia, lymphoid | | | 1 (25%) | 1 (25%) |
| Bronchial, hyperplasia, lymphoid | | | | 2 (50%) |
| Inguinal, angiectasis | 1 (25%) | | | |
| Inguinal, hyperplasia, lymphoid | 1 (25%) | | 1 (25%) | 1 (25%) |
| Mediastinal, hyperplasia, lymphoid | | | 1 (25%) | |
| Pancreatic, mineralization | 1 (25%) | | | |
| Renal, hyperplasia, lymphoid | | 1 (100%) | | |
| Lymph node, mandibular | (49) | (47) | (50) | (50) |
| Hyperplasia, lymphoid | 1 (2%) | | 1 (2%) | 3 (6%) |
| Infiltration cellular, mast cell | | | | 1 (2%) |
| Lymph node, mesenteric | (50) | (49) | (50) | (47) |
| Angiectasis | 17 (34%) | 20 (41%) | 20 (40%) | 17 (36%) |
| Atrophy | 1 (2%) | | | |
| Hematopoietic cell proliferation | | 1 (2%) | 2 (4%) | 1 (2%) |
| Hyperplasia, lymphoid | 2 (4%) | 2 (4%) | 2 (4%) | |
| Spleen | (50) | (49) | (50) | (49) |
| Atrophy | 1 (2%) | 2 (4%) | 3 (6%) | 5 (10%) |
| Hematopoietic cell proliferation | 11 (22%) | 8 (16%) | 10 (20%) | 6 (12%) |
| Hyperplasia, lymphoid | 1 (2%) | 1 (2%) | | 2 (4%) |
| Necrosis | | | 1 (2%) | |
| Thymus | (45) | (47) | (47) | (47) |
| Atrophy | 4 (9%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Cyst | 1 (2%) | 4 (9%) | 1 (2%) | |
| Necrosis | | | 1 (2%) | |
| Integumentary System | | | | |
| Skin | (50) | (49) | (50) | (50) |
| Ulcer | | 1 (2%) | | 1 (2%) |
| Dermis, inflammation, chronic | | 1 (2%) | | |
| Dermis, inflammation, subacute | | 1 (2%) | 1 (2%) | 1 (2%) |
| Subcutaneous tissue, abscess | 1 (2%) | | | |
| Subcutaneous tissue, inflammation, chronic | | | 1 (2%) | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Cranium, hyperostosis | | 1 (2%) | | |
| Femur, fibrous osteodystrophy | | | | 1 (2%) |
| Nervous System | | | | |
| None | | | | |

TABLE C5

Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | | | | 1 (2%) |
| Hemorrhage | | | 1 (2%) | |
| Infiltration cellular, histiocyte | | 1 (2%) | 1 (2%) | 3 (6%) |
| Inflammation, granulomatous | 1 (2%) | | | 3 (6%) |
| Inflammation, subacute | | | | 3 (6%) |
| Thrombosis | | | 1 (2%) | |
| Alveolar epithelium, hyperplasia | 2 (4%) | 7 (14%) | 7 (14%) | 8 (16%) |
| Bronchiole, hyperplasia | | | 1 (2%) | |
| Nose | (50) | (49) | (49) | (50) |
| Congestion | | 1 (2%) | | |
| Glands, inflammation, subacute | | | | 1 (2%) |
| Nasolacrimal duct, ectasia | | | 1 (2%) | |
| Nasolacrimal duct, inflammation, subacute | | | 1 (2%) | |
| Special Senses System | | | | |
| Eye | (2) | | | |
| Cataract | 1 (50%) | | | |
| Urinary System | | | | |
| Kidney | (50) | (49) | (50) | (48) |
| Fibrosis | 4 (8%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Glomerulosclerosis | 2 (4%) | 5 (10%) | 6 (12%) | 2 (4%) |
| Hemorrhage | | | | 1 (2%) |
| Infarct | 7 (14%) | 4 (8%) | 5 (10%) | 3 (6%) |
| Infiltration cellular, lymphocyte | 1 (2%) | | | |
| Inflammation, subacute | | | 1 (2%) | |
| Metaplasia, osseous | 1 (2%) | 1 (2%) | 1 (2%) | |
| Mineralization | 41 (82%) | 33 (67%) | 23 (46%) | 31 (65%) |
| Cortex, cyst | 11 (22%) | 6 (12%) | 5 (10%) | 3 (6%) |
| Renal tubule, casts | 7 (14%) | 7 (14%) | 7 (14%) | 3 (6%) |
| Renal tubule, regeneration | 43 (86%) | 37 (76%) | 35 (70%) | 32 (67%) |
| Urethra | | (1) | | |
| Bulbourethral gland, hemorrhage | | 1 (100%) | | |
| Urinary bladder | (50) | (50) | (50) | (48) |
| Dilatation | | | 1 (2%) | |

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE
IN THE 2-YEAR FEED STUDY
OF *p*-NITROBENZOIC ACID

| | | |
|-----------------|--|------------|
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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|---------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Accidental deaths | | | 1 | 2 |
| Moribund | 9 | 8 | 12 | 10 |
| Natural deaths | 3 | 5 | 4 | 8 |
| Survivors | | | | |
| Terminal sacrifice | 38 | 36 | 33 | 30 |
| Missing | | 1 | | |
| Animals examined microscopically | 60 | 59 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Hepatocellular carcinoma | 1 (10%) | | | |
| Hepatocellular adenoma | | 2 (20%) | 4 (40%) | 1 (10%) |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Hemangiosarcoma | | | 1 (10%) | |
| Lymph node | | | (2) | |
| Renal, hemangiosarcoma | | | 1 (50%) | |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| Endocrine System | | | | |
| General Body System | | | | |
| Genital System | | | | |
| Integumentary System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Respiratory System | | | | |
| Special Senses System | | | | |
| Urinary System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Gallbladder | (50) | (48) | (47) | (48) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Intestine small, duodenum | (49) | (46) | (47) | (46) |
| Polyp adenomatous | | | | 1 (2%) |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Intestine small, jejunum | (49) | (46) | (47) | (46) |
| Adenocarcinoma | | | 1 (2%) | |
| Intestine small, ileum | (49) | (46) | (47) | (45) |

TABLE D1

Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|---------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Liver | (50) | (49) | (50) | (50) |
| Hemangiosarcoma | 1 (2%) | | | |
| Hepatocellular carcinoma | 4 (8%) | 5 (10%) | 4 (8%) | 6 (12%) |
| Hepatocellular carcinoma, multiple | | | 1 (2%) | 1 (2%) |
| Hepatocellular adenoma | 8 (16%) | 9 (18%) | 11 (22%) | 4 (8%) |
| Hepatocellular adenoma, multiple | 3 (6%) | 4 (8%) | 2 (4%) | 1 (2%) |
| Histiocytic sarcoma | 1 (2%) | 2 (4%) | 3 (6%) | |
| Mesentery | (4) | (8) | (5) | (6) |
| Histiocytic sarcoma | | 1 (13%) | | |
| Osteosarcoma, metastatic, bone | | | | 1 (17%) |
| Sarcoma | | | | 1 (17%) |
| Pancreas | (50) | (47) | (49) | (49) |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Salivary glands | (50) | (49) | (50) | (50) |
| Stomach, forestomach | (50) | (49) | (49) | (49) |
| Hepatocellular carcinoma, metastatic, liver | | 1 (2%) | | |
| Squamous cell papilloma | 1 (2%) | 2 (4%) | 2 (4%) | 1 (2%) |
| Stomach, glandular | (49) | (49) | (49) | (49) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Tooth | | | (1) | |
| Histiocytic sarcoma | | | 1 (100%) | |
| Cardiovascular System | | | | |
| Heart | (50) | (49) | (50) | (50) |
| Hepatocellular carcinoma, metastatic, liver | | 1 (2%) | | |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (48) | (50) | (50) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Adrenal medulla | (49) | (48) | (50) | (50) |
| Pheochromocytoma benign | 1 (2%) | | | |
| Islets, pancreatic | (50) | (49) | (49) | (50) |
| Carcinoma | | | 1 (2%) | |
| Pituitary gland | (48) | (48) | (49) | (48) |
| Pars distalis, adenoma | 7 (15%) | 6 (13%) | 9 (18%) | 5 (10%) |
| Pars distalis, carcinoma | 1 (2%) | | | |
| Pars intermedia, adenoma | | 1 (2%) | | |
| Thyroid gland | (50) | (49) | (50) | (50) |
| Follicular cell, adenoma | | 1 (2%) | 2 (4%) | |
| Follicular cell, carcinoma | 1 (2%) | | | 1 (2%) |
| General Body System | | | | |
| None | | | | |

TABLE D1

Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Genital System | | | | |
| Ovary | (50) | (48) | (49) | (48) |
| Cystadenoma | 1 (2%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Histiocytic sarcoma | | 2 (4%) | 1 (2%) | |
| Luteoma | | 1 (2%) | | |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Uterus | (50) | (49) | (50) | (50) |
| Hemangiosarcoma | | | 1 (2%) | |
| Histiocytic sarcoma | 1 (2%) | 2 (4%) | 1 (2%) | |
| Leiomyoma | | | | 1 (2%) |
| Leiomyosarcoma | 1 (2%) | | | |
| Polyp stromal | | 1 (2%) | 2 (4%) | |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Sarcoma stromal | 1 (2%) | | | |
| Vagina | (1) | | (1) | |
| Squamous cell papilloma | 1 (100%) | | | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (49) | (48) | (50) |
| Hemangiosarcoma | 1 (2%) | 1 (2%) | 1 (2%) | |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | |
| Lymph node | (8) | (10) | (13) | (15) |
| Axillary, fibrosarcoma, metastatic, skin | | | 1 (8%) | |
| Iliac, histiocytic sarcoma | | 1 (10%) | 1 (8%) | |
| Inguinal, histiocytic sarcoma | | 1 (10%) | 1 (8%) | |
| Mediastinal, histiocytic sarcoma | | 1 (10%) | | |
| Pancreatic, histiocytic sarcoma | | | 1 (8%) | |
| Pancreatic, sarcoma, metastatic, mesentery | | | | 1 (7%) |
| Renal, histiocytic sarcoma | | 1 (10%) | 1 (8%) | |
| Lymph node, mandibular | (50) | (49) | (50) | (49) |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | |
| Lymph node, mesenteric | (50) | (49) | (48) | (48) |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | |
| Spleen | (50) | (49) | (49) | (50) |
| Hemangiosarcoma | 1 (2%) | 1 (2%) | 1 (2%) | |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Thymus | (50) | (48) | (48) | (46) |
| Fibrosarcoma, metastatic, skin | | | 1 (2%) | |
| Hepatocellular carcinoma, metastatic, liver | | 1 (2%) | | |
| Histiocytic sarcoma | | 1 (2%) | | |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Integumentary System | | | | |
| Mammary gland | (50) | (49) | (50) | (49) |
| Adenoma | 1 (2%) | | | 1 (2%) |
| Skin | (50) | (49) | (50) | (50) |
| Schwannoma NOS | | | 1 (2%) | |
| Subcutaneous tissue, basal cell carcinoma | | 1 (2%) | | |
| Subcutaneous tissue, fibrosarcoma | | 1 (2%) | 1 (2%) | |
| Subcutaneous tissue, hemangiosarcoma | | | 1 (2%) | |
| Subcutaneous tissue, histiocytic sarcoma | | | 1 (2%) | |
| Subcutaneous tissue, sarcoma | 1 (2%) | | 1 (2%) | 1 (2%) |
| Subcutaneous tissue, schwannoma malignant | 1 (2%) | | | |
| Subcutaneous tissue, thymoma malignant, metastatic, thymus | | 1 (2%) | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (49) | (49) | (50) |
| Femur, osteosarcoma | | | | 1 (2%) |
| Skeletal muscle | (1) | (1) | (2) | (2) |
| Fibrosarcoma | | | 2 (100%) | |
| Hemangiosarcoma | 1 (100%) | | | |
| Hepatocellular carcinoma, metastatic, liver | | 1 (100%) | | |
| Sarcoma, metastatic, mesentery | | | | 1 (50%) |
| Nervous System | | | | |
| Brain | (49) | (49) | (50) | (50) |
| Carcinoma, metastatic, pituitary gland | 1 (2%) | | | |
| Glioma NOS | | 1 (2%) | | |
| Meninges, histiocytic sarcoma | | 1 (2%) | 1 (2%) | |
| Olfactory lobe, histiocytic sarcoma | | 1 (2%) | | |
| Respiratory System | | | | |
| Lung | (50) | (49) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 3 (6%) | 5 (10%) | 3 (6%) | 8 (16%) |
| Alveolar/bronchiolar carcinoma | | 4 (8%) | 1 (2%) | |
| Alveolar/bronchiolar carcinoma, multiple | | 1 (2%) | | 1 (2%) |
| Fibrosarcoma, metastatic, skin | | | 1 (2%) | |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | 4 (8%) | 1 (2%) | |
| Osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Sarcoma, metastatic, mesentery | | | | 1 (2%) |
| Mediastinum, hemangiosarcoma, metastatic, spleen | 1 (2%) | | | |
| Nose | (50) | (49) | (48) | (50) |
| Glands, histiocytic sarcoma | | 1 (2%) | | |
| Special Senses System | | | | |
| Harderian gland | (3) | (2) | | |
| Adenoma | 3 (100%) | 1 (50%) | | |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|---------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Urinary System | | | | |
| Kidney | (50) | (49) | (50) | (50) |
| Histiocytic sarcoma | | 2 (4%) | 1 (2%) | |
| Osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Urinary bladder | (50) | (49) | (49) | (50) |
| Systemic Lesions | | | | |
| Multiple organs ^b | (50) | (49) | (50) | (50) |
| Histiocytic sarcoma | 1 (2%) | 2 (4%) | 3 (6%) | |
| Lymphoma malignant lymphocytic | 6 (12%) | 8 (16%) | 7 (14%) | 5 (10%) |
| Lymphoma malignant mixed | 3 (6%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 1 | 2 | 4 | 1 |
| 2-Year study | 36 | 38 | 35 | 32 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 1 | 2 | 6 | 1 |
| 2-Year study | 53 | 61 | 61 | 42 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | | 2 | 4 | 1 |
| 2-Year study | 22 | 22 | 23 | 20 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | | 2 | 4 | 1 |
| 2-Year study | 29 | 33 | 32 | 23 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | 1 | | 1 | |
| 2-Year study | 23 | 23 | 23 | 17 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | 1 | | 2 | |
| 2-Year study | 24 | 27 | 28 | 19 |
| Total animals with metastatic neoplasms | | | | |
| 2-Year study | 3 | 4 | 2 | 2 |
| Total metastatic neoplasms | | | | |
| 2-Year study | 3 | 9 | 4 | 12 |
| Total animals with uncertain neoplasms | | | | |
| benign or malignant | | | | |
| 2-Year study | | 1 | 1 | |
| Total uncertain neoplasms | | | | |
| 2-Year study | | 1 | 1 | |

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

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid: 0 ppm (continued)

| | | |
|--------------------------------|---|----------|
| Number of Days on Study | 7 | |
| | 3 | |
| | 5 | |
| Carcass ID Number | 2 | Total |
| | 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 8 8 8 8 8 8 | Tissues/ |
| | 0 1 2 3 4 5 6 7 8 0 1 2 4 5 6 7 9 0 1 2 3 4 5 8 9 | Tumors |
| Special Senses System | | |
| Eye | | 2 |
| Harderian gland | | 3 |
| Adenoma | | 3 |
| Urinary System | | |
| Kidney | + | 50 |
| Urinary bladder | + | 50 |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Histiocytic sarcoma | | 1 |
| Lymphoma malignant lymphocytic | X | 6 |
| Lymphoma malignant mixed | | 3 |
| | | |

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid: 1,250 ppm (continued)

| | |
|---|---|
| Number of Days on Study | 4 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 |
| | 0 2 5 9 9 2 5 7 8 8 8 0 0 3 3 3 3 3 3 3 3 3 3 |
| | 0 6 0 4 8 0 7 8 1 5 5 1 1 1 1 1 1 1 1 1 1 1 1 |
| Carcass ID Number | 3 |
| | 2 0 1 0 3 0 5 3 3 2 3 2 4 0 0 0 0 0 1 1 1 1 1 |
| | 0 6 7 8 7 2 0 2 3 5 5 3 3 1 3 4 5 9 0 1 2 3 4 5 |
| Nervous System | |
| Brain | + |
| Glioma NOS | X |
| Meninges, histiocytic sarcoma | X |
| Olfactory lobe, histiocytic sarcoma | X |
| Respiratory System | |
| Lung | + |
| Alveolar/bronchiolar adenoma | X X X |
| Alveolar/bronchiolar carcinoma | X X |
| Alveolar/bronchiolar carcinoma, multiple | X |
| Hepatocellular carcinoma, metastatic, liver | X |
| Nose | + |
| Glands, histiocytic sarcoma | X |
| Trachea | + |
| Special Senses System | |
| Ear | |
| Eye | + |
| Harderian gland | + |
| Adenoma | |
| Urinary System | |
| Kidney | + |
| Histiocytic sarcoma | X X |
| Urinary bladder | + |
| Systemic Lesions | |
| Multiple organs | + |
| Histiocytic sarcoma | X X |
| Lymphoma malignant lymphocytic | X X X |
| Lymphoma malignant mixed | |

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid: 2,500 ppm

| | |
|------------------------------------|---|
| Number of Days on Study | 0 4 4 5 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 |
| | 0 8 9 6 8 2 2 3 5 7 8 8 0 0 1 2 2 3 3 3 3 3 3 3 3 |
| | 7 0 0 9 9 4 9 8 1 8 0 9 7 7 5 0 0 1 1 1 1 1 1 1 1 |
| Carcass ID Number | 4 3 4 3 3 3 3 3 3 3 3 4 3 3 3 3 3 3 3 3 3 3 3 3 |
| | 0 6 0 7 6 9 7 8 7 9 9 0 6 9 8 7 9 6 6 6 6 6 6 7 7 |
| | 4 2 7 0 5 2 8 2 2 4 5 2 4 3 4 5 8 1 3 6 7 8 9 1 3 |
| Alimentary System | |
| Esophagus | + |
| Gallbladder | A A + A + |
| Intestine large, colon | A + + A + |
| Intestine large, rectum | A + + A + |
| Intestine large, cecum | A A + A + |
| Intestine small, duodenum | A A + A + |
| Intestine small, jejunum | A A + A + |
| Adenocarcinoma | |
| Intestine small, ileum | A A + A + |
| Liver | + |
| Hepatocellular carcinoma | |
| Hepatocellular carcinoma, multiple | |
| Hepatocellular adenoma | X X X X X |
| Hepatocellular adenoma, multiple | |
| Histiocytic sarcoma | X X |
| Mesentery | + + |
| Pancreas | A + |
| Histiocytic sarcoma | X |
| Salivary glands | + |
| Stomach, forestomach | A + |
| Squamous cell papilloma | |
| Stomach, glandular | A + |
| Tooth | + + |
| Histiocytic sarcoma | X |
| Cardiovascular System | |
| Heart | + |
| Endocrine System | |
| Adrenal cortex | + |
| Adrenal medulla | + |
| Islets, pancreatic | A + |
| Carcinoma | X |
| Parathyroid gland | M + |
| Pituitary gland | M + |
| Pars distalis, adenoma | X X X X |
| Thyroid gland | + |
| Follicular cell, adenoma | X X |
| General Body System | |
| None | |

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid: 5,000 ppm

| Number of Days on Study | 0 | 0 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | | | |
|------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--|--|--|
| Carcass ID Number | 0 | 0 | 5 | 7 | 9 | 1 | 6 | 7 | 8 | 1 | 3 | 3 | 3 | 4 | 6 | 7 | 0 | 0 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | | | |
| | 7 | 7 | 7 | 1 | 0 | 9 | 5 | 0 | 4 | 6 | 0 | 6 | 7 | 6 | 5 | 8 | 0 | 9 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Gallbladder | M | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Intestine large, colon | A | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Intestine large, rectum | A | + | + | + | + | + | A | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Intestine large, cecum | A | + | + | + | + | + | A | + | A | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Intestine small, duodenum | A | + | + | + | + | + | A | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | | | |
| Polyp adenomatous | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Sarcoma, metastatic, mesentery | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Intestine small, jejunum | A | + | + | + | + | + | A | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | | | |
| Intestine small, ileum | A | + | + | + | + | + | A | + | A | + | + | + | A | + | + | A | + | + | + | + | + | + | + | + | + | + | | | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Hepatocellular carcinoma, multiple | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Hepatocellular adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mesentery | | | | + | | | | + | | | | + | | | | + | | | | | | | | | | | | | |
| Osteosarcoma, metastatic, bone | | | | X | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sarcoma | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Pancreas | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Sarcoma, metastatic, mesentery | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Stomach, forestomach | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Stomach, glandular | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Sarcoma, metastatic, mesentery | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | | | |
| Pars distalis, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Follicular cell, carcinoma | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clitoral gland | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | | | |
| Cystadenoma | | | | | | | | | | | | | | | | | | X | | | | | | | | | | | |
| Sarcoma, metastatic, mesentery | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | |
| Leiomyoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sarcoma, metastatic, mesentery | | | | | | | | | | | | | | | | | X | | | | | | | | | | | | |

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------------|-------------|----------------|-------------|
| Harderian Gland: Adenoma | | | | |
| Overall rate ^a | 3/50 (6%) | 1/49 (2%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate ^b | 7.2% | 2.8% | 0.0% | 0.0% |
| Terminal rate ^c | 0/38 (0%) | 1/36 (3%) | 0/33 (0%) | 0/30 (0%) |
| First incidence (days) | 639 | 730 (T) | - ^e | - |
| Life table test ^d | P=0.061N | P=0.334N | P=0.149N | P=0.171N |
| Logistic regression test ^d | P=0.047N | P=0.312N | P=0.121N | P=0.128N |
| Cochran-Armitage test ^d | P=0.044N | | | |
| Fisher exact test ^d | | P=0.316N | P=0.121N | P=0.121N |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 11/50 (22%) | 13/49 (27%) | 13/50 (26%) | 5/50 (10%) |
| Adjusted rate | 27.2% | 31.9% | 34.0% | 15.6% |
| Terminal rate | 9/38 (24%) | 9/36 (25%) | 9/33 (27%) | 4/30 (13%) |
| First incidence (days) | 589 | 620 | 480 | 637 |
| Life table test | P=0.162N | P=0.359 | P=0.290 | P=0.192N |
| Logistic regression test | P=0.080N | P=0.382 | P=0.390 | P=0.131N |
| Cochran-Armitage test | P=0.055N | | | |
| Fisher exact test | | P=0.385 | P=0.408 | P=0.086N |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 5/49 (10%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted rate | 9.8% | 13.0% | 15.2% | 21.0% |
| Terminal rate | 3/38 (8%) | 3/36 (8%) | 5/33 (15%) | 5/30 (17%) |
| First incidence (days) | 533 | 685 | 730 (T) | 616 |
| Life table test | P=0.112 | P=0.466 | P=0.420 | P=0.163 |
| Logistic regression test | P=0.164 | P=0.487 | P=0.489 | P=0.232 |
| Cochran-Armitage test | P=0.210 | | | |
| Fisher exact test | | P=0.487 | P=0.500 | P=0.262 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 15/50 (30%) | 16/49 (33%) | 15/50 (30%) | 11/50 (22%) |
| Adjusted rate | 36.1% | 38.6% | 39.5% | 32.3% |
| Terminal rate | 12/38 (32%) | 11/36 (31%) | 11/33 (33%) | 8/30 (27%) |
| First incidence (days) | 533 | 620 | 480 | 616 |
| Life table test | P=0.418N | P=0.436 | P=0.433 | P=0.483N |
| Logistic regression test | P=0.240N | P=0.473 | P=0.570 | P=0.326N |
| Cochran-Armitage test | P=0.171N | | | |
| Fisher exact test | | P=0.473 | P=0.586N | P=0.247N |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 3/50 (6%) | 5/49 (10%) | 3/50 (6%) | 8/50 (16%) |
| Adjusted rate | 7.5% | 12.9% | 8.7% | 24.3% |
| Terminal rate | 2/38 (5%) | 3/36 (8%) | 2/33 (6%) | 6/30 (20%) |
| First incidence (days) | 689 | 685 | 715 | 570 |
| Life table test | P=0.035 | P=0.324 | P=0.599 | P=0.050 |
| Logistic regression test | P=0.052 | P=0.343 | P=0.643 | P=0.071 |
| Cochran-Armitage test | P=0.079 | | | |
| Fisher exact test | | P=0.346 | P=0.661N | P=0.100 |

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|------------|-------------|------------|------------|
| Lung: Alveolar/bronchiolar Carcinoma | | | | |
| Overall rate | 0/50 (0%) | 5/49 (10%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 13.9% | 2.9% | 3.3% |
| Terminal rate | 0/38 (0%) | 5/36 (14%) | 0/33 (0%) | 1/30 (3%) |
| First incidence (days) | — | 730 (T) | 720 | 730 (T) |
| Life table test | P=0.572N | P=0.029 | P=0.468 | P=0.453 |
| Logistic regression test | P=0.568N | P=0.029 | P=0.491 | P=0.453 |
| Cochran-Armitage test | P=0.471N | | | |
| Fisher exact test | | P=0.027 | P=0.500 | P=0.500 |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 10/49 (20%) | 4/50 (8%) | 9/50 (18%) |
| Adjusted rate | 7.5% | 26.1% | 11.3% | 27.5% |
| Terminal rate | 2/38 (5%) | 8/36 (22%) | 2/33 (6%) | 7/30 (23%) |
| First incidence (days) | 689 | 685 | 715 | 570 |
| Life table test | P=0.063 | P=0.031 | P=0.428 | P=0.027 |
| Logistic regression test | P=0.088 | P=0.031 | P=0.475 | P=0.039 |
| Cochran-Armitage test | P=0.147 | | | |
| Fisher exact test | | P=0.033 | P=0.500 | P=0.061 |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 7/48 (15%) | 6/48 (13%) | 9/49 (18%) | 5/48 (10%) |
| Adjusted rate | 18.3% | 16.7% | 24.1% | 17.9% |
| Terminal rate | 6/37 (16%) | 6/36 (17%) | 6/33 (18%) | 5/28 (18%) |
| First incidence (days) | 728 | 730 (T) | 589 | 730 (T) |
| Life table test | P=0.517 | P=0.526N | P=0.309 | P=0.583N |
| Logistic regression test | P=0.533N | P=0.564N | P=0.388 | P=0.615N |
| Cochran-Armitage test | P=0.374N | | | |
| Fisher exact test | | P=0.500N | P=0.410 | P=0.379N |
| Pituitary Gland (Pars Distalis): Adenoma or Carcinoma | | | | |
| Overall rate | 8/48 (17%) | 6/48 (13%) | 9/49 (18%) | 5/48 (10%) |
| Adjusted rate | 20.2% | 16.7% | 24.1% | 17.9% |
| Terminal rate | 6/37 (16%) | 6/36 (17%) | 6/33 (18%) | 5/28 (18%) |
| First incidence (days) | 689 | 730 (T) | 589 | 730 (T) |
| Life table test | P=0.504N | P=0.418N | P=0.406 | P=0.474N |
| Logistic regression test | P=0.429N | P=0.427N | P=0.501 | P=0.452N |
| Cochran-Armitage test | P=0.288N | | | |
| Fisher exact test | | P=0.387N | P=0.519 | P=0.276N |
| All Organs: Hemangiosarcoma | | | | |
| Overall rate | 4/50 (8%) | 1/49 (2%) | 2/50 (4%) | 0/50 (0%) |
| Adjusted rate | 10.1% | 2.5% | 5.6% | 0.0% |
| Terminal rate | 3/38 (8%) | 0/36 (0%) | 1/33 (3%) | 0/30 (0%) |
| First incidence (days) | 693 | 685 | 707 | — |
| Life table test | P=0.078N | P=0.204N | P=0.397N | P=0.098N |
| Logistic regression test | P=0.062N | P=0.186N | P=0.356N | P=0.082N |
| Cochran-Armitage test | P=0.052N | | | |
| Fisher exact test | | P=0.187N | P=0.339N | P=0.059N |

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------------|-------------|-------------|-------------|
| All Organs: Histiocytic Sarcoma | | | | |
| Overall rate | 1/50 (2%) | 2/49 (4%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted rate | 2.6% | 4.2% | 7.5% | 0.0% |
| Terminal rate | 1/38 (3%) | 0/36 (0%) | 1/33 (3%) | 0/30 (0%) |
| First incidence (days) | 730 (T) | 526 | 624 | — |
| Life table test | P=0.388N | P=0.496 | P=0.281 | P=0.547N |
| Logistic regression test | P=0.275N | P=0.444 | P=0.306 | P=0.547N |
| Cochran-Armitage test | P=0.335N | | | |
| Fisher exact test | | P=0.492 | P=0.309 | P=0.500N |
| All Organs: Malignant Lymphoma (Lymphocytic or Mixed) | | | | |
| Overall rate | 9/50 (18%) | 11/49 (22%) | 9/50 (18%) | 7/50 (14%) |
| Adjusted rate | 21.5% | 30.6% | 24.0% | 20.6% |
| Terminal rate | 6/38 (16%) | 11/36 (31%) | 6/33 (18%) | 3/30 (10%) |
| First incidence (days) | 423 | 730 (T) | 569 | 700 |
| Life table test | P=0.473N | P=0.347 | P=0.487 | P=0.586N |
| Logistic regression test | P=0.344N | P=0.378 | P=0.598 | P=0.458N |
| Cochran-Armitage test | P=0.262N | | | |
| Fisher exact test | | P=0.382 | P=0.602N | P=0.393N |
| All Organs: Malignant Lymphoma or Histiocytic Sarcoma | | | | |
| Overall rate | 10/50 (20%) | 13/49 (27%) | 11/50 (22%) | 7/50 (14%) |
| Adjusted rate | 24.0% | 33.4% | 27.5% | 20.6% |
| Terminal rate | 7/38 (18%) | 11/36 (31%) | 6/33 (18%) | 3/30 (10%) |
| First incidence (days) | 423 | 526 | 569 | 700 |
| Life table test | P=0.367N | P=0.274 | P=0.390 | P=0.495N |
| Logistic regression test | P=0.210N | P=0.297 | P=0.499 | P=0.363N |
| Cochran-Armitage test | P=0.175N | | | |
| Fisher exact test | | P=0.298 | P=0.500 | P=0.298N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 22/50 (44%) | 22/49 (45%) | 23/50 (46%) | 21/50 (42%) |
| Adjusted rate | 49.8% | 53.3% | 56.6% | 59.5% |
| Terminal rate | 16/38 (42%) | 17/36 (47%) | 16/33 (48%) | 16/30 (53%) |
| First incidence (days) | 589 | 620 | 480 | 570 |
| Life table test | P=0.244 | P=0.486 | P=0.314 | P=0.299 |
| Logistic regression test | P=0.452 | P=0.543 | P=0.471 | P=0.481 |
| Cochran-Armitage test | P=0.449N | | | |
| Fisher exact test | | P=0.545 | P=0.500 | P=0.500N |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 23/50 (46%) | 24/49 (49%) | 23/50 (46%) | 18/50 (36%) |
| Adjusted rate | 51.9% | 56.7% | 51.9% | 45.6% |
| Terminal rate | 17/38 (45%) | 18/36 (50%) | 12/33 (36%) | 9/30 (30%) |
| First incidence (days) | 423 | 526 | 569 | 471 |
| Life table test | P=0.472N | P=0.407 | P=0.387 | P=0.527N |
| Logistic regression test | P=0.193N | P=0.463 | P=0.560 | P=0.275N |
| Cochran-Armitage test | P=0.138N | | | |
| Fisher exact test | | P=0.462 | P=0.579N | P=0.208N |

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------------|-------------|-------------|-------------|
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 36/50 (72%) | 38/49 (78%) | 35/50 (70%) | 33/50 (66%) |
| Adjusted rate | 75.0% | 82.5% | 72.8% | 78.5% |
| Terminal rate | 26/38 (68%) | 28/36 (78%) | 20/33 (61%) | 21/30 (70%) |
| First incidence (days) | 423 | 526 | 480 | 471 |
| Life table test | P=0.294 | P=0.311 | P=0.376 | P=0.287 |
| Logistic regression test | P=0.211N | P=0.347 | P=0.390N | P=0.507N |
| Cochran-Armitage test | P=0.198N | | | |
| Fisher exact test | | P=0.343 | P=0.500N | P=0.333N |

(T) Terminal sacrifice

- ^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and pituitary gland; 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 exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test 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 an exposure group is indicated by N.
- ^e Not applicable; no neoplasms in animal group

TABLE D4
Historical Incidence of Lung Neoplasms in Untreated Female B6C3F₁ Mice^a

| Study | Incidence in Controls | | |
|--|---------------------------------|-----------------------------------|--|
| | Alveolar/bronchiolar Adenoma | Alveolar/bronchiolar Carcinoma | Alveolar/bronchiolar Adenoma or Carcinoma |
| Historical Incidence at Southern Research Institute | | | |
| Benzyl Acetate | 1/50 | 0/50 | 1/50 |
| C.I. Pigment Red 23 | 1/50 | 0/50 | 1/50 |
| C.I. Pigment Red 3 | 3/50 | 1/50 | 4/50 |
| Ethylene Glycol | 0/50 | 1/50 | 1/50 |
| Nitrofurantoin | 2/50 | 1/50 | 3/50 |
| <i>o</i> -Nitroanisole | 4/50 | 2/50 | 6/50 |
| Polysorbate 80 | 3/50 | 0/50 | 3/50 |
| Rhodamine 6G | 3/50 | 1/50 | 4/50 |
| Roxarsone | 1/50 | 2/50 | 3/50 |
| Total | 18/450 (4.0%) | 8/450 (1.8%) | 26/450 (5.8%) |
| Standard deviation | 2.7% | 1.6% | 3.4% |
| Range | 0%-8% | 0%-4% | 2%-12% |
| Overall Historical Incidence | | | |
| Total | 78/1,371 (5.7%) | 30/1,371 (2.2%) | 106/1,371 (7.7%) |
| Standard deviation | 4.9% | 2.3% | 5.0% |
| Range | 0%-24% | 0%-8% | 2%-26% |

^a Data as of 20 August 1992

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------------------------|----------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | | | | |
| Early deaths | | | | |
| Accidental deaths | | | 1 | 2 |
| Moribund | 9 | 8 | 12 | 10 |
| Natural deaths | 3 | 5 | 4 | 8 |
| Survivors | | | | |
| Terminal sacrifice | 38 | 36 | 33 | 30 |
| Missing | | 1 | | |
| Animals examined microscopically | 60 | 59 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Angiectasis | | | | 1 (10%) |
| Basophilic focus | | | | 1 (10%) |
| Eosinophilic focus | | 1 (10%) | | |
| Inflammation, subacute | 1 (10%) | | | 2 (20%) |
| Karyomegaly | | | 1 (10%) | |
| Necrosis | 1 (10%) | | | |
| Vacuolization cytoplasmic | 1 (10%) | | | |
| Bile duct, cyst | | | 1 (10%) | |
| Mesentery | (1) | | (1) | (1) |
| Cyst | 1 (100%) | | | |
| Fat, hemorrhage | 1 (100%) | | | |
| Fat, inflammation, suppurative | | | 1 (100%) | |
| Fat, necrosis | | | 1 (100%) | 1 (100%) |
| Pancreas | (10) | (10) | (10) | (10) |
| Acinus, atrophy | 1 (10%) | | | |
| Stomach, forestomach | (10) | (10) | (10) | (10) |
| Hyperplasia | | 1 (10%) | | |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Hyperplasia | | | 1 (10%) | |
| X-zone, vacuolization cytoplasmic | | 4 (40%) | 1 (10%) | 1 (10%) |
| Pituitary gland | (10) | (10) | (9) | (10) |
| Pars distalis, hyperplasia | 1 (10%) | | | 2 (20%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Follicle, cyst | 1 (10%) | | | |

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

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|---------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Genital System | | | | |
| Ovary | (10) | (10) | (10) | (10) |
| Angiectasis | 2 (20%) | | | |
| Cyst | | 4 (40%) | 3 (30%) | 1 (10%) |
| Follicle, hemorrhage | | | 1 (10%) | |
| Uterus | (10) | (10) | (10) | (10) |
| Dilatation | | 1 (10%) | 2 (20%) | 2 (20%) |
| Hyperplasia, cystic | 8 (80%) | 10 (100%) | 9 (90%) | 9 (90%) |
| Hematopoietic System | | | | |
| Lymph node | | | (2) | |
| Iliac, hyperplasia, lymphoid | | | 1 (50%) | |
| Lymph node, mesenteric | (9) | (10) | (10) | (10) |
| Necrosis | | | | 1 (10%) |
| Spleen | (10) | (10) | (10) | (10) |
| Hematopoietic cell proliferation | | | 1 (10%) | |
| Hemorrhage | | | 1 (10%) | |
| Hyperplasia, lymphoid | 1 (10%) | | | |
| Integumentary System | | | | |
| Skin | (10) | (10) | (10) | (10) |
| Inflammation, suppurative | | | 1 (10%) | |
| Dermis, inflammation, subacute | 1 (10%) | | | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Infiltration cellular, lymphocyte | | | 1 (10%) | 1 (10%) |
| Mineralization | 2 (20%) | 1 (10%) | 1 (10%) | |
| Renal tubule, casts | | 1 (10%) | 1 (10%) | |
| Renal tubule, regeneration | 4 (40%) | | 3 (30%) | 1 (10%) |
| Systems Examined With No Lesions Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Respiratory System | | | | |
| Special Senses System | | | | |

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|---------|-----------|-----------|-----------|
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Gallbladder | (50) | (48) | (47) | (48) |
| Dilatation | | | | 1 (2%) |
| Inflammation, subacute | | 1 (2%) | | |
| Intestine small, jejunum | (49) | (46) | (47) | (46) |
| Peyer's patch, hyperplasia, lymphoid | | | 1 (2%) | |
| Peyer's patch, necrosis | | | | 1 (2%) |
| Liver | (50) | (49) | (50) | (50) |
| Angiectasis | 1 (2%) | 1 (2%) | | |
| Basophilic focus | 1 (2%) | 5 (10%) | 1 (2%) | 1 (2%) |
| Clear cell focus | | | | 1 (2%) |
| Developmental malformation | | 1 (2%) | | |
| Eosinophilic focus | 4 (8%) | 5 (10%) | 6 (12%) | 2 (4%) |
| Fatty change, focal | | 1 (2%) | | |
| Fibrosis | | | 1 (2%) | |
| Hematopoietic cell proliferation | 1 (2%) | 3 (6%) | 1 (2%) | 2 (4%) |
| Infiltration cellular, lymphocyte | | 1 (2%) | | |
| Inflammation, subacute | 2 (4%) | | | 2 (4%) |
| Mineralization | | 1 (2%) | 1 (2%) | |
| Mixed cell focus | 3 (6%) | 3 (6%) | 1 (2%) | |
| Necrosis | 1 (2%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Thrombosis | | 1 (2%) | | |
| Vacuolization cytoplasmic | 2 (4%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Bile duct, hyperplasia | | | | 1 (2%) |
| Hepatocyte, hypertrophy, diffuse | | | 1 (2%) | |
| Mesentery | (4) | (8) | (5) | (6) |
| Inflammation, subacute | | 2 (25%) | 1 (20%) | 1 (17%) |
| Pigmentation, hemosiderin | 1 (25%) | | | |
| Polyarteritis | | 1 (13%) | | |
| Fat, hemorrhage | | | | 1 (17%) |
| Fat, inflammation, granulomatous | 1 (25%) | | | 1 (17%) |
| Fat, inflammation, subacute | | | | 1 (17%) |
| Fat, necrosis | 3 (75%) | 4 (50%) | 3 (60%) | 1 (17%) |
| Pancreas | (50) | (47) | (49) | (49) |
| Basophilic focus | | | | 1 (2%) |
| Congestion | | 1 (2%) | | |
| Edema | 1 (2%) | | 1 (2%) | 2 (4%) |
| Inflammation, suppurative | | 1 (2%) | | |
| Acinus, atrophy | | | 1 (2%) | 3 (6%) |
| Acinus, depletion secretory | 1 (2%) | 2 (4%) | 3 (6%) | 3 (6%) |
| Duct, cyst | 2 (4%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Duct, degeneration | | 1 (2%) | | |
| Duct, inflammation, chronic | | 1 (2%) | | |
| Salivary glands | (50) | (49) | (50) | (50) |
| Cytoplasmic alteration | | | | 1 (2%) |
| Stomach, forestomach | (50) | (49) | (49) | (49) |
| Diverticulum | 1 (2%) | | | |
| Hyperplasia | 7 (14%) | 4 (8%) | 4 (8%) | 1 (2%) |
| Inflammation, subacute | 2 (4%) | | 3 (6%) | 1 (2%) |
| Polyarteritis | | 1 (2%) | | |
| Ulcer | | 1 (2%) | | |

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Stomach, glandular | (49) | (49) | (49) | (49) |
| Cyst | 1 (2%) | 3 (6%) | 4 (8%) | 1 (2%) |
| Erosion | | 2 (4%) | 1 (2%) | 1 (2%) |
| Hyperplasia | | 1 (2%) | | |
| Inflammation, chronic | | | 1 (2%) | |
| Polyarteritis | | 1 (2%) | | |
| Cardiovascular System | | | | |
| Heart | (50) | (49) | (50) | (50) |
| Hemorrhage | | | | 1 (2%) |
| Infiltration cellular, histiocyte | 1 (2%) | | | 1 (2%) |
| Inflammation, subacute | 1 (2%) | | | |
| Coronary artery, amyloid deposition | | | | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (48) | (50) | (50) |
| Accessory adrenal cortical nodule | 1 (2%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Hematopoietic cell proliferation | 1 (2%) | | | |
| Hyperplasia | | | | 1 (2%) |
| Hypertrophy | 3 (6%) | 2 (4%) | 1 (2%) | |
| Vacuolization cytoplasmic | | | 1 (2%) | |
| Spindle cell, hyperplasia | | 1 (2%) | | |
| X-zone, vacuolization cytoplasmic | 2 (4%) | 1 (2%) | | 1 (2%) |
| Adrenal medulla | (49) | (48) | (50) | (50) |
| Hyperplasia | 2 (4%) | | | 1 (2%) |
| Islets, pancreatic | (50) | (49) | (49) | (50) |
| Hyperplasia | | 1 (2%) | | |
| Pituitary gland | (48) | (48) | (49) | (48) |
| Pars distalis, angiectasis | | 1 (2%) | 1 (2%) | 1 (2%) |
| Pars distalis, cyst | | 1 (2%) | 1 (2%) | |
| Pars distalis, hyperplasia | 10 (21%) | 10 (21%) | 14 (29%) | 9 (19%) |
| Pars intermedia, hyperplasia | 1 (2%) | | | |
| Thyroid gland | (50) | (49) | (50) | (50) |
| C-cell, hyperplasia | | | 1 (2%) | |
| Follicle, cyst | | 1 (2%) | 3 (6%) | 2 (4%) |
| Follicle, degeneration | | | | 1 (2%) |
| Follicular cell, hyperplasia | 17 (34%) | 14 (29%) | 24 (48%) | 8 (16%) |
| General Body System | | | | |
| None | | | | |

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|-------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Genital System | | | | |
| Clitoral gland | (50) | (49) | (50) | (49) |
| Inflammation, subacute | | | | 1 (2%) |
| Duct, cyst | 3 (6%) | 9 (18%) | 11 (22%) | 11 (22%) |
| Ovary | (50) | (48) | (49) | (48) |
| Abscess | 2 (4%) | 2 (4%) | | 6 (13%) |
| Atrophy | | | 1 (2%) | |
| Cyst | 17 (34%) | 9 (19%) | 15 (31%) | 14 (29%) |
| Hemorrhage | | | | 5 (10%) |
| Inflammation, subacute | | | | 1 (2%) |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | | |
| Pigmentation, hemosiderin | 1 (2%) | | | |
| Oviduct | | | (1) | |
| Cyst | | | 1 (100%) | |
| Uterus | (50) | (49) | (50) | (50) |
| Angiectasis | | 1 (2%) | | 1 (2%) |
| Atrophy | | | 1 (2%) | 2 (4%) |
| Cyst | | 1 (2%) | 2 (4%) | |
| Dilatation | 7 (14%) | 10 (20%) | 6 (12%) | 4 (8%) |
| Hemorrhage | 3 (6%) | 1 (2%) | | |
| Hyperplasia, cystic | 43 (86%) | 45 (92%) | 48 (96%) | 37 (74%) |
| Inflammation, subacute | | | | 1 (2%) |
| Thrombosis | | 1 (2%) | | |
| Vagina | (1) | | (1) | |
| Inflammation, granulomatous | | | 1 (100%) | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (49) | (48) | (50) |
| Atrophy | | | 1 (2%) | |
| Hypercellularity | 5 (10%) | 1 (2%) | 1 (2%) | 4 (8%) |
| Myeloid cell, depletion cellular | | | | 1 (2%) |
| Lymph node | (8) | (10) | (13) | (15) |
| Axillary, hyperplasia, lymphoid | | 1 (10%) | | |
| Iliac, hyperplasia, lymphoid | 2 (25%) | | 2 (15%) | 3 (20%) |
| Inguinal, hyperplasia, lymphoid | 1 (13%) | | | 3 (20%) |
| Mediastinal, edema | | | | 1 (7%) |
| Mediastinal, hyperplasia, lymphoid | 3 (38%) | 2 (20%) | 1 (8%) | 1 (7%) |
| Mediastinal, inflammation, subacute | | | | 2 (13%) |
| Pancreatic, hyperplasia, lymphoid | | | 1 (8%) | |
| Renal, hyperplasia | 1 (13%) | | | |
| Renal, hyperplasia, lymphoid | 4 (50%) | 2 (20%) | | 3 (20%) |
| Renal, inflammation, subacute | | | | 1 (7%) |
| Lymph node, mandibular | (50) | (49) | (50) | (49) |
| Angiectasis | | 2 (4%) | | |
| Cyst | | 1 (2%) | | |
| Hyperplasia, lymphoid | 1 (2%) | 1 (2%) | | 2 (4%) |
| Infiltration cellular, mast cell | 1 (2%) | | 1 (2%) | |

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of p-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System (continued) | | | | |
| Lymph node, mesenteric | (50) | (49) | (48) | (48) |
| Angiectasis | 3 (6%) | 4 (8%) | 5 (10%) | 5 (10%) |
| Cyst | 1 (2%) | | | |
| Hematopoietic cell proliferation | 1 (2%) | | | |
| Hyperplasia, lymphoid | 2 (4%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Spleen | (50) | (49) | (49) | (50) |
| Atrophy | | | | 2 (4%) |
| Congestion | 1 (2%) | | | |
| Hematopoietic cell proliferation | 12 (24%) | 18 (37%) | 14 (29%) | 16 (32%) |
| Hyperplasia, lymphoid | 12 (24%) | 12 (24%) | 9 (18%) | 13 (26%) |
| Necrosis | | 1 (2%) | | |
| Pigmentation, hemosiderin | 1 (2%) | | 1 (2%) | 1 (2%) |
| Thymus | (50) | (48) | (48) | (46) |
| Atrophy | 1 (2%) | | 1 (2%) | 4 (9%) |
| Integumentary System | | | | |
| Mammary gland | (50) | (49) | (50) | (49) |
| Hyperplasia, lobular | 1 (2%) | | | |
| Duct, cyst | | | 1 (2%) | 2 (4%) |
| Skin | (50) | (49) | (50) | (50) |
| Parakeratosis | | 1 (2%) | | |
| Dermis, inflammation, subacute | 1 (2%) | 3 (6%) | 2 (4%) | 3 (6%) |
| Dermis, subcutaneous tissue, inflammation, subacute | | 1 (2%) | | |
| Epidermis, necrosis | | 1 (2%) | | |
| Subcutaneous tissue, edema | | 1 (2%) | | |
| Subcutaneous tissue, hemorrhage | | | 1 (2%) | |
| Subcutaneous tissue, inflammation, subacute | | 1 (2%) | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (49) | (49) | (50) |
| Osteopetrosis | | | 1 (2%) | |
| Cranium, fibrous osteodystrophy | | 3 (6%) | | 1 (2%) |
| Femur, fibrous osteodystrophy | 2 (4%) | 2 (4%) | 3 (6%) | 5 (10%) |
| Nervous System | | | | |
| Brain | (49) | (49) | (50) | (50) |
| Compression | 1 (2%) | | 4 (8%) | |
| Cyst | 1 (2%) | | | |
| Vacuolization cytoplasmic | | 1 (2%) | | |
| Meninges, infiltration cellular | | | 1 (2%) | |

TABLE D5

Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid
(continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|-------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Respiratory System | | | | |
| Lung | (50) | (49) | (50) | (50) |
| Congestion | | | 1 (2%) | |
| Edema | | | | 1 (2%) |
| Hemorrhage | | 1 (2%) | | 1 (2%) |
| Infiltration cellular, lymphocyte | | 1 (2%) | | |
| Infiltration cellular, histiocyte | 2 (4%) | 2 (4%) | | |
| Inflammation, subacute | | | | 1 (2%) |
| Alveolar epithelium, hyperplasia | 3 (6%) | 3 (6%) | | 1 (2%) |
| Mediastinum, inflammation, subacute | | 1 (2%) | | |
| Nose | (50) | (49) | (48) | (50) |
| Glands, cyst | 1 (2%) | | | 1 (2%) |
| Lumen, fungus | | 1 (2%) | | |
| Special Senses System | | | | |
| Eye | (2) | (1) | | |
| Atrophy | | 1 (100%) | | |
| Cataract | 1 (50%) | | | |
| Inflammation, subacute | 1 (50%) | | | |
| Cornea, inflammation, subacute | 1 (50%) | | | |
| Harderian gland | (3) | (2) | | |
| Inflammation, chronic | | 1 (50%) | | |
| Urinary System | | | | |
| Kidney | (50) | (49) | (50) | (50) |
| Fibrosis | | 1 (2%) | | |
| Glomerulosclerosis | 4 (8%) | 3 (6%) | | 1 (2%) |
| Infarct | 2 (4%) | | 1 (2%) | |
| Infiltration cellular, lymphocyte | 3 (6%) | 1 (2%) | 3 (6%) | 1 (2%) |
| Inflammation, subacute | 1 (2%) | | | 1 (2%) |
| Metaplasia, osseous | | | 2 (4%) | 2 (4%) |
| Mineralization | 15 (30%) | 7 (14%) | 7 (14%) | 5 (10%) |
| Polyarteritis | | 1 (2%) | | |
| Capsule, fibrosis | | | | 1 (2%) |
| Glomerulus, amyloid deposition | | | | 2 (4%) |
| Renal tubule, casts | 4 (8%) | 2 (4%) | 1 (2%) | 4 (8%) |
| Renal tubule, degeneration | 1 (2%) | | | |
| Renal tubule, dilatation | 1 (2%) | | 1 (2%) | 4 (8%) |
| Renal tubule, hyperplasia | 1 (2%) | | | |
| Renal tubule, pigmentation | | | | 1 (2%) |
| Renal tubule, regeneration | 18 (36%) | 14 (29%) | 12 (24%) | 9 (18%) |
| Urinary bladder | (50) | (49) | (49) | (50) |
| Dilatation | | 1 (2%) | 1 (2%) | |

APPENDIX E

GENETIC TOXICOLOGY

| | |
|--|-----|
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GENETIC TOXICOLOGY

***SALMONELLA TYPHIMURIUM* MUTAGENICITY TEST PROTOCOL**

Testing was performed as reported by Zeiger *et al.* (1987). *p*-Nitrobenzoic acid 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, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of *p*-nitrobenzoic acid. The high dose was limited to 3,333 µg/plate. All positive trials were repeated under the conditions that elicited the positive response.

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

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1985) and Zeiger *et al.* (1987). *p*-Nitrobenzoic acid was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of *p*-nitrobenzoic acid; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with *p*-nitrobenzoic acid in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing *p*-nitrobenzoic acid was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for approximately 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with *p*-nitrobenzoic acid, serum-free medium, and S9 for approximately 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no *p*-nitrobenzoic acid, 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. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant chemical-induced cell cycle delay was seen in the absence of S9 at doses of 498 µg/mL and above, incubation time was lengthened for these cultures to ensure a sufficient number of scorable (second-division metaphase) cells.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was

chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with *p*-nitrobenzoic acid for 18.5 to 19.5 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*-nitrobenzoic acid and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test: because cell cycle delay was anticipated in the absence of S9, 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. Generally, 200 first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay can be found in MacGregor *et al.* (1990). Peripheral blood samples were obtained from male and female B6C3F₁ mice at the end of the 13-week study. Smears were immediately prepared and fixed in absolute methanol, stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983), and coded. Slides were scanned to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in each of 10 animals per dose group. The criteria of Schmid (1976) were used to define micronuclei, with the additional requirement that the micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm and orange with 540 nm ultraviolet illumination); the minimum size limit was approximately one-twentieth the diameter of the NCE cell.

The frequency of micronucleated cells among NCEs was analyzed by a statistical software package (ILS, 1990) which employed a one-tailed trend test across dose groups and a *t*-test for pairwise comparisons of each dose group to the concurrent control.

RESULTS

p-Nitrobenzoic acid, tested in a preincubation protocol at concentrations of 1 to 3,333 $\mu\text{g}/\text{plate}$, with and without induced rat or hamster S9, was mutagenic in strain TA100 (Table E1; Zeiger *et al.*, 1987). No mutagenicity was detected in strains TA1535, TA1537, or TA98, with or without S9.

In cytogenetic tests with cultured CHO cells, *p*-nitrobenzoic acid induced significant increases in SCEs (Table E2; Zeiger *et al.*, 1987) and Abs (Table E3; Zeiger *et al.*, 1987) at dose levels which induced cell cycle delay in the absence of S9; no increases in either endpoint were observed in the presence of S9. In the SCE test without S9, doses ranging from 498 to 1,000 $\mu\text{g}/\text{mL}$ produced positive responses, induced cell cycle delay, and required use of an extended harvest protocol to allow accumulation of sufficient cells for metaphase analysis. Doses producing positive responses in the Abs assay without S9 ranged from 875 to 1,750 $\mu\text{g}/\text{mL}$ *p*-nitrobenzoic acid. As with the SCE test, cell harvest was delayed to permit a sufficient number of cells to progress to metaphase for analysis.

Despite the positive results obtained in the *in vitro* studies, results of a single NTP *in vivo* genotoxicity study were negative. In this study, the frequencies of micronucleated normochromatic erythrocytes in the peripheral blood of male and female mice were found to be unaffected by administration of *p*-nitrobenzoic acid in feed for 13 weeks (Table E4).

TABLE E1
Mutagenicity of *p*-Nitrobenzoic Acid in *Salmonella typhimurium*^a

| Strain | Dose ($\mu\text{g}/\text{plate}$) | Revertants/plate ^b | | | | | |
|-------------------------------|--|-------------------------------|-----------------|------------------|-------------------|------------------|----------|
| | | -S9 | | +10% hamster S9 | | | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 3 | |
| TA100 | 0.0 | 98 \pm 5.8 | 106 \pm 13.9 | 144 \pm 11.5 | 140 \pm 7.8 | 146 \pm 14.4 | |
| | 1.0 | | | | | | |
| | 3.3 | | 95 \pm 15.6 | | | | |
| | 10.0 | 83 \pm 4.4 | 121 \pm 9.6 | | 159 \pm 11.0 | 86 \pm 5.8 | |
| | 33.0 | 126 \pm 6.1 | 118 \pm 6.7 | 214 \pm 17.2 | 173 \pm 7.6 | 109 \pm 12.0 | |
| | 100.0 | 169 \pm 12.8 | 146 \pm 7.8 | 241 \pm 13.8 | 228 \pm 5.9 | 151 \pm 16.2 | |
| | 333.0 | Toxic | 283 \pm 19.3 | 329 \pm 7.4 | 321 \pm 17.0 | 244 \pm 5.6 | |
| | 1,000.0 | Toxic | | Toxic | 542 \pm 29.7 | 373 \pm 65.2 | |
| | 3,333.0 | | 5 \pm 2.9 | | | | |
| | Trial summary | | Weakly Positive | Positive | Positive | Positive | Positive |
| Positive control ^c | | 1,417 \pm 47.1 | 765 \pm 76.9 | 1,987 \pm 60.5 | 1,688 \pm 111.4 | 1,314 \pm 35.7 | |

| Strain | Dose ($\mu\text{g}/\text{plate}$) | Revertants/plate | | |
|----------------------|--|------------------|------------------|-------------------|
| | | +10% rat S9 | | |
| | | Trial 1 | Trial 2 | Trial 3 |
| TA100 (continued) | 0.0 | 130 \pm 10.5 | 163 \pm 5.2 | 133 \pm 10.2 |
| | 1.0 | | | |
| | 3.3 | | | |
| | 10.0 | | 168 \pm 3.7 | 116 \pm 8.2 |
| | 33.0 | 164 \pm 12.1 | 182 \pm 9.2 | 126 \pm 1.2 |
| | 100.0 | 232 \pm 17.1 | 224 \pm 9.0 | 169 \pm 5.8 |
| | 333.0 | 274 \pm 9.5 | 351 \pm 15.6 | 227 \pm 2.7 |
| | 1,000.0 | Toxic | 501 \pm 19.4 | 401 \pm 26.5 |
| | 3,333.0 | 17 \pm 7.2 | | |
| | Trial summary | | Positive | Positive |
| Positive control | | 1,675 \pm 99.1 | 2,329 \pm 49.4 | 2,086 \pm 106.3 |

TABLE E1
Mutagenicity of *p*-Nitrobenzoic Acid in *Salmonella typhimurium* (continued)

| Strain | Dose ($\mu\text{g}/\text{plate}$) | Revertants/plate | | | | | |
|------------------|--|------------------|----------------|------------------|------------------|-------------------|------------------|
| | | -S9 | | +10% hamster S9 | | +10% rat S9 | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| TA1535 | 0.0 | 11 \pm 1.2 | | 16 \pm 0.9 | 8 \pm 0.7 | 11 \pm 0.7 | |
| | 1.0 | | | | | | |
| | 3.3 | | | | 6 \pm 1.3 | | |
| | 10.0 | 5 \pm 1.3 | | | 5 \pm 1.5 | | |
| | 33.0 | 7 \pm 0.7 | | 14 \pm 1.2 | 7 \pm 0.9 | 16 \pm 1.2 | |
| | 100.0 | 9 \pm 0.7 | | 17 \pm 1.5 | 8 \pm 1.2 | 15 \pm 0.9 | |
| | 333.0 | 7 \pm 1.8 | | 3 \pm 0.7 | 7 \pm 1.2 | 4 \pm 0.9 | |
| | 1,000.0 | 5 \pm 1.5 | | 1 \pm 1.0 | | 2 \pm 1.5 | |
| | 3,333.0 | | | 0 \pm 0.0 | | 0 \pm 0.0 | |
| | Trial summary | | Negative | | Negative | Negative | Negative |
| Positive control | | 319 \pm 66.5 | | 145 \pm 9.7 | 148 \pm 12.8 | 115 \pm 13.2 | |
| TA1537 | 0.0 | 8 \pm 1.2 | 6 \pm 0.9 | 18 \pm 0.9 | | 14 \pm 0.7 | |
| | 1.0 | | 4 \pm 1.5 | | | | |
| | 3.3 | | 5 \pm 0.9 | | | | |
| | 10.0 | 10 \pm 0.7 | 5 \pm 2.7 | | | | |
| | 33.0 | 5 \pm 1.7 | 5 \pm 0.6 | 14 \pm 0.6 | | 14 \pm 3.0 | |
| | 100.0 | 8 \pm 0.3 | 6 \pm 0.9 | 17 \pm 2.3 | | 16 \pm 1.5 | |
| | 333.0 | 0 \pm 0.3 | | 15 \pm 0.3 | | 10 \pm 3.9 | |
| | 1,000.0 | 1 \pm 0.6 | | 10 \pm 0.9 | | 3 \pm 1.7 | |
| | 3,333.0 | | | 4 \pm 0.7 | | 1 \pm 0.3 | |
| | Trial summary | | Negative | Negative | Negative | | Negative |
| Positive control | | 228 \pm 35.3 | 166 \pm 75.8 | 335 \pm 42.9 | | 323 \pm 44.3 | |
| TA98 | 0.0 | 21 \pm 1.2 | | 28 \pm 1.2 | 17 \pm 4.7 | 16 \pm 2.0 | 14 \pm 0.3 |
| | 1.0 | | | | | | |
| | 3.3 | | | | 22 \pm 0.9 | | 18 \pm 2.6 |
| | 10.0 | 25 \pm 4.0 | | | 18 \pm 2.0 | | 25 \pm 5.2 |
| | 33.0 | 22 \pm 2.9 | | 30 \pm 1.5 | 20 \pm 2.2 | 24 \pm 2.9 | 24 \pm 3.1 |
| | 100.0 | 22 \pm 0.3 | | 25 \pm 5.8 | 20 \pm 4.0 | 20 \pm 6.5 | 14 \pm 0.3 |
| | 333.0 | 21 \pm 0.9 | | 20 \pm 3.3 | 18 \pm 3.6 | 8 \pm 2.0 | 19 \pm 3.5 |
| | 1,000.0 | 16 \pm 0.3 | | 19 \pm 3.8 | | 10 \pm 2.9 | |
| | 3,333.0 | | | 18 \pm 1.8 | | 17 \pm 3.0 | |
| | Trial summary | | Negative | | Negative | Negative | Negative |
| Positive control | | 535 \pm 50.4 | | 1,484 \pm 80.3 | 1,323 \pm 73.5 | 1,837 \pm 135.5 | 1,793 \pm 49.9 |

^a Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Zeiger *et al.* (1987).

^b Revertants are presented as mean \pm 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.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells
by *p*-Nitrobenzoic Acid^a

| Compound | Dose (µg/mL) | Total Cells | No. of Chromosomes | No. of SCEs | SCEs/Chromosome | SCEs/Cell | Hrs in BrdU | Relative SCEs/Chromosome (%) ^b |
|-----------------------------|--------------|-------------|--------------------|-------------|----------------------|-----------|-------------------|---|
| -S9 | | | | | | | | |
| Trial 1 | | | | | | | | |
| Summary: Weak positive | | | | | | | | |
| Dimethylsulfoxide | | 50 | 1,045 | 398 | 0.38 | 8.0 | 25.7 | |
| Mitomycin-C | 0.001 | 50 | 1,050 | 584 | 0.55 | 11.7 | 25.7 | 46.04 |
| | 0.010 | 5 | 105 | 245 | 2.33 | 49.0 | 25.7 | 512.65 |
| <i>p</i> -Nitrobenzoic acid | 58.3 | 50 | 1,031 | 382 | 0.37 | 7.6 | 25.7 | -2.72 |
| | 175.0 | 50 | 1,041 | 429 | 0.41 | 8.6 | 25.7 | 8.20 |
| | 583.0 | 50 | 1,033 | 570 | 0.55 | 11.4 | 31.3 ^c | 44.88* |
| | 1,750.0 | 0 | | | | | 31.3 | |
| | | | | | P<0.001 ^d | | | |
| Trial 2 | | | | | | | | |
| Summary: Positive | | | | | | | | |
| Dimethylsulfoxide | | 50 | 1,044 | 380 | 0.36 | 7.6 | 25.5 | |
| Mitomycin-C | 0.001 | 50 | 1,042 | 580 | 0.55 | 11.6 | 25.5 | 52.93 |
| | 0.010 | 5 | 105 | 251 | 2.39 | 50.2 | 25.5 | 556.76 |
| <i>p</i> -Nitrobenzoic acid | 498 | 50 | 1,034 | 505 | 0.48 | 10.1 | 32.4 ^c | 34.18* |
| | 753 | 50 | 1,045 | 604 | 0.57 | 12.1 | 32.4 ^c | 58.80* |
| | 1,000 | 50 | 1,049 | 629 | 0.59 | 12.6 | 32.4 ^c | 64.74* |
| | 1,510 | 0 | | | | | | |
| | | | | | P<0.001 | | | |

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells
by *p*-Nitrobenzoic Acid (continued)

| Compound | Dose ($\mu\text{g/mL}$) | Total Cells | No. of Chromo- somes | No. of SCEs | SCEs/ Chromo- some | SCEs/ Cell | Hrs in BrdU | Relative SCEs/ Chromosome (%) |
|-----------------------------|------------------------------|----------------|----------------------------|----------------|--------------------------|---------------|----------------|-------------------------------------|
| +S9 | | | | | | | | |
| Summary: Negative | | | | | | | | |
| Dimethylsulfoxide | | 50 | 1,041 | 346 | 0.33 | 6.9 | 25.5 | |
| Cyclophosphamide | 0.4 | 25 | 519 | 312 | 0.60 | 12.5 | 25.5 | 80.87 |
| | 2.0 | 5 | 105 | 157 | 1.49 | 31.4 | 25.5 | 349.87 |
| <i>p</i> -Nitrobenzoic acid | | | | | | | | |
| | 175 | 50 | 1,044 | 393 | 0.37 | 7.9 | 25.5 | 13.26 |
| | 583 | 50 | 1,037 | 328 | 0.31 | 6.6 | 25.5 | -4.84 |
| | 1,750 | 50 | 1,027 | 364 | 0.35 | 7.3 | 25.5 | 6.64 |
| P=0.485 | | | | | | | | |

* Positive ($P \leq 0.01$)

^a Study performed at Litton Bionetics, Inc. The protocol is presented in detail by Galloway *et al.* (1985); data published in Zeiger *et al.* (1987). SCE = sister chromatid exchange; BrdU = bromodeoxyuridine.

^b SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells.

^c Because of chemical-induced cell cycle delay, incubation time was lengthened to ensure sufficient metaphase cells at harvest.

^d Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *p*-Nitrobenzoic Acid^a

| -S9 | | | | | +S9 | | | | |
|--|----------------|---------------|--------------|--------------------------|---|----------------|---------------|--------------|--------------------------|
| Dose ($\mu\text{g/mL}$) | Total Cells | No. of Abs | Abs/ Cell | Cells with Abs (%) | Dose ($\mu\text{g/mL}$) | Total Cells | No. of Abs | Abs/ Cell | Cells with Abs (%) |
| Trial 1 - Harvest time: 20.5 hours^b Summary: Positive | | | | | Trial 1 - Harvest time: 12.5 hours Summary: Equivocal | | | | |
| Dimethylsulfoxide | | | | | Dimethylsulfoxide | | | | |
| | 200 | 8 | 0.04 | 3.0 | | 200 | 10 | 0.05 | 3.5 |
| Mitomycin-C | | | | | Cyclophosphamide | | | | |
| 0.05 | 50 | 22 | 0.44 | 30.0 | 7.5 | 200 | 30 | 0.15 | 12.0 |
| 0.08 | 25 | 37 | 1.48 | 56.0 | 37.5 | 25 | 38 | 1.52 | 84.0 |
| <i>p</i> -Nitrobenzoic acid | | | | | <i>p</i> -Nitrobenzoic acid | | | | |
| 875 | 200 | 21 | 0.11 | 8.5* | 875 | 200 | 13 | 0.07 | 5.5 |
| 1,313 | 200 | 30 | 0.15 | 12.5* | 1,313 | 200 | 14 | 0.07 | 5.5 |
| 1,750 | 50 | 20 | 0.40 | 26.0* | 1,750 | 200 | 24 | 0.12 | 9.0* |
| P < 0.001 ^c | | | | | P = 0.015 | | | | |
| Trial 2 - Harvest time: 21.5 hours^b Summary: Positive | | | | | Trial 2 - Harvest time: 12.5 hours Summary: Negative | | | | |
| Dimethylsulfoxide | | | | | Dimethylsulfoxide | | | | |
| | 200 | 1 | 0.01 | 0.5 | | 200 | 1 | 0.01 | 0.5 |
| Mitomycin-C | | | | | Cyclophosphamide | | | | |
| 0.05 | 200 | 48 | 0.24 | 15.0 | 7.5 | 200 | 16 | 0.08 | 7.0 |
| 0.08 | 25 | 28 | 1.12 | 48.0 | 37.5 | 25 | 12 | 0.48 | 36.0 |
| <i>p</i> -Nitrobenzoic acid | | | | | <i>p</i> -Nitrobenzoic acid | | | | |
| 439.5 | 200 | 3 | 0.02 | 1.5 | 1,249 | 200 | 2 | 0.01 | 1.0 |
| 879.0 | 200 | 7 | 0.04 | 3.5* | 1,505 | 200 | 10 | 0.05 | 4.5* |
| 1,313.0 | 200 | 17 | 0.09 | 7.5* | 1,750 | 200 | 3 | 0.02 | 1.5 |
| P < 0.001 | | | | | P = 0.052 | | | | |

* Positive ($P \leq 0.05$)

^a Study performed at Litton Bionetics, Inc. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985); data published in Zeiger *et al.* (1987). Abs = aberrations.

^b Because of chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to ensure sufficient metaphase cells at harvest.

^c Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

TABLE E4
Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes Following Administration of *p*-Nitrobenzoic Acid in Feed for 13 Weeks^a

| Dose (ppm) | Micronucleated Normochromatic Erythrocytes (%) ^b | Pairwise Significance |
|---------------|---|-----------------------|
| Male | | |
| 0 | 0.1412 ± 0.0132 | |
| 1,250 | 0.1466 ± 0.0119 | 0.365 |
| 2,500 | 0.1313 ± 0.0096 | 0.750 |
| 5,000 | 0.1600 ± 0.0111 | 0.114 |
| 10,000 | 0.1250 ± 0.0119 | 0.869 |
| 20,000 | 0.1492 ± 0.0092 | 0.300 |
| | | P=0.424 |
| Female | | |
| 0 | 0.1031 ± 0.0127 | |
| 1,250 | 0.0992 ± 0.0062 | 0.590 |
| 2,500 | 0.0997 ± 0.0086 | 0.583 |
| 5,000 | 0.1146 ± 0.0078 | 0.242 |
| 10,000 | 0.1453 ± 0.0238 | 0.009 |
| 20,000 | 0.1032 ± 0.0058 | 0.497 |
| | | P=0.215 |

^a Ten thousand normochromatic erythrocytes scored per animal. A detailed description of the protocol is found in MacGregor *et al.* (1990).

^b Data presented as mean ± standard error. Pairwise comparison of treated group to concurrent control by Student's *t*-test. One-tailed trend test performed across all doses (ILS, 1990).

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

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TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|------------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|
| Male | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Necropsy body wt | 210 ± 4 | 205 ± 6 | 198 ± 3 | 173 ± 4** | 127 ± 5** | 110 ± 6** |
| Brain | | | | | | |
| Absolute | 1.866 ± 0.013 | 1.840 ± 0.041 | 1.847 ± 0.015 | 1.785 ± 0.017* | 1.727 ± 0.013** | 1.674 ± 0.032** |
| Relative | 8.89 ± 0.14 | 8.99 ± 0.12 | 9.34 ± 0.12 | 10.32 ± 0.16** | 13.68 ± 0.47** | 15.33 ± 0.58** |
| Heart | | | | | | |
| Absolute | 0.856 ± 0.019 | 0.859 ± 0.061 | 0.843 ± 0.024 | 0.777 ± 0.030 | 0.649 ± 0.022** | 0.550 ± 0.041** |
| Relative | 4.08 ± 0.09 | 4.18 ± 0.22 | 4.26 ± 0.11 | 4.49 ± 0.15 | 5.13 ± 0.20** | 4.99 ± 0.25** |
| R. Kidney | | | | | | |
| Absolute | 1.005 ± 0.031 | 1.073 ± 0.068 | 1.093 ± 0.043 | 0.992 ± 0.045 | 0.722 ± 0.029** | 0.631 ± 0.032** |
| Relative | 4.79 ± 0.11 | 5.22 ± 0.19 | 5.52 ± 0.19** | 5.72 ± 0.13** | 5.69 ± 0.12** | 5.75 ± 0.17** |
| Liver | | | | | | |
| Absolute | 12.380 ± 0.156 | 12.272 ± 0.420 | 12.555 ± 0.502 | 11.831 ± 0.452 | 7.733 ± 0.313** | 6.145 ± 0.268** |
| Relative | 58.97 ± 0.62 | 59.86 ± 0.51 | 63.39 ± 1.93 | 68.25 ± 1.31** | 60.96 ± 1.09 | 56.11 ± 2.14 |
| Lungs | | | | | | |
| Absolute | 1.204 ± 0.041 | 1.321 ± 0.092 | 1.350 ± 0.059 | 1.325 ± 0.081 | 0.908 ± 0.033** | 0.881 ± 0.022** |
| Relative | 5.73 ± 0.15 | 6.43 ± 0.30 | 6.82 ± 0.30* | 7.65 ± 0.42** | 7.16 ± 0.07** | 8.08 ± 0.40** |
| Spleen | | | | | | |
| Absolute | 0.533 ± 0.017 | 0.556 ± 0.020 | 0.553 ± 0.018 | 0.640 ± 0.038* | 0.856 ± 0.051** | 0.779 ± 0.018** |
| Relative | 2.54 ± 0.10 | 2.71 ± 0.06 | 2.80 ± 0.09 | 3.69 ± 0.19** | 6.73 ± 0.25** | 7.14 ± 0.30** |
| R. Testis | | | | | | |
| Absolute | 1.208 ± 0.039 | 1.156 ± 0.050 | 1.147 ± 0.016 | 1.100 ± 0.027 | 0.866 ± 0.062** | 0.775 ± 0.063** |
| Relative | 5.76 ± 0.19 | 5.64 ± 0.17 | 5.80 ± 0.12 | 6.35 ± 0.08 | 6.79 ± 0.24** | 7.00 ± 0.30** |
| Thymus | | | | | | |
| Absolute | 0.450 ± 0.029 | 0.382 ± 0.022 | 0.402 ± 0.018 | 0.306 ± 0.016** | 0.158 ± 0.023** | 0.076 ± 0.008** |
| Relative | 2.15 ± 0.18 | 1.88 ± 0.16 | 2.03 ± 0.07 | 1.77 ± 0.09 | 1.26 ± 0.18** | 0.69 ± 0.07** |

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|------------------|---------------|---------------|---------------|-----------------|-----------------|-----------------|
| Female | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Necropsy body wt | 140 ± 3 | 139 ± 4 | 134 ± 3 | 123 ± 3** | 95 ± 4** | 91 ± 4** |
| Brain | | | | | | |
| Absolute | 1.750 ± 0.018 | 1.719 ± 0.016 | 1.878 ± 0.105 | 1.680 ± 0.012 | 1.633 ± 0.007 | 1.638 ± 0.026 |
| Relative | 12.48 ± 0.20 | 12.38 ± 0.42 | 14.07 ± 1.09 | 13.73 ± 0.33 | 17.29 ± 0.65** | 18.07 ± 0.62** |
| Heart | | | | | | |
| Absolute | 0.653 ± 0.019 | 0.623 ± 0.022 | 0.635 ± 0.017 | 0.625 ± 0.022 | 0.473 ± 0.027** | 0.477 ± 0.034** |
| Relative | 4.65 ± 0.12 | 4.48 ± 0.12 | 4.74 ± 0.10 | 5.10 ± 0.11 | 4.97 ± 0.15 | 5.21 ± 0.17** |
| R. Kidney | | | | | | |
| Absolute | 0.734 ± 0.010 | 0.704 ± 0.015 | 0.694 ± 0.026 | 0.654 ± 0.022* | 0.507 ± 0.022** | 0.510 ± 0.020** |
| Relative | 5.23 ± 0.08 | 5.06 ± 0.07 | 5.17 ± 0.18 | 5.33 ± 0.09 | 5.33 ± 0.10 | 5.60 ± 0.05* |
| Liver | | | | | | |
| Absolute | 7.497 ± 0.312 | 7.018 ± 0.308 | 7.256 ± 0.158 | 7.100 ± 0.415 | 5.221 ± 0.365** | 5.228 ± 0.231** |
| Relative | 53.34 ± 1.52 | 50.34 ± 1.47 | 54.16 ± 1.55 | 57.75 ± 2.12 | 54.81 ± 2.39 | 57.41 ± 1.45 |
| Lungs | | | | | | |
| Absolute | 0.968 ± 0.026 | 0.988 ± 0.043 | 1.021 ± 0.023 | 0.911 ± 0.033 | 0.778 ± 0.031** | 0.737 ± 0.027** |
| Relative | 6.89 ± 0.09 | 7.12 ± 0.35 | 7.61 ± 0.15 | 7.42 ± 0.16 | 8.21 ± 0.29** | 8.14 ± 0.43** |
| Spleen | | | | | | |
| Absolute | 0.404 ± 0.008 | 0.395 ± 0.014 | 0.406 ± 0.011 | 0.522 ± 0.023** | 0.516 ± 0.032** | 0.485 ± 0.038** |
| Relative | 2.88 ± 0.10 | 2.84 ± 0.13 | 3.03 ± 0.07 | 4.26 ± 0.17** | 5.44 ± 0.33** | 5.32 ± 0.35** |
| Thymus | | | | | | |
| Absolute | 0.346 ± 0.010 | 0.361 ± 0.020 | 0.331 ± 0.007 | 0.295 ± 0.012* | 0.146 ± 0.011** | 0.110 ± 0.028** |
| Relative | 2.47 ± 0.09 | 2.60 ± 0.17 | 2.47 ± 0.09 | 2.42 ± 0.13 | 1.54 ± 0.10** | 1.17 ± 0.26** |

* 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)

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 630 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm |
|------------------|----------------|----------------|----------------|----------------|-----------------|-----------------|
| Male | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 362 ± 5 | 367 ± 5 | 348 ± 6 | 347 ± 4 | 330 ± 7** | 271 ± 3** |
| Brain | | | | | | |
| Absolute | 1.943 ± 0.018 | 1.923 ± 0.021 | 1.925 ± 0.019 | 1.941 ± 0.022 | 1.882 ± 0.026 | 1.863 ± 0.018* |
| Relative | 5.38 ± 0.07 | 5.24 ± 0.05 | 5.55 ± 0.11 | 5.59 ± 0.06 | 5.73 ± 0.15* | 6.88 ± 0.11** |
| Heart | | | | | | |
| Absolute | 1.156 ± 0.027 | 1.114 ± 0.020 | 1.107 ± 0.038 | 1.110 ± 0.015 | 1.039 ± 0.019** | 0.918 ± 0.021** |
| Relative | 3.20 ± 0.07 | 3.04 ± 0.05 | 3.18 ± 0.09 | 3.20 ± 0.04 | 3.15 ± 0.07 | 3.39 ± 0.07 |
| R. Kidney | | | | | | |
| Absolute | 1.284 ± 0.032 | 1.331 ± 0.029 | 1.297 ± 0.031 | 1.341 ± 0.024 | 1.318 ± 0.027 | 1.118 ± 0.013** |
| Relative | 3.55 ± 0.05 | 3.62 ± 0.05 | 3.73 ± 0.06* | 3.86 ± 0.06** | 4.00 ± 0.05** | 4.13 ± 0.05** |
| Liver | | | | | | |
| Absolute | 12.899 ± 0.312 | 13.802 ± 0.241 | 12.765 ± 0.256 | 13.263 ± 0.218 | 12.713 ± 0.252 | 12.131 ± 0.185* |
| Relative | 35.63 ± 0.40 | 37.61 ± 0.40* | 36.72 ± 0.37* | 38.18 ± 0.42** | 38.54 ± 0.44** | 44.80 ± 0.67** |
| Lungs | | | | | | |
| Absolute | 1.693 ± 0.077 | 2.003 ± 0.136 | 1.741 ± 0.111 | 1.734 ± 0.067 | 1.792 ± 0.112 | 1.522 ± 0.075 |
| Relative | 4.68 ± 0.19 | 5.45 ± 0.36 | 5.01 ± 0.31 | 4.99 ± 0.17 | 5.46 ± 0.39 | 5.63 ± 0.29* |
| Spleen | | | | | | |
| Absolute | 0.785 ± 0.027 | 0.822 ± 0.023 | 0.776 ± 0.015 | 0.800 ± 0.011 | 0.806 ± 0.016 | 1.073 ± 0.023** |
| Relative | 2.17 ± 0.06 | 2.24 ± 0.05 | 2.23 ± 0.03 | 2.30 ± 0.02 | 2.45 ± 0.07** | 3.96 ± 0.09** |
| R. Testis | | | | | | |
| Absolute | 1.488 ± 0.024 | 1.478 ± 0.032 | 1.455 ± 0.027 | 1.435 ± 0.026 | 1.373 ± 0.020** | 0.408 ± 0.024** |
| Relative | 4.12 ± 0.04 | 4.03 ± 0.08 | 4.18 ± 0.03 | 4.13 ± 0.06 | 4.17 ± 0.06 | 1.50 ± 0.08** |
| Thymus | | | | | | |
| Absolute | 0.333 ± 0.020 | 0.355 ± 0.022 | 0.312 ± 0.015 | 0.314 ± 0.014 | 0.304 ± 0.018 | 0.233 ± 0.011** |
| Relative | 0.92 ± 0.04 | 0.97 ± 0.06 | 0.90 ± 0.04 | 0.90 ± 0.03 | 0.92 ± 0.05 | 0.86 ± 0.04 |

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 630 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm |
|------------------|---------------|---------------|---------------|----------------|-----------------|-----------------|
| Female | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 205 ± 3 | 206 ± 3 | 202 ± 3 | 201 ± 3 | 185 ± 3** | 171 ± 2** |
| Brain | | | | | | |
| Absolute | 1.789 ± 0.025 | 1.822 ± 0.015 | 1.805 ± 0.013 | 1.797 ± 0.026 | 1.805 ± 0.011 | 1.780 ± 0.027 |
| Relative | 8.72 ± 0.13 | 8.86 ± 0.14 | 8.95 ± 0.16 | 8.94 ± 0.11 | 9.79 ± 0.12** | 10.41 ± 0.20** |
| Heart | | | | | | |
| Absolute | 0.705 ± 0.011 | 0.714 ± 0.017 | 0.703 ± 0.018 | 0.711 ± 0.017 | 0.691 ± 0.010 | 0.656 ± 0.012* |
| Relative | 3.44 ± 0.05 | 3.48 ± 0.10 | 3.48 ± 0.07 | 3.54 ± 0.08 | 3.74 ± 0.05** | 3.83 ± 0.06** |
| R. Kidney | | | | | | |
| Absolute | 0.763 ± 0.015 | 0.747 ± 0.016 | 0.736 ± 0.015 | 0.717 ± 0.017* | 0.683 ± 0.014** | 0.681 ± 0.007** |
| Relative | 3.72 ± 0.08 | 3.63 ± 0.06 | 3.64 ± 0.06 | 3.56 ± 0.05 | 3.70 ± 0.05 | 3.98 ± 0.04** |
| Liver | | | | | | |
| Absolute | 6.582 ± 0.104 | 7.030 ± 0.138 | 6.626 ± 0.125 | 6.675 ± 0.114 | 6.257 ± 0.101 | 6.187 ± 0.104* |
| Relative | 32.10 ± 0.56 | 34.14 ± 0.48 | 32.79 ± 0.54 | 33.20 ± 0.42 | 33.89 ± 0.34* | 36.16 ± 0.69** |
| Lungs | | | | | | |
| Absolute | 1.157 ± 0.052 | 1.124 ± 0.042 | 1.139 ± 0.050 | 1.255 ± 0.105 | 1.098 ± 0.031 | 1.007 ± 0.034 |
| Relative | 5.62 ± 0.18 | 5.46 ± 0.20 | 5.65 ± 0.27 | 6.22 ± 0.49 | 5.94 ± 0.13 | 5.88 ± 0.17 |
| Spleen | | | | | | |
| Absolute | 0.524 ± 0.020 | 0.522 ± 0.007 | 0.498 ± 0.016 | 0.545 ± 0.017 | 0.549 ± 0.006 | 0.676 ± 0.011** |
| Relative | 2.55 ± 0.10 | 2.54 ± 0.04 | 2.46 ± 0.07 | 2.71 ± 0.05 | 2.98 ± 0.05** | 3.95 ± 0.05** |
| Thymus | | | | | | |
| Absolute | 0.277 ± 0.011 | 0.287 ± 0.009 | 0.280 ± 0.009 | 0.295 ± 0.016 | 0.266 ± 0.012 | 0.242 ± 0.008* |
| Relative | 1.35 ± 0.05 | 1.40 ± 0.06 | 1.38 ± 0.05 | 1.46 ± 0.07 | 1.44 ± 0.06 | 1.41 ± 0.04 |

* 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).

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------|----------------|----------------|-----------------|-----------------|
| Male | | | | |
| n | 10 | 9 | 10 | 10 |
| Necropsy body wt | 483 ± 7 | 488 ± 9 | 490 ± 8 | 475 ± 5 |
| R. Kidney | | | | |
| Absolute | 1.697 ± 0.045 | 1.720 ± 0.042 | 1.700 ± 0.037 | 1.744 ± 0.042 |
| Relative | 3.51 ± 0.07 | 3.52 ± 0.07 | 3.47 ± 0.07 | 3.67 ± 0.08 |
| Liver | | | | |
| Absolute | 17.192 ± 0.398 | 16.933 ± 0.554 | 16.238 ± 0.393 | 17.289 ± 0.353 |
| Relative | 35.58 ± 0.44 | 34.64 ± 0.76 | 33.14 ± 0.69* | 36.37 ± 0.67 |
| Spleen | | | | |
| Absolute | 1.115 ± 0.058 | 1.112 ± 0.069 | 1.065 ± 0.031 | 1.046 ± 0.020 |
| Relative | 2.31 ± 0.12 | 2.28 ± 0.13 | 2.18 ± 0.07 | 2.20 ± 0.04 |
| Female | | | | |
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt | 297 ± 7 | 297 ± 7 | 280 ± 5 | 260 ± 4** |
| R. Kidney | | | | |
| Absolute | 1.000 ± 0.026 | 0.984 ± 0.016 | 0.920 ± 0.019** | 0.897 ± 0.017** |
| Relative | 3.37 ± 0.07 | 3.32 ± 0.05 | 3.29 ± 0.05 | 3.46 ± 0.06 |
| Liver | | | | |
| Absolute | 9.103 ± 0.257 | 9.575 ± 0.280 | 8.842 ± 0.174 | 9.480 ± 0.279 |
| Relative | 30.69 ± 0.63 | 32.17 ± 0.30 | 31.60 ± 0.56 | 36.49 ± 0.81** |
| Spleen | | | | |
| Absolute | 0.512 ± 0.021 | 0.585 ± 0.024* | 0.583 ± 0.018* | 0.671 ± 0.022** |
| Relative | 1.73 ± 0.07 | 1.97 ± 0.07* | 2.08 ± 0.05** | 2.59 ± 0.08** |

* 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).

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|------------------|---------------|---------------|-----------------|---------------|----------------|----------------|
| Male | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 2 |
| Necropsy body wt | 19.5 ± 0.9 | 19.1 ± 0.7 | 18.4 ± 0.5 | 19.0 ± 0.4 | 17.9 ± 0.5 | 18.0 ± 0.4 |
| Brain | | | | | | |
| Absolute | 0.469 ± 0.008 | 0.455 ± 0.006 | 0.447 ± 0.008 | 0.457 ± 0.007 | 0.455 ± 0.003 | 0.469 ± 0.020 |
| Relative | 24.30 ± 1.00 | 23.92 ± 0.98 | 24.42 ± 0.66 | 24.02 ± 0.35 | 25.51 ± 0.81 | 26.12 ± 0.60 |
| Heart | | | | | | |
| Absolute | 0.159 ± 0.011 | 0.149 ± 0.006 | 0.154 ± 0.007 | 0.151 ± 0.005 | 0.153 ± 0.008 | 0.142 ± 0.006 |
| Relative | 8.17 ± 0.44 | 7.77 ± 0.14 | 8.37 ± 0.24 | 7.96 ± 0.39 | 8.56 ± 0.31 | 7.89 ± 0.46 |
| R. Kidney | | | | | | |
| Absolute | 0.246 ± 0.012 | 0.210 ± 0.016 | 0.185 ± 0.007** | 0.205 ± 0.010 | 0.196 ± 0.009* | 0.226 ± 0.013 |
| Relative | 12.74 ± 0.78 | 10.94 ± 0.63 | 10.06 ± 0.31* | 10.76 ± 0.60 | 11.03 ± 0.74 | 12.58 ± 0.48 |
| Liver | | | | | | |
| Absolute | 1.334 ± 0.046 | 1.111 ± 0.054 | 1.084 ± 0.025 | 1.265 ± 0.027 | 1.337 ± 0.031 | 1.518 ± 0.069* |
| Relative | 68.77 ± 1.78 | 58.08 ± 1.51 | 59.07 ± 0.93 | 66.46 ± 1.35 | 75.00 ± 2.54* | 84.53 ± 2.20** |
| Lungs | | | | | | |
| Absolute | 0.216 ± 0.009 | 0.192 ± 0.011 | 0.192 ± 0.007 | 0.203 ± 0.011 | 0.226 ± 0.007 | 0.227 ± 0.051 |
| Relative | 11.22 ± 0.67 | 10.00 ± 0.26 | 10.48 ± 0.41 | 10.70 ± 0.61 | 12.70 ± 0.76 | 12.57 ± 2.57 |
| Spleen | | | | | | |
| Absolute | 0.058 ± 0.002 | 0.060 ± 0.002 | 0.055 ± 0.002 | 0.062 ± 0.003 | 0.060 ± 0.006 | 0.048 ± 0.005 |
| Relative | 2.99 ± 0.10 | 3.12 ± 0.09 | 3.02 ± 0.02 | 3.26 ± 0.13 | 3.35 ± 0.28 | 2.64 ± 0.20 |
| R. Testis | | | | | | |
| Absolute | 0.100 ± 0.004 | 0.103 ± 0.004 | 0.100 ± 0.005 | 0.100 ± 0.003 | 0.097 ± 0.005 | 0.093 ± 0.005 |
| Relative | 5.18 ± 0.17 | 5.42 ± 0.29 | 5.47 ± 0.23 | 5.27 ± 0.08 | 5.41 ± 0.17 | 5.15 ± 0.15 |
| Thymus | | | | | | |
| Absolute | 0.065 ± 0.004 | 0.051 ± 0.006 | 0.057 ± 0.005 | 0.067 ± 0.010 | 0.039 ± 0.006* | 0.037 ± 0.012* |
| Relative | 3.36 ± 0.30 | 2.70 ± 0.31 | 3.14 ± 0.32 | 3.57 ± 0.56 | 2.16 ± 0.31* | 2.05 ± 0.68 |

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|------------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Female | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 3 |
| Necropsy body wt | 16.8 ± 0.7 | 16.5 ± 0.4 | 15.9 ± 0.4 | 15.3 ± 0.2* | 14.6 ± 0.3** | 15.3 ± 0.7* |
| Brain | | | | | | |
| Absolute | 0.468 ± 0.007 | 0.444 ± 0.008 | 0.453 ± 0.007 | 0.441 ± 0.003* | 0.443 ± 0.008* | 0.446 ± 0.011* |
| Relative | 28.00 ± 0.85 | 27.00 ± 0.69 | 28.48 ± 0.44 | 28.86 ± 0.22 | 30.32 ± 0.60 | 29.36 ± 1.90 |
| Heart | | | | | | |
| Absolute | 0.140 ± 0.005 | 0.126 ± 0.005 | 0.120 ± 0.003** | 0.115 ± 0.005** | 0.111 ± 0.004** | 0.117 ± 0.012** |
| Relative | 8.39 ± 0.27 | 7.65 ± 0.21 | 7.56 ± 0.30* | 7.50 ± 0.31 | 7.59 ± 0.14 | 7.66 ± 0.49 |
| R. Kidney | | | | | | |
| Absolute | 0.197 ± 0.013 | 0.143 ± 0.006** | 0.144 ± 0.005** | 0.143 ± 0.009** | 0.151 ± 0.012** | 0.164 ± 0.010 |
| Relative | 11.76 ± 0.76 | 8.70 ± 0.28** | 9.06 ± 0.19** | 9.33 ± 0.53* | 10.27 ± 0.72 | 10.76 ± 0.29 |
| Liver | | | | | | |
| Absolute | 1.128 ± 0.059 | 0.967 ± 0.025 | 1.001 ± 0.026 | 1.130 ± 0.008 | 1.180 ± 0.040 | 1.243 ± 0.070 |
| Relative | 67.10 ± 1.42 | 58.71 ± 0.89 | 63.05 ± 2.45 | 73.92 ± 1.11* | 80.58 ± 2.11** | 81.68 ± 4.96** |
| Lungs | | | | | | |
| Absolute | 0.214 ± 0.014 | 0.182 ± 0.012 | 0.165 ± 0.005* | 0.177 ± 0.010 | 0.184 ± 0.012 | 0.197 ± 0.006 |
| Relative | 12.67 ± 0.29 | 11.01 ± 0.63 | 10.40 ± 0.29* | 11.56 ± 0.56 | 12.59 ± 0.74 | 13.02 ± 1.01 |
| Spleen | | | | | | |
| Absolute | 0.065 ± 0.005 | 0.063 ± 0.005 | 0.061 ± 0.003 | 0.058 ± 0.004 | 0.051 ± 0.006* | 0.050 ± 0.005 |
| Relative | 3.86 ± 0.19 | 3.81 ± 0.26 | 3.80 ± 0.13 | 3.79 ± 0.26 | 3.43 ± 0.37 | 3.26 ± 0.20 |
| Thymus | | | | | | |
| Absolute | 0.082 ± 0.006 | 0.071 ± 0.008 | 0.069 ± 0.009 | 0.046 ± 0.004** | 0.035 ± 0.005** | 0.038 ± 0.002** |
| Relative | 4.88 ± 0.36 | 4.29 ± 0.42 | 4.38 ± 0.65 | 2.98 ± 0.29** | 2.35 ± 0.34** | 2.49 ± 0.22** |

* 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)

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm |
|------------------|---------------|---------------|----------------|----------------------------|-----------------|-----------------|
| Male | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 34.0 ± 0.7 | 31.7 ± 0.6* | 31.8 ± 1.2 | 30.8 ± 0.8** | 27.6 ± 0.4** | 24.1 ± 0.6** |
| Brain | | | | | | |
| Absolute | 0.466 ± 0.007 | 0.481 ± 0.012 | 0.487 ± 0.009 | 0.494 ± 0.008 | 0.482 ± 0.012 | 0.465 ± 0.005 |
| Relative | 13.78 ± 0.36 | 15.20 ± 0.43* | 15.51 ± 0.57** | 16.11 ± 0.44** | 17.50 ± 0.44** | 19.35 ± 0.44** |
| Heart | | | | | | |
| Absolute | 0.160 ± 0.007 | 0.157 ± 0.003 | 0.156 ± 0.005 | 0.155 ± 0.004 | 0.137 ± 0.004** | 0.126 ± 0.006** |
| Relative | 4.71 ± 0.16 | 4.96 ± 0.05 | 4.94 ± 0.18 | 5.03 ± 0.10 | 4.98 ± 0.11 | 5.23 ± 0.27 |
| R. Kidney | | | | | | |
| Absolute | 0.295 ± 0.007 | 0.310 ± 0.008 | 0.316 ± 0.010 | 0.302 ± 0.012 | 0.264 ± 0.011* | 0.211 ± 0.005** |
| Relative | 8.74 ± 0.33 | 9.76 ± 0.17* | 10.01 ± 0.25** | 9.78 ± 0.25* | 9.57 ± 0.35 | 8.77 ± 0.16 |
| Liver | | | | | | |
| Absolute | 1.431 ± 0.050 | 1.416 ± 0.048 | 1.470 ± 0.071 | 1.524 ± 0.050 | 1.346 ± 0.032 | 1.130 ± 0.047** |
| Relative | 42.09 ± 1.02 | 44.58 ± 1.05 | 46.22 ± 1.21** | 49.47 ± 1.11** | 48.81 ± 1.08** | 46.68 ± 0.92** |
| Lungs | | | | | | |
| Absolute | 0.203 ± 0.006 | 0.227 ± 0.010 | 0.221 ± 0.013 | 0.217 ± 0.012 | 0.222 ± 0.018 | 0.216 ± 0.012 |
| Relative | 6.00 ± 0.23 | 7.21 ± 0.38 | 7.05 ± 0.51 | 7.07 ± 0.39 | 8.02 ± 0.61** | 9.04 ± 0.66** |
| Spleen | | | | | | |
| Absolute | 0.071 ± 0.002 | 0.071 ± 0.003 | 0.072 ± 0.004 | 0.077 ± 0.005 | 0.075 ± 0.005 | 0.052 ± 0.004** |
| Relative | 2.08 ± 0.05 | 2.23 ± 0.09 | 2.26 ± 0.08 | 2.50 ± 0.14* | 2.73 ± 0.17* | 2.11 ± 0.13 |
| R. Testis | | | | | | |
| Absolute | 0.124 ± 0.001 | 0.123 ± 0.002 | 0.139 ± 0.003 | 0.120 ± 0.003 ^b | 0.127 ± 0.006 | 0.097 ± 0.002** |
| Relative | 3.65 ± 0.09 | 3.88 ± 0.08 | 4.41 ± 0.19* | 3.89 ± 0.10* ^b | 4.60 ± 0.19** | 4.05 ± 0.11** |
| Thymus | | | | | | |
| Absolute | 0.055 ± 0.003 | 0.048 ± 0.003 | 0.051 ± 0.006 | 0.051 ± 0.005 | 0.041 ± 0.003 | 0.044 ± 0.004 |
| Relative | 1.60 ± 0.07 | 1.51 ± 0.09 | 1.58 ± 0.17 | 1.63 ± 0.14 | 1.48 ± 0.13 | 1.78 ± 0.12 |

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of *p*-Nitrobenzoic Acid
 (continued)

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm |
|------------------|---------------|---------------|---------------|----------------------------|----------------|-----------------|
| Female | | | | | | |
| n | 10 | 9 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 26.4 ± 0.6 | 26.2 ± 0.4 | 26.8 ± 0.6 | 25.2 ± 0.3 | 22.8 ± 0.3** | 20.6 ± 0.4** |
| Brain | | | | | | |
| Absolute | 0.520 ± 0.012 | 0.495 ± 0.011 | 0.511 ± 0.010 | 0.500 ± 0.010 | 0.499 ± 0.011 | 0.473 ± 0.011** |
| Relative | 19.77 ± 0.45 | 18.95 ± 0.60 | 19.16 ± 0.50 | 19.85 ± 0.51 | 21.96 ± 0.44** | 23.05 ± 0.71** |
| Heart | | | | | | |
| Absolute | 0.143 ± 0.004 | 0.128 ± 0.004 | 0.139 ± 0.005 | 0.131 ± 0.004 ^b | 0.125 ± 0.003* | 0.123 ± 0.008** |
| Relative | 5.45 ± 0.19 | 4.89 ± 0.12 | 5.18 ± 0.15 | 5.19 ± 0.16 ^b | 5.51 ± 0.16 | 5.98 ± 0.40 |
| R. Kidney | | | | | | |
| Absolute | 0.212 ± 0.009 | 0.212 ± 0.007 | 0.226 ± 0.008 | 0.208 ± 0.005 | 0.188 ± 0.005* | 0.171 ± 0.005** |
| Relative | 8.06 ± 0.31 | 8.10 ± 0.28 | 8.45 ± 0.23 | 8.25 ± 0.20 | 8.25 ± 0.20 | 8.29 ± 0.19 |
| Liver | | | | | | |
| Absolute | 1.345 ± 0.057 | 1.167 ± 0.098 | 1.401 ± 0.049 | 1.272 ± 0.037 | 1.230 ± 0.041 | 1.095 ± 0.024** |
| Relative | 50.95 ± 1.45 | 44.82 ± 3.86 | 52.30 ± 1.17 | 50.40 ± 1.16 | 54.05 ± 1.59 | 53.23 ± 0.69 |
| Lungs | | | | | | |
| Absolute | 0.198 ± 0.012 | 0.200 ± 0.007 | 0.207 ± 0.011 | 0.241 ± 0.025 | 0.223 ± 0.013 | 0.239 ± 0.012 |
| Relative | 7.49 ± 0.40 | 7.65 ± 0.34 | 7.76 ± 0.36 | 9.50 ± 0.88** | 9.81 ± 0.53** | 11.56 ± 0.42** |
| Spleen | | | | | | |
| Absolute | 0.090 ± 0.005 | 0.090 ± 0.004 | 0.103 ± 0.005 | 0.095 ± 0.004 | 0.084 ± 0.007 | 0.061 ± 0.005** |
| Relative | 3.41 ± 0.16 | 3.45 ± 0.14 | 3.85 ± 0.14 | 3.75 ± 0.14 | 3.68 ± 0.26 | 2.95 ± 0.21 |
| Thymus | | | | | | |
| Absolute | 0.065 ± 0.005 | 0.054 ± 0.003 | 0.064 ± 0.004 | 0.049 ± 0.002* | 0.056 ± 0.005* | 0.043 ± 0.003** |
| Relative | 2.46 ± 0.16 | 2.07 ± 0.13 | 2.40 ± 0.14 | 1.94 ± 0.10 | 2.47 ± 0.25 | 2.06 ± 0.14 |

* 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=9

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluation in the 2-Year Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------|---------------|---------------|---------------|----------------|
| Male | | | | |
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt | 47.8 ± 1.5 | 44.3 ± 1.7 | 47.9 ± 1.6 | 46.2 ± 1.5 |
| R. Kidney | | | | |
| Absolute | 0.397 ± 0.015 | 0.356 ± 0.007 | 0.379 ± 0.013 | 0.363 ± 0.014 |
| Relative | 8.32 ± 0.24 | 8.11 ± 0.26 | 7.94 ± 0.24 | 7.86 ± 0.23 |
| Liver | | | | |
| Absolute | 2.083 ± 0.157 | 2.427 ± 0.377 | 2.001 ± 0.129 | 1.906 ± 0.098 |
| Relative | 43.40 ± 2.57 | 57.08 ± 11.55 | 41.64 ± 1.88 | 41.05 ± 0.93 |
| Spleen | | | | |
| Absolute | 0.085 ± 0.013 | 0.081 ± 0.009 | 0.082 ± 0.006 | 0.072 ± 0.005 |
| Relative | 1.78 ± 0.24 | 1.90 ± 0.31 | 1.73 ± 0.15 | 1.55 ± 0.09 |
| Female | | | | |
| n | 10 | 10 | 9 | 10 |
| Necropsy body wt | 48.9 ± 1.2 | 46.3 ± 2.3 | 43.1 ± 1.5* | 38.9 ± 1.0** |
| R. Kidney | | | | |
| Absolute | 0.272 ± 0.005 | 0.275 ± 0.005 | 0.268 ± 0.008 | 0.279 ± 0.021 |
| Relative | 5.59 ± 0.16 | 6.06 ± 0.28 | 6.29 ± 0.32 | 7.32 ± 0.78* |
| Liver | | | | |
| Absolute | 1.734 ± 0.111 | 1.714 ± 0.038 | 1.662 ± 0.045 | 1.649 ± 0.049 |
| Relative | 35.54 ± 2.22 | 37.66 ± 1.60 | 38.95 ± 1.55 | 42.58 ± 1.56** |
| Spleen | | | | |
| Absolute | 0.108 ± 0.007 | 0.093 ± 0.003 | 0.116 ± 0.012 | 0.103 ± 0.004 |
| Relative | 2.21 ± 0.14 | 2.08 ± 0.17 | 2.80 ± 0.44 | 2.67 ± 0.13 |

* 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).

APPENDIX G

HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

| | | |
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TABLE G1
Hematology Data for Rats in the 14-Day Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|--|-------------|-------------|-------------|---------------|----------------|----------------|
| Male | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Hematocrit (%) | 43.7 ± 0.8 | 44.2 ± 0.5 | 44.1 ± 0.6 | 42.3 ± 1.0 | 36.9 ± 0.6** | 37.4 ± 0.9** |
| Hemoglobin (g/dL) | 15.5 ± 0.2 | 15.5 ± 0.1 | 15.3 ± 0.2 | 14.8 ± 0.3 | 13.5 ± 0.3** | 13.5 ± 0.3** |
| Erythrocytes (10 ⁶ /μL) | 8.13 ± 0.16 | 8.09 ± 0.10 | 8.22 ± 0.10 | 7.77 ± 0.15 | 6.51 ± 0.18** | 6.22 ± 0.31** |
| Mean cell volume (fL) | 53.8 ± 0.5 | 54.8 ± 1.0 | 53.6 ± 0.4 | 54.6 ± 0.8 | 56.6 ± 0.8* | 60.6 ± 2.0** |
| Reticulocytes (10 ⁶ /μL) | 0.2 ± 0.0 | 0.4 ± 0.1 | 0.4 ± 0.0* | 0.5 ± 0.1** | 0.9 ± 0.1** | 1.3 ± 0.2** |
| Leukocytes (10 ³ /μL) | 9.84 ± 0.57 | 9.42 ± 0.51 | 9.38 ± 1.14 | 10.84 ± 0.73 | 19.44 ± 1.38** | 15.78 ± 1.62** |
| Segmented neutrophils (10 ³ /μL) | 1.16 ± 0.20 | 1.24 ± 0.25 | 1.31 ± 0.16 | 1.73 ± 0.16 | 3.48 ± 0.92** | 2.58 ± 0.39** |
| Lymphocytes (10 ³ /μL) | 8.36 ± 0.57 | 7.95 ± 0.58 | 7.90 ± 1.08 | 8.95 ± 0.69 | 15.66 ± 0.78** | 12.86 ± 1.22* |
| Monocytes (10 ³ /μL) | 0.25 ± 0.03 | 0.19 ± 0.05 | 0.16 ± 0.03 | 0.17 ± 0.04 | 0.25 ± 0.09 | 0.34 ± 0.13 |
| Eosinophils (10 ³ /μL) | 0.06 ± 0.03 | 0.04 ± 0.02 | 0.02 ± 0.02 | 0.00 ± 0.00* | 0.05 ± 0.05 | 0.00 ± 0.00* |
| Nucleated erythrocytes (10 ³ /μL) | 0.12 ± 0.05 | 0.06 ± 0.04 | 0.17 ± 0.08 | 2.88 ± 0.84** | 37.14 ± 4.60** | 47.76 ± 6.91** |
| Methemoglobin (% hemoglobin) | 0.14 ± 0.05 | 0.11 ± 0.01 | 0.27 ± 0.01 | 0.38 ± 0.13 | 3.06 ± 0.20** | 2.48 ± 0.18** |

TABLE G1
Hematology Data for Rats in the 14-Day Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 2,500 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|--|--------------|--------------|---------------|---------------|---------------|----------------|
| Female | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Hematocrit (%) | 44.7 ± 0.5 | 45.0 ± 0.3 | 44.8 ± 0.4 | 40.6 ± 0.7* | 37.8 ± 0.6** | 38.5 ± 1.2** |
| Hemoglobin (g/dL) | 15.8 ± 0.2 | 16.0 ± 0.1 | 15.8 ± 0.2 | 14.4 ± 0.2* | 13.4 ± 0.2** | 13.7 ± 0.4** |
| Erythrocytes (10 ⁶ /μL) | 8.27 ± 0.12 | 8.36 ± 0.08 | 8.45 ± 0.10 | 7.47 ± 0.11* | 7.12 ± 0.10** | 7.24 ± 0.32* |
| Mean cell volume (fL) | 54.0 ± 0.3 | 53.8 ± 0.4 | 53.2 ± 0.2 | 54.4 ± 0.5 | 53.2 ± 0.8 | 53.2 ± 0.7 |
| Reticulocytes (10 ⁶ /μL) | 0.2 ± 0.0 | 0.3 ± 0.0* | 0.2 ± 0.0 | 0.8 ± 0.1** | 0.7 ± 0.1** | 0.3 ± 0.1* |
| Leukocytes (10 ³ /μL) | 10.38 ± 0.64 | 10.42 ± 0.64 | 10.50 ± 1.07 | 10.28 ± 0.70 | 13.50 ± 1.25 | 10.92 ± 0.79 |
| Segmented neutrophils (10 ³ /μL) | 1.35 ± 0.12 | 1.48 ± 0.28 | 1.46 ± 0.14 | 1.47 ± 0.07 | 2.14 ± 0.25* | 1.81 ± 0.33 |
| Lymphocytes (10 ³ /μL) | 8.69 ± 0.82 | 8.64 ± 0.48 | 8.66 ± 0.96 | 8.50 ± 0.69 | 11.04 ± 1.18 | 8.89 ± 0.80 |
| Monocytes (10 ³ /μL) | 0.29 ± 0.07 | 0.26 ± 0.10 | 0.33 ± 0.08 | 0.26 ± 0.07 | 0.31 ± 0.10 | 0.22 ± 0.10 |
| Eosinophils (10 ³ /μL) | 0.05 ± 0.04 | 0.04 ± 0.02 | 0.04 ± 0.03 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Nucleated erythrocytes (10 ³ /μL) | 0.10 ± 0.04 | 0.14 ± 0.08 | 0.06 ± 0.02 | 2.42 ± 0.45* | 9.03 ± 2.38** | 12.26 ± 5.35** |
| Methemoglobin (% hemoglobin) | 0.09 ± 0.02 | 0.16 ± 0.02* | 0.35 ± 0.02** | 0.53 ± 0.03** | 1.63 ± 0.13** | 1.27 ± 0.15** |

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

** P≤0.01

^a Mean ± standard error

TABLE G2
Hematology and Clinical Chemistry Data for Special Study Rats in the 13-Week Feed Study
of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 630 ppm | 2,500 ppm | 10,000 ppm |
|---|--------------------------|---------------------------|--------------------------|----------------------------|
| Male | | | | |
| n | 10 | 10 | 10 | 10 |
| Hematology | | | | |
| Hematocrit (%) | | | | |
| Day 7 | 42.8 ± 0.9 | 42.0 ± 0.5 | 43.9 ± 0.6 | 43.7 ± 0.5 ^b |
| Day 30 | 48.0 ± 0.7 | 46.6 ± 0.8 | 46.6 ± 0.9 | 44.5 ± 0.7 ^{**} |
| Day 60 | 45.6 ± 0.3 ^c | 45.1 ± 0.6 ^c | 44.8 ± 0.7 ^c | 43.6 ± 0.5 [*] |
| Day 90 | 45.0 ± 0.7 | 44.3 ± 0.5 | 43.7 ± 0.6 | 42.9 ± 0.6 |
| Hemoglobin (g/dL) | | | | |
| Day 7 | 15.1 ± 0.1 | 15.1 ± 0.2 | 15.5 ± 0.2 | 15.4 ± 0.2 ^b |
| Day 30 | 16.9 ± 0.2 | 16.6 ± 0.2 | 16.5 ± 0.2 | 15.5 ± 0.1 ^{**} |
| Day 60 | 16.8 ± 0.2 ^c | 16.8 ± 0.3 ^c | 16.8 ± 0.3 ^c | 15.5 ± 0.2 ^{**} |
| Day 90 | 16.5 ± 0.2 | 16.6 ± 0.2 | 16.4 ± 0.2 | 15.5 ± 0.2 ^{**} |
| Erythrocytes (10 ⁶ /μL) | | | | |
| Day 7 | 7.00 ± 0.15 | 6.92 ± 0.11 | 7.11 ± 0.12 | 7.18 ± 0.08 ^b |
| Day 30 | 9.12 ± 0.13 | 8.84 ± 0.12 | 8.89 ± 0.15 | 7.83 ± 0.13 ^{**} |
| Day 60 | 9.62 ± 0.09 ^c | 9.28 ± 0.12 ^{*c} | 9.46 ± 0.13 ^c | 8.32 ± 0.17 ^{**} |
| Day 90 | 9.37 ± 0.13 | 9.09 ± 0.14 | 9.08 ± 0.13 | 8.18 ± 0.12 ^{**} |
| Mean cell volume (fL) | | | | |
| Day 7 | 61.2 ± 1.1 | 60.9 ± 1.3 | 61.9 ± 1.0 | 60.9 ± 0.6 ^b |
| Day 30 | 52.5 ± 0.4 | 52.6 ± 0.3 | 52.5 ± 0.4 | 56.8 ± 0.4 ^{**} |
| Day 60 | 47.4 ± 0.2 ^c | 48.6 ± 0.3 ^{*c} | 47.4 ± 0.2 ^c | 52.5 ± 0.7 ^{**} |
| Day 90 | 48.0 ± 0.2 | 48.7 ± 0.3 [*] | 48.2 ± 0.3 | 52.4 ± 0.3 ^{**} |
| Mean cell hemoglobin (pg) | | | | |
| Day 7 | 21.7 ± 0.5 | 21.9 ± 0.4 | 21.9 ± 0.4 | 21.4 ± 0.3 ^b |
| Day 30 | 18.6 ± 0.2 | 18.8 ± 0.1 | 18.7 ± 0.3 | 19.9 ± 0.2 ^{**} |
| Day 60 | 17.5 ± 0.2 ^c | 18.1 ± 0.1 ^{*c} | 17.7 ± 0.2 ^c | 18.6 ± 0.2 ^{**} |
| Day 90 | 17.6 ± 0.1 | 18.2 ± 0.2 [*] | 18.1 ± 0.2 [*] | 18.9 ± 0.2 ^{**} |
| Mean cell hemoglobin concentration (g/dL) | | | | |
| Day 7 | 35.5 ± 0.8 | 36.0 ± 0.2 | 35.4 ± 0.2 | 35.2 ± 0.3 ^b |
| Day 30 | 35.3 ± 0.4 | 35.7 ± 0.4 | 35.6 ± 0.6 | 35.0 ± 0.4 |
| Day 60 | 36.9 ± 0.4 ^c | 37.2 ± 0.4 ^c | 37.4 ± 0.4 ^c | 35.6 ± 0.3 [*] |
| Day 90 | 36.7 ± 0.4 | 37.4 ± 0.4 | 37.7 ± 0.5 | 36.1 ± 0.3 |
| Reticulocytes (10 ⁶ /μL) | | | | |
| Day 7 | 0.16 ± 0.02 | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.23 ± 0.03 ^{*b} |
| Day 30 | 0.07 ± 0.01 | 0.11 ± 0.01 ^{**} | 0.09 ± 0.01 | 0.26 ± 0.03 ^{**} |
| Day 60 | 0.09 ± 0.02 ^c | 0.08 ± 0.01 ^c | 0.11 ± 0.01 ^c | 0.20 ± 0.03 ^{**} |
| Day 90 | 0.08 ± 0.01 | 0.06 ± 0.01 | 0.09 ± 0.01 | 0.15 ± 0.02 [*] |
| Leukocytes (10 ³ /μL) | | | | |
| Day 7 | 5.48 ± 0.39 | 5.60 ± 0.14 | 5.81 ± 0.41 | 5.30 ± 0.33 ^b |
| Day 30 | 7.98 ± 0.29 | 7.55 ± 0.24 | 7.59 ± 0.28 | 9.35 ± 0.45 |
| Day 60 | 7.06 ± 0.49 ^c | 7.41 ± 0.51 ^c | 7.17 ± 0.22 ^c | 7.34 ± 0.39 |
| Day 90 | 7.46 ± 0.55 | 7.34 ± 0.54 | 8.14 ± 0.46 | 8.00 ± 0.69 |
| Segmented neutrophils (10 ³ /μL) | | | | |
| Day 7 | 0.49 ± 0.06 | 0.56 ± 0.07 | 0.60 ± 0.05 | 0.76 ± 0.07 ^{**b} |
| Day 30 | 0.90 ± 0.09 | 0.92 ± 0.08 | 1.09 ± 0.12 | 2.01 ± 0.12 ^{**} |
| Day 60 | 1.44 ± 0.20 ^c | 1.31 ± 0.11 ^c | 1.24 ± 0.16 ^c | 1.82 ± 0.12 |
| Day 90 | 1.54 ± 0.07 | 1.61 ± 0.13 | 1.83 ± 0.15 | 2.31 ± 0.20 ^{**} |

TABLE G2
Hematology and Clinical Chemistry Data for Special Study Rats in the 13-Week Feed Study
of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 630 ppm | 2,500 ppm | 10,000 ppm |
|------------------------------------|--------------------------|--------------------------|---------------------------|----------------------------|
| Male (continued) | | | | |
| n | 10 | 10 | 10 | 10 |
| Hematology (continued) | | | | |
| Lymphocytes ($10^3/\mu\text{L}$) | | | | |
| Day 7 | 4.91 ± 0.39 | 4.96 ± 0.13 | 5.11 ± 0.40 | 4.46 ± 0.27 ^b |
| Day 30 | 6.95 ± 0.27 | 6.40 ± 0.23 | 6.37 ± 0.30 | 7.22 ± 0.37 |
| Day 60 | 5.40 ± 0.43 ^c | 5.98 ± 0.47 ^c | 5.76 ± 0.20 ^c | 5.47 ± 0.35 |
| Day 90 | 5.86 ± 0.52 | 5.61 ± 0.47 | 6.22 ± 0.45 | 5.64 ± 0.65 |
| Monocytes ($10^3/\mu\text{L}$) | | | | |
| Day 7 | 0.06 ± 0.02 | 0.04 ± 0.02 | 0.06 ± 0.02 | 0.03 ± 0.02 ^b |
| Day 30 | 0.13 ± 0.03 | 0.12 ± 0.03 | 0.08 ± 0.03 | 0.10 ± 0.03 |
| Day 60 | 0.10 ± 0.03 ^c | 0.06 ± 0.02 ^c | 0.08 ± 0.03 ^c | 0.03 ± 0.02* |
| Day 90 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.00 ± 0.00 |
| Eosinophils ($10^3/\mu\text{L}$) | | | | |
| Day 7 | 0.01 ± 0.01 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.04 ± 0.02 ^b |
| Day 30 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.03 | 0.02 ± 0.01 |
| Day 60 | 0.11 ± 0.04 ^c | 0.09 ± 0.02 ^c | 0.09 ± 0.02 ^c | 0.03 ± 0.02* |
| Day 90 | 0.07 ± 0.02 | 0.12 ± 0.04 | 0.09 ± 0.02 | 0.06 ± 0.02 |
| Heinz bodies (% RBC) | | | | |
| Day 7 | 0.22 ± 0.03 | 0.26 ± 0.03 | 0.23 ± 0.03 | 0.40 ± 0.05 ^{**b} |
| Day 30 | 0.10 ± 0.02 | 0.19 ± 0.04 | 0.08 ± 0.03 | 0.37 ± 0.06 ^{**} |
| Day 60 | 0.09 ± 0.04 ^c | 0.17 ± 0.09 ^c | 0.26 ± 0.07 ^{*c} | 0.48 ± 0.06 ^{**} |
| Day 90 | 0.10 ± 0.02 | 0.05 ± 0.02 | 0.15 ± 0.03 | 0.47 ± 0.12 ^{**} |
| Methemoglobin (% hemoglobin) | | | | |
| Day 7 | 0.39 ± 0.06 | 0.43 ± 0.08 | 0.39 ± 0.12 | 1.04 ± 0.22* |
| Day 30 | 0.49 ± 0.21 | 1.21 ± 0.24* | 1.92 ± 0.40 ^{**} | 4.03 ± 0.18 ^{**} |
| Day 60 | 0.72 ± 0.09 ^c | 0.59 ± 0.06 ^c | 0.69 ± 0.10 | 3.20 ± 0.25 ^{**} |
| Day 90 | 0.79 ± 0.09 | 0.66 ± 0.09 | 0.89 ± 0.13 | 3.33 ± 0.25 ^{**} |
| Clinical Chemistry | | | | |
| Alkaline phosphatase (IU/L) | | | | |
| Day 7 | 326 ± 7 | 313 ± 5 | 328 ± 6 | 283 ± 4 ^{**} |
| Day 30 | 252 ± 11 | 259 ± 8 | 247 ± 5 | 191 ± 6 ^{**} |
| Day 60 | 146 ± 6 | 170 ± 15 | 137 ± 4 | 116 ± 4 ^{**} |
| Day 90 | 129 ± 9 | 117 ± 4 | 111 ± 3 | 101 ± 4 ^{**} |
| Alanine aminotransferase (IU/L) | | | | |
| Day 7 | 26 ± 1 | 28 ± 1 | 30 ± 1 | 33 ± 2 ^{**} |
| Day 30 | 40 ± 2 | 35 ± 1 | 34 ± 1* | 40 ± 1 |
| Day 60 | 42 ± 2 | 43 ± 2 | 40 ± 2 | 49 ± 6 |
| Day 90 | 42 ± 2 | 41 ± 1 | 42 ± 1 | 39 ± 2 |
| Sorbitol dehydrogenase (IU/L) | | | | |
| Day 7 | 63 ± 6 | 64 ± 7 | 63 ± 7 | 61 ± 7 |
| Day 30 | 83 ± 9 | 70 ± 6 | 70 ± 5 | 67 ± 5 |
| Day 60 | 81 ± 6 | 68 ± 4 | 70 ± 5 | 73 ± 6 |
| Day 90 | 65 ± 6 | 56 ± 7 | 57 ± 6 | 49 ± 6* |

TABLE G2
Hematology and Clinical Chemistry Data for Special Study Rats in the 13-Week Feed Study
of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 630 ppm | 2,500 ppm | 10,000 ppm |
|--|-------------|-------------|-------------|---------------|
| Female | | | | |
| n | 10 | 10 | 10 | 10 |
| Hematology | | | | |
| Hematocrit (%) | | | | |
| Day 7 | 42.3 ± 0.5 | 43.2 ± 0.9 | 44.7 ± 1.1 | 43.8 ± 0.7 |
| Day 30 | 46.8 ± 0.4 | 46.4 ± 0.7 | 45.5 ± 0.3* | 44.0 ± 0.7** |
| Day 60 | 45.2 ± 0.4 | 45.7 ± 0.4 | 45.6 ± 0.5 | 42.5 ± 0.5** |
| Day 90 | 44.3 ± 0.6 | 43.4 ± 0.6 | 44.0 ± 0.5 | 43.0 ± 0.6 |
| Hemoglobin (g/dL) | | | | |
| Day 7 | 15.5 ± 0.2 | 15.6 ± 0.2 | 15.9 ± 0.2 | 15.6 ± 0.2 |
| Day 30 | 16.8 ± 0.1 | 16.9 ± 0.2 | 16.7 ± 0.1 | 15.7 ± 0.2** |
| Day 60 | 16.9 ± 0.2 | 16.9 ± 0.1 | 16.7 ± 0.1 | 15.5 ± 0.1** |
| Day 90 | 16.6 ± 0.1 | 16.3 ± 0.2 | 16.5 ± 0.1 | 15.9 ± 0.1** |
| Erythrocytes (10⁶/μL) | | | | |
| Day 7 | 7.33 ± 0.13 | 7.56 ± 0.18 | 7.72 ± 0.21 | 7.74 ± 0.20 |
| Day 30 | 8.77 ± 0.08 | 8.73 ± 0.17 | 8.62 ± 0.06 | 8.01 ± 0.14** |
| Day 60 | 9.03 ± 0.12 | 9.08 ± 0.11 | 9.05 ± 0.09 | 8.15 ± 0.08** |
| Day 90 | 8.65 ± 0.11 | 8.39 ± 0.12 | 8.67 ± 0.09 | 8.27 ± 0.10 |
| Mean cell volume (fL) | | | | |
| Day 7 | 57.9 ± 0.7 | 57.3 ± 0.6 | 58.0 ± 0.7 | 56.8 ± 0.7 |
| Day 30 | 53.5 ± 0.3 | 53.4 ± 0.3 | 52.9 ± 0.4 | 55.0 ± 0.4* |
| Day 60 | 50.0 ± 0.5 | 50.3 ± 0.3 | 50.5 ± 0.2 | 52.2 ± 0.3** |
| Day 90 | 51.3 ± 0.4 | 51.8 ± 0.3 | 50.6 ± 0.4 | 51.9 ± 0.3 |
| Mean cell hemoglobin (pg) | | | | |
| Day 7 | 21.2 ± 0.3 | 20.7 ± 0.3 | 20.6 ± 0.4 | 20.3 ± 0.3 |
| Day 30 | 19.2 ± 0.1 | 19.3 ± 0.2 | 19.4 ± 0.1 | 19.6 ± 0.2 |
| Day 60 | 18.7 ± 0.1 | 18.6 ± 0.1 | 18.5 ± 0.2 | 19.0 ± 0.2 |
| Day 90 | 19.2 ± 0.1 | 19.5 ± 0.2 | 19.0 ± 0.2 | 19.3 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | | | | |
| Day 7 | 36.7 ± 0.2 | 36.2 ± 0.4 | 35.6 ± 0.5* | 35.7 ± 0.3* |
| Day 30 | 36.0 ± 0.2 | 36.3 ± 0.4 | 36.8 ± 0.1 | 35.7 ± 0.3 |
| Day 60 | 37.3 ± 0.2 | 36.9 ± 0.2 | 36.7 ± 0.3 | 36.4 ± 0.3* |
| Day 90 | 37.5 ± 0.3 | 37.6 ± 0.3 | 37.5 ± 0.4 | 37.1 ± 0.4 |
| Reticulocytes (10⁶/μL) | | | | |
| Day 7 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0** |
| Day 30 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0** |
| Day 60 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0** |
| Day 90 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.0 ± 0.0* | 0.1 ± 0.0 |
| Leukocytes (10³/μL) | | | | |
| Day 7 | 5.36 ± 0.42 | 6.04 ± 0.40 | 5.65 ± 0.24 | 6.63 ± 0.38* |
| Day 30 | 6.95 ± 0.30 | 6.53 ± 0.31 | 6.19 ± 0.42 | 8.82 ± 0.31** |
| Day 60 | 5.43 ± 0.26 | 5.60 ± 0.30 | 5.58 ± 0.35 | 6.09 ± 0.54 |
| Day 90 | 6.64 ± 0.35 | 6.44 ± 0.44 | 5.58 ± 0.37 | 7.14 ± 0.90 |
| Segmented neutrophils (10³/μL) | | | | |
| Day 7 | 0.62 ± 0.07 | 0.72 ± 0.11 | 0.63 ± 0.06 | 0.78 ± 0.14 |
| Day 30 | 0.72 ± 0.15 | 0.77 ± 0.09 | 0.86 ± 0.13 | 1.45 ± 0.13** |
| Day 60 | 0.99 ± 0.08 | 0.93 ± 0.13 | 1.08 ± 0.16 | 1.22 ± 0.11 |
| Day 90 | 1.46 ± 0.14 | 1.65 ± 0.19 | 1.28 ± 0.12 | 1.40 ± 0.17 |

TABLE G2
Hematology and Clinical Chemistry Data for Special Study Rats in the 13-Week Feed Study
of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 630 ppm | 2,500 ppm | 10,000 ppm |
|------------------------------------|--------------------------|-------------|--------------------------|--------------------------|
| Female (continued) | | | | |
| n | 10 | 10 | 10 | 10 |
| Hematology (continued) | | | | |
| Lymphocytes ($10^3/\mu\text{L}$) | | | | |
| Day 7 | 4.65 ± 0.35 | 5.24 ± 0.41 | 4.87 ± 0.19 | 5.66 ± 0.34* |
| Day 30 | 5.95 ± 0.27 | 5.68 ± 0.27 | 5.20 ± 0.32 | 7.23 ± 0.29 |
| Day 60 | 4.35 ± 0.26 | 4.51 ± 0.21 | 4.36 ± 0.24 | 4.74 ± 0.48 |
| Day 90 | 5.07 ± 0.33 | 4.69 ± 0.33 | 4.18 ± 0.32 | 5.64 ± 0.77 |
| Monocytes ($10^3/\mu\text{L}$) | | | | |
| Day 7 | 0.04 ± 0.02 | 0.03 ± 0.02 | 0.07 ± 0.02 | 0.04 ± 0.02 |
| Day 30 | 0.10 ± 0.04 | 0.06 ± 0.02 | 0.06 ± 0.03 | 0.10 ± 0.03 |
| Day 60 | 0.03 ± 0.02 | 0.07 ± 0.03 | 0.07 ± 0.03 | 0.07 ± 0.03 |
| Day 90 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| Eosinophils ($10^3/\mu\text{L}$) | | | | |
| Day 7 | 0.06 ± 0.02 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.04 ± 0.02 |
| Day 30 | 0.07 ± 0.03 | 0.05 ± 0.02 | 0.08 ± 0.03 | 0.06 ± 0.02 |
| Day 60 | 0.05 ± 0.02 | 0.11 ± 0.02 | 0.08 ± 0.03 | 0.05 ± 0.02 |
| Day 90 | 0.09 ± 0.02 | 0.12 ± 0.02 | 0.09 ± 0.02 | 0.03 ± 0.02 ^c |
| Heinz bodies (% RBC) | | | | |
| Day 7 | 0.18 ± 0.04 | 0.19 ± 0.04 | 0.13 ± 0.05 | 0.37 ± 0.03** |
| Day 30 | 0.10 ± 0.03 | 0.09 ± 0.03 | 0.11 ± 0.03 | 0.24 ± 0.03** |
| Day 60 | 0.11 ± 0.02 | 0.12 ± 0.04 | 0.16 ± 0.03 | 0.37 ± 0.15** |
| Day 90 | 0.08 ± 0.03 | 0.11 ± 0.04 | 0.08 ± 0.02 | 0.25 ± 0.16** |
| Methemoglobin (% hemoglobin) | | | | |
| Day 7 | 0.48 ± 0.10 | 0.56 ± 0.12 | 0.36 ± 0.10 | 1.29 ± 0.19** |
| Day 30 | 1.24 ± 0.23 | 2.34 ± 0.66 | 0.62 ± 0.24 | 3.10 ± 0.21** |
| Day 60 | 0.64 ± 0.11 ^c | 0.85 ± 0.08 | 0.73 ± 0.09 ^c | 1.92 ± 0.21** |
| Day 90 | 0.78 ± 0.10 ^c | 0.86 ± 0.06 | 0.95 ± 0.14 | 2.08 ± 0.15** |
| Clinical Chemistry | | | | |
| Alkaline phosphatase (IU/L) | | | | |
| Day 7 | 240 ± 7 | 235 ± 6 | 238 ± 5 | 208 ± 5** |
| Day 30 | 183 ± 7 | 169 ± 6 | 191 ± 6 | 164 ± 5 |
| Day 60 | 105 ± 5 | 109 ± 5 | 107 ± 3 | 102 ± 4 |
| Day 90 | 75 ± 2 | 77 ± 4 | 88 ± 3** | 101 ± 5** |
| Alanine aminotransferase (IU/L) | | | | |
| Day 7 | 26 ± 1 | 26 ± 1 | 30 ± 2 | 37 ± 2** |
| Day 30 | 32 ± 2 | 31 ± 1 | 33 ± 2 | 47 ± 2** |
| Day 60 | 45 ± 3 | 37 ± 2 | 47 ± 7 | 63 ± 10 |
| Day 90 | 33 ± 1 | 34 ± 1 | 34 ± 1 | 43 ± 2** |
| Sorbitol dehydrogenase (IU/L) | | | | |
| Day 7 | 70 ± 4 | 55 ± 6 | 58 ± 4 | 60 ± 8 |
| Day 30 | 58 ± 5 | 59 ± 4 | 74 ± 7 | 69 ± 7 |
| Day 60 | 72 ± 2 | 69 ± 5 | 73 ± 6 | 76 ± 5 |
| Day 90 | 52 ± 6 | 61 ± 8 | 54 ± 6 | 52 ± 4 |

* 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 ^c n=9

TABLE G3
Hematology Data for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study
of p-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------|--------------------------|--------------------------|--------------------------|
| Male | | | | |
| n | 10 | 8 | 10 | 10 |
| Hematocrit (%) | 47.1 ± 0.7 | 48.1 ± 0.6 | 48.6 ± 0.6 | 47.1 ± 0.8 |
| Hemoglobin (g/dL) | 15.5 ± 0.2 | 15.9 ± 0.2 | 16.0 ± 0.2 | 15.4 ± 0.2 |
| Erythrocytes (10 ⁶ /μL) | 8.81 ± 0.10 | 9.04 ± 0.15 | 9.18 ± 0.12* | 9.21 ± 0.12* |
| Mean cell volume (fL) | 53.5 ± 0.2 | 53.3 ± 0.5 | 53.0 ± 0.5 | 51.2 ± 0.3** |
| Mean cell hemoglobin (pg) | 17.6 ± 0.2 | 17.7 ± 0.2 | 17.5 ± 0.2 | 16.7 ± 0.1** |
| Mean cell hemoglobin concentration (g/dL) | 32.9 ± 0.3 | 33.1 ± 0.3 | 32.9 ± 0.2 | 32.6 ± 0.2 |
| Platelets (10 ³ /μL) | 602.1 ± 14.6 | 579.6 ± 18.2 | 593.7 ± 13.2 | 647.3 ± 15.1 |
| Reticulocytes (10 ⁶ /μL) | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 ^b |
| Leukocytes (10 ³ /μL) | 8.55 ± 0.57 | 8.65 ± 0.41 | 8.58 ± 0.45 | 10.55 ± 0.85* |
| Segmented neutrophils (10 ³ /μL) | 2.24 ± 0.36 | 2.20 ± 0.24 | 2.73 ± 0.36 | 2.67 ± 0.23 ^b |
| Lymphocytes (10 ³ /μL) | 5.93 ± 0.34 | 5.99 ± 0.35 | 5.50 ± 0.40 | 6.88 ± 0.32 |
| Atypical lymphocytes (10 ³ /μL) | 0.02 ± 0.01 | 0.06 ± 0.03 | 0.02 ± 0.01 | 0.02 ± 0.02 |
| Monocytes (10 ³ /μL) | 0.21 ± 0.06 | 0.28 ± 0.09 | 0.18 ± 0.05 | 0.15 ± 0.03 |
| Eosinophils (10 ³ /μL) | 0.14 ± 0.03 | 0.14 ± 0.04 | 0.16 ± 0.03 | 0.08 ± 0.03 |
| Nucleated erythrocytes (10 ³ /μL) | 0.09 ± 0.02 | 0.14 ± 0.03 | 0.18 ± 0.04 | 0.20 ± 0.03* |
| Methemoglobin (% hemoglobin) | 0.22 ± 0.02 | 0.24 ± 0.02 ^b | 0.26 ± 0.02 | 0.28 ± 0.02* |
| Female | | | | |
| n | 10 | 10 | 9 | 10 |
| Hematocrit (%) | 46.3 ± 0.3 | 44.9 ± 0.4* | 45.8 ± 0.4 | 44.1 ± 0.4** |
| Hemoglobin (g/dL) | 16.0 ± 0.2 | 15.5 ± 0.2 | 15.7 ± 0.1 | 14.9 ± 0.2** |
| Erythrocytes (10 ⁶ /μL) | 8.24 ± 0.06 | 8.00 ± 0.12 | 8.20 ± 0.07 | 7.84 ± 0.07** |
| Mean cell volume (fL) | 56.3 ± 0.3 | 56.1 ± 0.6 | 55.8 ± 0.3 | 56.3 ± 0.3 |
| Mean cell hemoglobin (pg) | 19.4 ± 0.1 | 19.4 ± 0.1 | 19.2 ± 0.1* | 19.0 ± 0.1** |
| Mean cell hemoglobin concentration (g/dL) | 34.5 ± 0.2 | 34.6 ± 0.3 | 34.4 ± 0.2 | 33.8 ± 0.1* |
| Platelets (10 ³ /μL) | 560.3 ± 16.8 | 510.1 ± 29.2 | 576.7 ± 12.7 | 652.3 ± 21.8** |
| Reticulocytes (10 ⁶ /μL) | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.3 ± 0.0 |
| Leukocytes (10 ³ /μL) | 4.09 ± 0.16 | 4.58 ± 0.40 | 4.52 ± 0.22 | 6.05 ± 0.49** |
| Segmented neutrophils (10 ³ /μL) | 1.11 ± 0.08 | 1.42 ± 0.12* | 1.10 ± 0.08 | 1.87 ± 0.26* |
| Lymphocytes (10 ³ /μL) | 2.86 ± 0.12 | 3.02 ± 0.34 | 3.32 ± 0.21 | 4.06 ± 0.28** |
| Atypical lymphocytes (10 ³ /μL) | 0.02 ± 0.02 | 0.03 ± 0.02 | 0.01 ± 0.01 | 0.02 ± 0.01 |
| Monocytes (10 ³ /μL) | 0.02 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.03 ± 0.02 |
| Eosinophils (10 ³ /μL) | 0.05 ± 0.02 | 0.09 ± 0.02 | 0.07 ± 0.02 | 0.09 ± 0.02 |
| Nucleated erythrocytes (10 ³ /μL) | 0.09 ± 0.04 | 0.19 ± 0.04 ^b | 0.18 ± 0.05 | 0.42 ± 0.08** |
| Methemoglobin (% hemoglobin) | 0.26 ± 0.03 | 0.26 ± 0.02 | 0.28 ± 0.03 ^c | 0.31 ± 0.03 |

* 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=9 ^c n=10

TABLE G4
Hematology Data for Mice in the 14-Day Feed Study of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|---|-------------|--------------|--------------------------|--------------------------|--------------|----------------|
| Male | | | | | | |
| n | 5 | 4 | 5 | 4 | 5 | 2 |
| Hematocrit (%) | 47.1 ± 1.4 | 48.8 ± 1.6 | 50.5 ± 0.8 | 48.3 ± 0.5 | 50.3 ± 1.5 | 49.8 ± 1.0 |
| Hemoglobin (g/dL) | 15.8 ± 0.4 | 16.3 ± 0.1 | 16.7 ± 0.1 | 16.3 ± 0.1 | 15.8 ± 0.3 | 16.1 ± 0.2 |
| Erythrocytes (10 ⁶ /μL) | 9.89 ± 0.41 | 10.18 ± 0.32 | 10.29 ± 0.14 | 10.21 ± 0.13 | 10.31 ± 0.30 | 10.24 ± 0.10 |
| Mean cell volume (fL) | 47.8 ± 0.6 | 48.0 ± 0.0 | 49.2 ± 0.6 | 46.8 ± 0.5 | 48.8 ± 0.4 | 48.5 ± 0.5 |
| Reticulocytes (10 ⁶ /μL) | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.0 |
| Leukocytes (10 ³ /μL) | 4.60 ± 0.68 | 5.13 ± 0.48 | 5.38 ± 0.38 | 4.40 ± 0.84 | 3.10 ± 0.38 | 3.70 ± 1.00 |
| Segmented neutrophils (10 ³ /μL) | 0.55 ± 0.07 | 0.44 ± 0.08 | 0.60 ± 0.12 | 0.37 ± 0.07 | 0.62 ± 0.03 | 0.53 ± 0.23 |
| Lymphocytes (10 ³ /μL) | 3.87 ± 0.59 | 4.51 ± 0.48 | 4.62 ± 0.28 | 3.92 ± 0.75 | 2.43 ± 0.37 | 3.02 ± 0.70 |
| Monocytes (10 ³ /μL) | 0.13 ± 0.08 | 0.09 ± 0.03 | 0.08 ± 0.02 | 0.06 ± 0.03 | 0.04 ± 0.02 | 0.05 ± 0.00 |
| Eosinophils (10 ³ /μL) | 0.05 ± 0.05 | 0.09 ± 0.04 | 0.08 ± 0.04 | 0.05 ± 0.03 | 0.02 ± 0.01 | 0.11 ± 0.08 |
| Methemoglobin (% hemoglobin) | 0.06 ± 0.04 | 0.00 ± 0.00 | 0.02 ± 0.01 ^b | 0.00 ± 0.00 ^c | 0.05 ± 0.03 | — ^d |

TABLE G4
Hematology Data for Mice in the 14-Day Feed Study of *p*-Nitrobenzoic Acid (continued)

| | 0 ppm | 1,250 ppm | 5,000 ppm | 10,000 ppm | 20,000 ppm | 40,000 ppm |
|---|-------------|--------------|--------------|--------------------------|--------------|-------------|
| Female | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 3 |
| Hematocrit (%) | 48.5 ± 1.0 | 48.3 ± 1.2 | 48.6 ± 0.9 | 49.2 ± 1.5 | 46.7 ± 0.9 | 46.6 ± 1.2 |
| Hemoglobin (g/dL) | 16.3 ± 0.3 | 16.0 ± 0.2 | 16.4 ± 0.1 | 16.1 ± 0.4 | 15.4 ± 0.1* | 15.3 ± 0.3 |
| Erythrocytes (10 ⁶ /μL) | 9.99 ± 0.26 | 10.06 ± 0.27 | 10.17 ± 0.21 | 10.34 ± 0.47 | 9.71 ± 0.21 | 9.61 ± 0.28 |
| Mean cell volume (fL) | 48.4 ± 0.4 | 48.0 ± 0.0 | 47.8 ± 0.6 | 47.6 ± 0.9 | 48.2 ± 0.4 | 48.3 ± 0.3 |
| Reticulocytes (10 ⁶ /μL) | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.1 ^b | 0.3 ± 0.1 | 0.3 ± 0.0 |
| Leukocytes (10 ³ /μL) | 3.80 ± 0.67 | 6.70 ± 0.50 | 6.58 ± 0.31 | 5.16 ± 0.92 | 3.40 ± 0.27 | 5.30 ± 1.40 |
| Segmented neutrophils (10 ³ /μL) | 0.37 ± 0.14 | 0.59 ± 0.11 | 0.85 ± 0.15* | 0.69 ± 0.12 ^b | 0.81 ± 0.18* | 0.61 ± 0.40 |
| Lymphocytes (10 ³ /μL) | 3.37 ± 0.61 | 5.80 ± 0.54 | 5.43 ± 0.34 | 4.41 ± 1.01 ^b | 2.56 ± 0.20 | 3.30 ± 1.76 |
| Monocytes (10 ³ /μL) | 0.01 ± 0.01 | 0.05 ± 0.01 | 0.17 ± 0.05 | 0.13 ± 0.05 ^b | 0.01 ± 0.01 | 0.11 ± 0.07 |
| Eosinophils (10 ³ /μL) | 0.05 ± 0.02 | 0.26 ± 0.10 | 0.02 ± 0.02 | 0.10 ± 0.03 ^b | 0.02 ± 0.01 | 0.18 ± 0.12 |
| Methemoglobin (% hemoglobin) | 0.01 ± 0.01 | 0.07 ± 0.04 | 0.16 ± 0.04 | 0.00 ± 0.00 ^c | 0.27 ± 0.16 | 0.15 ± 0.02 |

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test

^a Mean ± standard error

^b n=4

^c n=3

^d n=0; no data reported

TABLE G5
Hematology Data for Mice at the 15-Month Interim Evaluation in the 2-Year Feed Study
of *p*-Nitrobenzoic Acid^a

| | 0 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------------------|--------------------------|--------------------------|------------------------|
| Male | | | | |
| n | 9 | 10 | 10 | 9 |
| Hematocrit (%) | 47.1 ± 0.5 | 48.2 ± 0.9 | 47.6 ± 0.5 | 48.3 ± 0.5 |
| Hemoglobin (g/dL) | 16.0 ± 0.1 | 16.2 ± 0.4 | 16.2 ± 0.1 | 16.3 ± 0.1* |
| Erythrocytes (10 ⁶ /μL) | 9.94 ± 0.16 | 10.12 ± 0.30 | 10.04 ± 0.16 | 10.16 ± 0.14 |
| Mean cell volume (fL) | 47.3 ± 0.4 | 47.8 ± 0.5 | 47.5 ± 0.7 | 47.6 ± 0.5 |
| Mean cell hemoglobin (pg) | 16.1 ± 0.3 | 16.1 ± 0.3 | 16.2 ± 0.2 | 16.1 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | 34.0 ± 0.4 | 33.7 ± 0.4 | 34.1 ± 0.3 | 33.9 ± 0.3 |
| Platelets (10 ³ /μL) | 1,279 ± 53 | 1,405 ± 68 | 1,310 ± 57 | 1,309 ± 30 |
| Reticulocytes (10 ⁶ /μL) | 0.1 ± 0.0 ^b | 0.2 ± 0.0 ^c | 0.2 ± 0.0 ^c | 0.2 ± 0.0 |
| Leukocytes (10 ³ /μL) | 4.35 ± 0.29 ^d | 4.54 ± 0.21 | 3.79 ± 0.17 ^d | 4.26 ± 0.34 |
| Segmented neutrophils (10 ³ /μL) | 1.13 ± 0.15 ^d | 1.40 ± 0.11 | 0.96 ± 0.08 ^d | 1.21 ± 0.15 |
| Lymphocytes (10 ³ /μL) | 3.14 ± 0.21 ^d | 3.06 ± 0.24 | 2.89 ± 0.22 ^c | 2.90 ± 0.25 |
| Atypical lymphocytes (10 ³ /μL) | 0.00 ± 0.00 ^d | 0.00 ± 0.00 | 0.00 ± 0.00 ^c | 0.00 ± 0.00 |
| Monocytes (10 ³ /μL) | 0.00 ± 0.00 ^d | 0.01 ± 0.01 | 0.00 ± 0.00 ^c | 0.01 ± 0.01 |
| Eosinophils (10 ³ /μL) | 0.08 ± 0.04 ^d | 0.09 ± 0.02 | 0.13 ± 0.03 ^c | 0.13 ± 0.04 |
| Nucleated erythrocytes (10 ³ /μL) | 0.00 ± 0.00 ^d | 0.00 ± 0.00 ^c | 0.00 ± 0.00 ^c | 0.00 ± 0.00 |
| Methemoglobin (% hemoglobin) | 0.16 ± 0.03 ^d | 0.17 ± 0.02 | 0.16 ± 0.02 ^c | 0.14 ± 0.02 |
| Female | | | | |
| n | 10 | 9 | 9 | 10 |
| Hematocrit (%) | 46.3 ± 0.7 | 46.6 ± 0.5 | 47.1 ± 0.7 | 45.2 ± 0.5 |
| Hemoglobin (g/dL) | 16.0 ± 0.2 | 16.0 ± 0.1 | 16.2 ± 0.2 | 15.7 ± 0.1 |
| Erythrocytes (10 ⁶ /μL) | 9.83 ± 0.22 | 9.81 ± 0.11 | 10.11 ± 0.10 | 9.59 ± 0.12 |
| Mean cell volume (fL) | 47.2 ± 0.5 | 47.6 ± 0.3 | 46.8 ± 0.3 | 47.3 ± 0.3 |
| Mean cell hemoglobin (pg) | 16.3 ± 0.2 | 16.3 ± 0.1 | 16.0 ± 0.2 | 16.4 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | 34.5 ± 0.2 | 34.4 ± 0.3 | 34.4 ± 0.4 | 34.8 ± 0.2 |
| Platelets (10 ³ /μL) | 947.4 ± 42.9 | 1,060.8 ± 37.1 | 1,074.2 ± 61.7 | 1,032.6 ± 44.9 |
| Reticulocytes (10 ⁶ /μL) | 0.2 ± 0.0 ^c | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 ^c |
| Leukocytes (10 ³ /μL) | 3.30 ± 0.33 | 3.62 ± 0.31 | 4.19 ± 0.54 | 3.81 ± 0.30 |
| Segmented neutrophils (10 ³ /μL) | 0.92 ± 0.15 | 1.08 ± 0.18 | 1.32 ± 0.40 | 1.00 ± 0.13 |
| Lymphocytes (10 ³ /μL) | 2.29 ± 0.23 | 2.43 ± 0.14 | 2.74 ± 0.21 | 2.73 ± 0.22 |
| Atypical lymphocytes (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Monocytes (10 ³ /μL) | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.02 ± 0.02 | 0.00 ± 0.00 |
| Eosinophils (10 ³ /μL) | 0.10 ± 0.02 | 0.11 ± 0.04 | 0.12 ± 0.04 | 0.07 ± 0.02 |
| Nucleated erythrocytes (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Methemoglobin (% hemoglobin) | 0.16 ± 0.03 | 0.13 ± 0.02 | 0.12 ± 0.02 | 0.11 ± 0.02 |

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

^a Mean ± standard error

^b n=7

^c n=9

^d n=8

APPENDIX H

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF *p*-NITROBENZOIC ACID

p-Nitrobenzoic acid was obtained from E.I. du Pont de Nemours and Company, Inc. (Wilmington, DE), in one lot (40) which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of *p*-nitrobenzoic acid studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a light yellow, crystalline solid, was identified as *p*-nitrobenzoic acid by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra (*Sadtler Standard Spectra*) of *p*-nitrobenzoic acid (Figures H1 and H2). The observed melting point of 239.7° to 241.2° C was consistent with the literature reference (*Merck Index*, 1983).

The purity of *p*-nitrobenzoic acid was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography, and high-performance liquid chromatography. Functional group titration was performed by dissolving a sample of *p*-nitrobenzoic acid in methanol and titrating with 0.1 N aqueous sodium hydroxide. The titration was monitored potentiometrically using a combination pH/mV electrode filled with 4 M potassium chloride. Thin-layer chromatography was performed on Silica Gel 60 F-254 plates using two solvent systems: A) toluene:ethyl acetate:glacial acetic acid (70:25:5) and B) diethylamine:methanol:*N,N*-dimethylformamide (48:40:12). The reference standard used was 10 µg of 1-nitronaphthalene (1 µL of a 10 µg/µL solution in methanol). Visualization was accomplished with ultraviolet light (254 and 366 nm) and a spray of 5% titanous chloride in 1 N hydrochloric acid. High-performance liquid chromatography was performed using a Fisher Scientific Resolvex C₁₈ column (250 × 4.6 mm ID) and a solvent system of water with 1% (v/v) phosphoric acid:methanol with 1% phosphoric acid (61:39). The flow rate was 1.0 mL/minute. Detection was with ultraviolet light at 254 nm.

Elemental analysis for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for *p*-nitrobenzoic acid. Karl Fischer analysis indicated 0.08% ± 0.01% water. Functional group titration indicated a purity of 100.1% ± 0.4%. Thin-layer chromatography using system A detected one major spot and one slight trace impurity; using system B, one major spot and one trace impurity were detected. High-performance liquid chromatography indicated no impurities with areas greater than 0.1% relative to the major peak area. The overall purity was determined to be greater than 99%.

Stability studies were performed by the analytical chemistry laboratory. High-performance liquid chromatography was performed using the system described above except with a solvent ratio of 52:48. These studies indicated that *p*-nitrobenzoic acid was stable as a bulk chemical when stored in the dark for 2 weeks at temperatures up to 60° C. The study laboratory stored the bulk chemical in sealed containers, protected from light, at room temperature. Purity and stability were monitored during the 2-year study by high-performance liquid chromatography and functional group titration. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared weekly by mixing *p*-nitrobenzoic acid and feed to give the required concentrations (Table H1). Mixtures were made by preparing a *p*-nitrobenzoic acid/feed premix with a spatula, which was then blended with feed in a twin shell blender for 15 minutes using an intensifier bar for the initial 5 minutes. Formulations were stored in doubled sealed plastic bags at -22°C or less for up to 3 weeks.

Homogeneity and stability studies of the dose formulations were performed by the analytical chemistry laboratory. For the homogeneity studies at the 400 ppm concentration, aliquots were extracted with methanol containing 0.5% phosphoric acid and centrifuged. Aliquots of the extracts were mixed with an internal standard solution (propiphenone diluted with mobile phase). High-performance liquid chromatography was then performed using a Brownlee RP-18 column and a mobile phase of methanol:water:phosphoric acid (42:57.5:0.5) at a flow rate of 1.0 mL/minute. Homogeneity was confirmed and the stability of the dose formulations was confirmed for at least 3 weeks when stored in the dark at room temperature. Dose formulations open to air and light were stable for 1 week.

Periodic analyses of the dose formulations of *p*-nitrobenzoic acid were conducted at the study laboratory and analytical chemistry laboratory using high-performance liquid chromatography. Dose formulations were analyzed once during the 14-day studies and were within 10% of the target concentrations (Table H2). Dose formulations for the 13-week studies were analyzed prestudy, during week 1, at study mid-point, and at the final mix (Table H3). During the 2-year studies, the dose formulations were analyzed approximately every 2 months (Table H4). All dose formulations were within 10% of the target concentrations during the 13-week studies; 95% (160/168) of the formulations were within 10% of the target concentration during the 2-year studies. 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 H5).

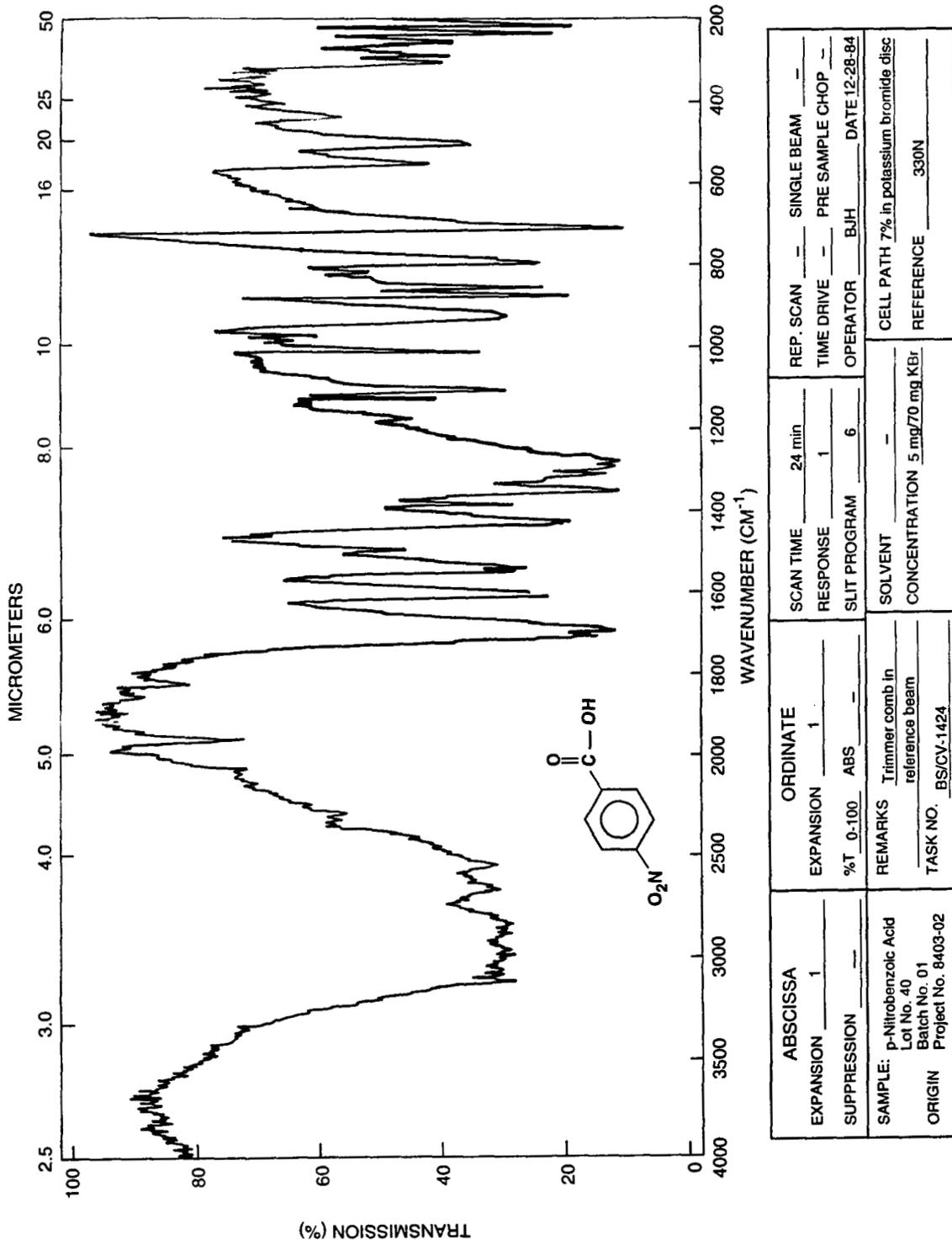


FIGURE H1
Infrared Absorption Spectrum of *p*-Nitrobenzoic Acid

TABLE H1
Preparation and Storage of Dose Formulations in the Feed Studies of *p*-Nitrobenzoic Acid

| 14-Day Studies | 13-Week Studies | 2-Year Studies |
|---|--|---|
| Preparation | | |
| Premix was prepared by mixing <i>p</i> -nitrobenzoic acid and feed with a spatula; premix and feed were then layered in a twin shell blender and mixed for 15 minutes with the intensifier bar on for the first 5 minutes. Doses were prepared at study initiation. | Same as 14-day studies. Doses were prepared every 2 weeks. | Same as 14-day studies. Doses were prepared weekly. |
| Lot Number | | |
| 40 | 40 | 40 |
| Maximum Storage Time | | |
| 3 weeks | 3 weeks | 2 weeks |
| Storage | | |
| In double, sealed plastic bags at -22° C or less for 2 weeks | Same as 14-day studies | Same as 14-day studies |
| Study Laboratory | | |
| Microbiological Associates, Incorporated, Bethesda, MD | Same as 14-day studies | Southern Research Institute, Birmingham, AL |
| Analytical Chemistry Laboratory | | |
| Midwest Research Institute, Kansas City, MO | Same as 14-day studies | Same as 14-day studies |

TABLE H2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 14-Day Feed Studies of *p*-Nitrobenzoic Acid

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|-----------------|--------------------|----------------------------|---|----------------------------|
| 6 December 1985 | 9-10 December 1985 | 2,500 | 2,580 | +3 |
| | | 5,000 | 4,990 | 0 |
| | | 10,000 | 9,520 | -5 |
| | | 20,000 | 20,700 | +4 |
| | | 40,000 | 37,000 | -7 |

^a Results of duplicate analyses

TABLE H3
Results of Analysis of Dose Formulations Administered to Rats and Mice
in the 13-Week Feed Studies of *p*-Nitrobenzoic Acid

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|---------------|-----------------|----------------------------|---|----------------------------|
| Rats | | | | |
| 5 May 1986 | 6-8 May 1986 | 630 | 703 ^b | +10 |
| | | 630 | 702 ^c | +10 |
| | | 630 | 660 ^d | +5 |
| 12 May 1986 | 12-14 May 1986 | 630 | 677 | +7 |
| | | 1,250 | 1,330 | +6 |
| | | 2,500 | 2,470 | -1 |
| | | 5,000 | 4,930 | -1 |
| | | 10,000 | 9,710 | -3 |
| 25 June 1986 | 26-30 June 1986 | 630 | 582 | -8 |
| | | 1,250 | 1,270 | +2 |
| | | 2,500 | 2,470 | -1 |
| | | 5,000 | 5,290 | +6 |
| | | 10,000 | 10,300 | +3 |
| 6 August 1986 | 7-9 August 1986 | 630 | 578 | -8 |
| | | 1,250 | 1,180 | -6 |
| | | 2,500 | 2,440 | -2 |
| | | 5,000 | 5,200 | +4 |
| | | 10,000 | 9,980 | 0 |
| Mice | | | | |
| 5 May 1986 | 6-8 May 1986 | 20,000 | 19,500 ^b | -3 |
| | | 20,000 | 19,900 ^c | -1 |
| | | 20,000 | 20,700 ^d | +3 |
| 12 May 1986 | 12-14 May 1986 | 1,250 | 1,330 | +6 |
| | | 2,500 | 2,470 | -1 |
| | | 5,000 | 4,930 | -1 |
| | | 10,000 | 9,710 | -3 |
| | | 20,000 | 19,600 | -2 |
| 25 June 1986 | 26-30 June 1986 | 1,250 | 1,270 | +2 |
| | | 2,500 | 2,470 | -1 |
| | | 5,000 | 5,290 | +6 |
| | | 10,000 | 10,300 | +3 |
| | | 20,000 | 20,000 | 0 |
| 6 August 1986 | 7-9 August 1986 | 1,250 | 1,180 | -6 |
| | | 2,500 | 2,440 | -2 |
| | | 5,000 | 5,200 | +4 |
| | | 10,000 | 9,980 | 0 |
| | | 20,000 | 20,800 | +4 |

^a Results of duplicate analyses

^b Sample taken from top right of blender

^c Sample taken from top left of blender

^d Sample taken from bottom of blender

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice
in the 2-Year Feed Studies of *p*-Nitrobenzoic Acid

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|----------------------------|------------------|----------------------------|---|----------------------------|
| 27 April 1988 ^b | 27-29 April 1988 | 1,250 | 1,270 ^c | +2 |
| | | 1,250 | 1,220 ^d | -2 |
| | | 1,250 | 1,130 ^e | -10 |
| | | 5,000 | 5,150 ^c | +3 |
| | | 5,000 | 5,080 ^d | +2 |
| | | 5,000 | 5,000 ^e | 0 |
| 4 May 1988 ^b | 4-6 May 1988 | 1,250 | 1,240 ^c | -1 |
| | | 1,250 | 1,300 ^d | +4 |
| | | 1,250 | 1,320 ^e | +6 |
| | | 1,250 | 1,380 ^c | +10 |
| | | 1,250 | 1,290 ^d | +3 |
| | | 1,250 | 1,230 ^e | -2 |
| 4 May 1988 ^f | 4-6 May 1988 | 1,250 | 1,270 | +1 |
| | | 1,250 | 1,300 | +4 |
| | | 2,500 | 2,630 | +5 |
| | | 2,500 | 2,540 | +2 |
| | | 5,000 | 5,420 | +8 |
| | | 5,000 | 5,260 | +5 |
| 18 May 1988 | 18-20 May 1988 | 1,250 | 1,190 | -5 |
| | | 1,250 | 1,250 | 0 |
| | | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,280 | +2 |
| | | 2,500 | 2,500 | 0 |
| | | 2,500 | 2,580 | +3 |
| | | 2,500 | 2,540 | +2 |
| | | 2,500 | 2,600 | +4 |
| | | 5,000 | 5,180 | +4 |
| | | 5,000 | 5,320 | +6 |
| | | 5,000 | 5,210 | +4 |
| | | 5,000 | 5,150 | +3 |
| 6 July 1988 | 7-11 July 1988 | 1,250 | 1,310 | +5 |
| | | 1,250 | 1,260 | +1 |
| | | 1,250 | 1,260 | +1 |
| | | 1,250 | 1,310 | +5 |
| | | 2,500 | 2,530 | +1 |
| | | 2,500 | 2,540 | +2 |
| | | 2,500 | 2,620 | +5 |
| | | 2,500 | 2,580 | +3 |
| | | 5,000 | 5,070 | +1 |
| | | 5,000 | 5,140 | +3 |
| | | 5,000 | 5,020 | 0 |
| | | 5,000 | 5,270 | +5 |

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice
in the 2-Year Feed Studies of *p*-Nitrobenzoic Acid (continued)

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|------------------|---------------------|----------------------------|---|----------------------------|
| 7 September 1988 | 8-12 September 1988 | 1,250 | 1,270 | +2 |
| | | 1,250 | 1,310 | +5 |
| | | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,300 | +4 |
| | | 2,500 | 2,680 | +7 |
| | | 2,500 | 2,520 | +1 |
| | | 2,500 | 2,550 | +2 |
| | | 2,500 | 2,610 | +4 |
| | | 5,000 | 4,920 | -2 |
| | | 5,000 | 5,020 | 0 |
| | | 5,000 | 4,980 | 0 |
| | | 5,000 | 5,200 | +4 |
| | | 9 November 1988 | 10-11 November 1988 | 1,250 |
| 1,250 | 1,300 | | | +4 |
| 1,250 | 1,340 | | | +7 |
| 1,250 | 1,240 | | | -1 |
| 2,500 | 2,640 | | | +6 |
| 2,500 | 2,480 | | | -1 |
| 2,500 | 2,570 | | | +3 |
| 2,500 | 2,600 | | | +4 |
| 5,000 | 5,160 | | | +3 |
| 5,000 | 5,140 | | | +3 |
| 5,000 | 4,990 | | | 0 |
| 5,000 | 4,990 | | | 0 |
| 18 January 1989 | 19-20 January 1989 | | | 1,250 |
| | | 1,250 | 1,360 | +9 |
| | | 1,250 | 1,350 | +8 |
| | | 1,250 | 1,370 | +10 |
| | | 2,500 | 2,590 | +4 |
| | | 2,500 | 2,700 | +8 |
| | | 2,500 | 2,540 | +2 |
| | | 2,500 | 2,860 ^g | +14 |
| | | 5,000 | 5,190 | +4 |
| | | 5,000 | 5,360 | +7 |
| | | 5,000 | 5,120 | +2 |
| | | 5,000 | 5,580 ^g | +12 |
| | | 23 January 1989 | 23-24 January 1989 | 2,500 |
| 5,000 | 5,120 ^h | | | +2 |

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice
in the 2-Year Feed Studies of *p*-Nitrobenzoic Acid (continued)

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|----------------|--------------------|----------------------------|---|----------------------------|
| 22 March 1989 | 23-24 March 1989 | 1,250 | 1,250 | 0 |
| | | 1,250 | 1,250 | 0 |
| | | 1,250 | 1,260 | +1 |
| | | 1,250 | 1,350 | +8 |
| | | 2,500 | 2,630 | +5 |
| | | 2,500 | 2,720 | +9 |
| | | 2,500 | 2,580 | +3 |
| | | 2,500 | 2,530 | +1 |
| | | 5,000 | 5,190 | +4 |
| | | 5,000 | 5,080 | +2 |
| | | 5,000 | 5,260 | +5 |
| | | 5,000 | 5,370 | +7 |
| | | 10 May 1989 | 11-12 May 1989 | 1,250 |
| 1,250 | 1,340 | | | +7 |
| 1,250 | 1,360 | | | +9 |
| 2,500 | 2,550 | | | +2 |
| 2,500 | 2,640 | | | +6 |
| 2,500 | 2,830 ^g | | | +13 |
| 5,000 | 5,450 | | | +9 |
| 5,000 | 5,410 | | | +8 |
| 5,000 | 5,320 | | | +6 |
| 17 May 1989 | 18 May 1989 | 2,500 | 2,820 ^h | +13 |
| 5 July 1989 | 7 July 1989 | 1,250 | 1,260 | +1 |
| | | 1,250 | 1,220 | -2 |
| | | 1,250 | 1,290 | +3 |
| | | 2,500 | 2,470 | -1 |
| | | 2,500 | 2,480 | -1 |
| | | 2,500 | 2,480 | -1 |
| | | 5,000 | 5,050 | +1 |
| | | 5,000 | 5,010 | 0 |
| 16 August 1989 | 16-22 August 1989 | 1,250 | 1,230 | -2 |
| | | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,240 | -1 |
| | | 2,500 | 2,520 | +1 |
| | | 2,500 | 2,500 | 0 |
| | | 2,500 | 2,540 | +2 |
| | | 5,000 | 5,350 | +7 |
| | | 5,000 | 4,920 | -2 |
| | | 5,000 | 5,210 | +4 |

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice
in the 2-Year Feed Studies of *p*-Nitrobenzoic Acid (continued)

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|-----------------|------------------------|----------------------------|---|----------------------------|
| 27 October 1989 | 30-31 October 1989 | 1,250 | 1,320 | +6 |
| | | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,310 | +5 |
| | | 2,500 | 2,630 | +5 |
| | | 2,500 | 2,510 | 0 |
| | | 2,500 | 2,580 | +3 |
| | | 5,000 | 5,170 | +3 |
| | | 5,000 | 4,900 | -2 |
| | | 5,000 | 5,160 | +3 |
| 8 December 1989 | 9, 11-12 December 1989 | 1,250 | 1,270 | +2 |
| | | 1,250 | 1,160 | -7 |
| | | 1,250 | 1,230 | -2 |
| | | 2,500 | 2,560 | +2 |
| | | 2,500 | 2,500 | 0 |
| | | 2,500 | 2,530 | +1 |
| | | 5,000 | 4,860 | -3 |
| | | 5,000 | 5,000 | 0 |
| | | 5,000 | 4,980 | 0 |
| 2 February 1990 | 5-6 February 1990 | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,390 | +11 |
| | | 2,500 | 2,520 | +1 |
| | | 2,500 | 2,660 | +6 |
| | | 2,500 | 2,470 | -1 |
| | | 5,000 | 5,320 | +6 |
| | | 5,000 | 5,220 | +4 |
| | | 5,000 | 5,020 | 0 |
| 30 March 1990 | 2-3 April 1990 | 1,250 | 1,100 ^g | -12 |
| | | 1,250 | 1,480 ^{g,i} | +18 |
| | | 1,250 | 1,390 ^g | +11 |
| | | 2,500 | 2,740 | +10 |
| | | 2,500 | 2,470 | -1 |
| | | 2,500 | 2,590 ⁱ | +4 |
| | | 5,000 | 4,600 | -8 |
| | | 5,000 | 5,230 | +5 |
| | | 5,000 | 4,680 ⁱ | -6 |
| 3 April 1990 | 4 April 1990 | 1,250 | 1,280 ^h | +2 |
| | | 1,250 | 1,280 ^h | +2 |
| | | 1,250 | 1,360 ^h | +9 |

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice
in the 2-Year Feed Studies of *p*-Nitrobenzoic Acid (continued)

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration ^a (ppm) | Difference from Target (%) |
|----------------------------|---------------|----------------------------|---|----------------------------|
| 27 April 1990 ^j | 30 April 1990 | 1,250 | 1,190 | -5 |
| | | 1,250 | 1,240 | -1 |
| | | 1,250 | 1,290 ⁱ | +3 |
| | | 2,500 | 2,530 | +1 |
| | | 2,500 | 2,470 | -1 |
| | | 2,500 | 2,340 | -6 |
| | | 5,000 | 4,870 ⁱ | -3 |
| | | 5,000 | 5,070 | +1 |
| | | 5,000 | 4,950 ⁱ | -1 |

^a Results of duplicate analyses except where indicated

^b Samples not used for dosing

^c Sample taken from top right of blender

^d Sample taken from top left of blender

^e Sample taken from bottom of blender

^f Used only for rats

^g Sample remixed

^h Results of remix

ⁱ Results of triplicate analyses

^j Used only for mice

TABLE H5
Results of Referee Analysis of Dose Formulations in the 13-Week and 2-Year Feed Studies
of *p*-Nitrobenzoic Acid

| Date Prepared | Target Concentration (ppm) | Determined Concentration (ppm) | |
|------------------------|----------------------------|--------------------------------|---------------------------------|
| | | Study Laboratory ^a | Referee Laboratory ^b |
| 13-Week Studies | | | |
| 12 May 1986 | 630 | 677 | 633 ± 29 |
| 6 August 1986 | 2,500 | 2,440 | 2,410 ± 40 |
| 2-Year Studies | | | |
| 4 May 1988 | 1,250 | 1,260 | 1,135 ± 36 |
| 9 November 1988 | 5,000 | 5,140 | 4,900 ± 200 |
| 10 May 1989 | 2,500 | 2,550 | 2,590 ± 30 |
| 27 October 1989 | 1,250 | 1,240 | 1,210 ± 4 |

^a Results of duplicate analyses

^b Results of triplicate analyses (mean ± standard deviation)

APPENDIX I

FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES

| | | |
|----------|---|-----|
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TABLE II
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Week | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|------------------------------|-----------------------|-----------------|-----------------------|--|-----------------|-----------------------|-----------------------------|-----------------|-----------------------|-----------------------------|
| | Feed (g/day) ^a | Body Weight (g) | Feed (g/day) | Body Weight (g) | Dose/ Day ^b (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) |
| 3 | 17.4 | 207 | 17.6 | 206 | 107 | 17.7 | 200 | 222 | 17.4 | 198 | 437 |
| 6 | 20.7 | 283 | 19.5 | 281 | 87 | 19.3 | 280 | 172 | 18.7 | 262 | 356 |
| 10 | 18.8 | 338 | 19.0 | 337 | 70 | 19.2 | 337 | 142 | 17.5 | 315 | 278 |
| 13 | 17.2 | 362 | 17.2 | 360 | 60 | 16.6 | 359 | 116 | 17.4 | 338 | 258 |
| 17 | 20.2 | 387 | 18.6 | 381 | 61 | 17.5 | 391 | 112 | 16.4 | 366 | 224 |
| 21 | 18.3 | 409 | 18.9 | 409 | 58 | 18.6 | 412 | 113 | 17.8 | 389 | 229 |
| 25 | 17.3 | 426 | 19.0 | 421 | 56 | 18.9 | 427 | 110 | 18.5 | 400 | 232 |
| 28 | 18.3 | 435 | 18.0 | 431 | 52 | 18.4 | 435 | 106 | 16.6 | 410 | 202 |
| 33 | 19.1 | 450 | 17.4 | 450 | 48 | 18.0 | 447 | 101 | 18.6 | 426 | 218 |
| 37 | 17.3 | 460 | 18.4 | 461 | 50 | 18.4 | 464 | 99 | 17.8 | 442 | 201 |
| 41 | 16.2 | 469 | 16.2 | 470 | 43 | 17.7 | 466 | 95 | 16.7 | 446 | 187 |
| 45 | 17.2 | 475 | 16.9 | 475 | 44 | 17.6 | 480 | 91 | 17.3 | 459 | 188 |
| 49 | 19.0 | 478 | 17.1 | 485 | 44 | 18.8 | 484 | 97 | 17.9 | 464 | 194 |
| 53 | 16.2 | 480 | 17.4 | 488 | 45 | 17.7 | 485 | 91 | 17.3 | 463 | 186 |
| 57 | 17.8 | 482 | 17.5 | 491 | 45 | 16.6 | 486 | 86 | 18.0 | 464 | 194 |
| 61 | 16.0 | 484 | 18.4 | 484 | 48 | 17.2 | 480 | 89 | 16.4 | 461 | 178 |
| 65 | 15.8 | 483 | 16.4 | 487 | 42 | 17.1 | 486 | 88 | 17.7 | 466 | 191 |
| 69 | 15.7 | 482 | 16.6 | 490 | 42 | 15.7 | 485 | 81 | 16.2 | 466 | 174 |
| 73 | 15.2 | 474 | 14.3 | 478 | 37 | 14.2 | 472 | 75 | 16.5 | 458 | 180 |
| 77 | 15.7 | 475 | 16.1 | 480 | 42 | 16.3 | 474 | 86 | 16.9 | 465 | 182 |
| 80 | 17.0 | 472 | 17.1 | 471 | 45 | 16.2 | 469 | 87 | 17.7 | 458 | 193 |
| 85 | 17.0 | 463 | 17.6 | 463 | 48 | 16.7 | 469 | 89 | 17.9 | 459 | 195 |
| 89 | 16.6 | 462 | 14.6 | 468 | 39 | 15.8 | 473 | 84 | 16.8 | 462 | 182 |
| 93 | 16.7 | 455 | 15.6 | 458 | 43 | 14.9 | 470 | 79 | 15.9 | 457 | 174 |
| 97 | 15.2 | 442 | 15.6 | 447 | 44 | 14.3 | 451 | 79 | 14.8 | 447 | 165 |
| 101 | 15.8 | 449 | 16.3 | 441 | 46 | 16.6 | 460 | 90 | 16.3 | 444 | 184 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 18.5 | 297 | 18.3 | 296 | 81 | 18.2 | 294 | 163 | 17.7 | 278 | 332 |
| 14-52 | 18.1 | 443 | 17.8 | 443 | 51 | 18.2 | 445 | 103 | 17.5 | 422 | 208 |
| 53-101 | 16.2 | 470 | 16.4 | 473 | 43 | 16.1 | 474 | 85 | 16.8 | 459 | 183 |

^a Grams of feed consumed per animal per day

^b Milligrams of *p*-nitrobenzoic acid consumed per day per kilogram body weight

TABLE I2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Week | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|------------------------------|-----------------------|-----------------|-----------------------|--|-----------------|-----------------------|-----------------------------|-----------------|-----------------------|-----------------------------|
| | Feed (g/day) ^a | Body Weight (g) | Feed (g/day) | Body Weight (g) | Dose/ Day ^b (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) |
| 3 | 12.1 | 137 | 11.7 | 137 | 107 | 11.7 | 134 | 219 | 11.8 | 131 | 449 |
| 6 | 12.3 | 166 | 12.9 | 168 | 96 | 12.5 | 164 | 190 | 12.1 | 160 | 378 |
| 10 | 11.8 | 184 | 11.8 | 185 | 80 | 11.7 | 183 | 160 | 11.3 | 179 | 316 |
| 13 | 10.8 | 192 | 11.2 | 192 | 73 | 11.0 | 188 | 147 | 11.2 | 184 | 303 |
| 17 | 10.9 | 203 | 10.6 | 205 | 64 | 11.9 | 193 | 153 | 10.1 | 199 | 253 |
| 21 | 11.2 | 211 | 11.3 | 211 | 67 | 11.2 | 207 | 136 | 10.8 | 203 | 266 |
| 25 | 11.1 | 218 | 10.7 | 218 | 61 | 10.5 | 212 | 124 | 10.5 | 206 | 254 |
| 29 | 11.0 | 217 | 11.0 | 220 | 62 | 10.8 | 216 | 125 | 10.5 | 208 | 251 |
| 33 | 10.9 | 228 | 10.5 | 226 | 58 | 10.9 | 221 | 123 | 10.7 | 212 | 252 |
| 37 | 11.8 | 236 | 11.6 | 235 | 62 | 11.2 | 227 | 123 | 11.1 | 219 | 253 |
| 41 | 10.8 | 242 | 10.6 | 243 | 55 | 10.9 | 234 | 116 | 10.6 | 224 | 237 |
| 45 | 11.6 | 249 | 11.6 | 249 | 58 | 11.7 | 242 | 121 | 11.4 | 230 | 249 |
| 50 | 11.8 | 262 | 12.4 | 261 | 59 | 11.8 | 254 | 117 | 11.7 | 237 | 246 |
| 53 | 12.4 | 271 | 12.2 | 269 | 57 | 11.8 | 262 | 113 | 11.6 | 243 | 239 |
| 57 | 12.9 | 280 | 12.7 | 276 | 58 | 12.3 | 267 | 115 | 12.1 | 245 | 247 |
| 61 | 12.7 | 290 | 12.2 | 282 | 54 | 12.0 | 273 | 110 | 11.8 | 253 | 234 |
| 65 | 11.6 | 298 | 12.4 | 289 | 53 | 12.2 | 280 | 109 | 11.8 | 257 | 229 |
| 69 | 12.1 | 306 | 12.2 | 297 | 51 | 11.5 | 286 | 101 | 11.3 | 267 | 211 |
| 73 | 11.7 | 308 | 10.9 | 299 | 46 | 11.8 | 290 | 102 | 10.7 | 264 | 203 |
| 77 | 12.9 | 316 | 13.3 | 304 | 55 | 12.5 | 292 | 107 | 11.8 | 270 | 219 |
| 81 | 13.2 | 321 | 13.7 | 308 | 55 | 13.1 | 298 | 110 | 12.6 | 274 | 229 |
| 85 | 13.7 | 324 | 13.6 | 313 | 54 | 13.2 | 296 | 112 | 13.3 | 270 | 245 |
| 89 | 12.8 | 334 | 12.8 | 323 | 49 | 12.7 | 306 | 104 | 12.6 | 282 | 224 |
| 93 | 12.0 | 332 | 12.5 | 324 | 48 | 12.5 | 305 | 103 | 12.5 | 283 | 220 |
| 97 | 12.9 | 337 | 12.6 | 327 | 48 | 13.0 | 316 | 103 | 12.6 | 289 | 218 |
| 101 | 12.4 | 341 | 13.9 | 331 | 52 | 13.5 | 314 | 107 | 12.8 | 288 | 223 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 11.8 | 170 | 11.9 | 170 | 89 | 11.7 | 167 | 179 | 11.6 | 164 | 362 |
| 14-52 | 11.2 | 230 | 11.1 | 230 | 61 | 11.2 | 223 | 126 | 10.8 | 215 | 251 |
| 53-101 | 12.6 | 312 | 12.7 | 303 | 52 | 12.5 | 291 | 107 | 12.1 | 268 | 226 |

^a Grams of feed consumed per animal per day

^b Milligrams of *p*-nitrobenzoic acid consumed per day per kilogram body weight

TABLE I3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Week | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|------------------------------|-----------------------|-----------------|-----------------------|--|-----------------|-----------------------|-----------------------------|-----------------|-----------------------|-----------------------------|
| | Feed (g/day) ^a | Body Weight (g) | Feed (g/day) | Body Weight (g) | Dose/ Day ^b (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) |
| 2 | 5.1 | 24.8 | 4.7 | 24.3 | 244 | 4.8 | 24.4 | 491 | 4.8 | 23.9 | 1,001 |
| 6 | 5.3 | 28.1 | 5.2 | 27.9 | 231 | 5.2 | 27.9 | 462 | 6.0 | 27.3 | 1,091 |
| 10 | 5.2 | 31.1 | 5.3 | 30.1 | 219 | 4.9 | 30.8 | 398 | 5.3 | 29.5 | 893 |
| 13 | 5.8 | 33.1 | 6.0 | 32.8 | 228 | 5.5 | 33.1 | 416 | 6.0 | 31.7 | 949 |
| 17 | 5.0 | 35.3 | 5.1 | 34.9 | 183 | 5.2 | 34.9 | 375 | 5.6 | 33.2 | 836 |
| 21 | 4.5 | 37.2 | 4.6 | 36.5 | 156 | 4.4 | 36.4 | 303 | 4.8 | 34.5 | 698 |
| 25 | 4.3 | 38.4 | 4.6 | 38.0 | 151 | 4.5 | 37.8 | 299 | 4.7 | 35.3 | 672 |
| 29 | 4.0 | 40.8 | 4.3 | 40.2 | 133 | 4.3 | 39.8 | 272 | 4.6 | 37.5 | 620 |
| 33 | 4.6 | 41.9 | 4.6 | 41.9 | 138 | 4.8 | 41.6 | 286 | 4.8 | 38.7 | 620 |
| 37 | 5.1 | 43.4 | 5.1 | 43.5 | 145 | 4.8 | 43.3 | 279 | 5.3 | 40.8 | 654 |
| 41 | 4.4 | 45.5 | 4.7 | 45.1 | 130 | 4.5 | 44.8 | 254 | 5.0 | 41.8 | 601 |
| 45 | 4.9 | 46.1 | 4.8 | 45.4 | 133 | 4.9 | 45.4 | 270 | 5.0 | 41.9 | 599 |
| 49 | 5.1 | 47.5 | 5.0 | 46.9 | 135 | 5.0 | 46.7 | 265 | 5.3 | 43.3 | 610 |
| 53 | 4.6 | 47.4 | 4.7 | 47.3 | 123 | 4.6 | 46.7 | 247 | 4.8 | 43.7 | 553 |
| 57 | 5.0 | 47.6 | 5.0 | 46.8 | 134 | 4.9 | 46.3 | 267 | 5.5 | 42.9 | 640 |
| 61 | 4.9 | 47.3 | 5.0 | 46.5 | 135 | 5.1 | 46.0 | 277 | 5.2 | 42.8 | 607 |
| 65 | 5.0 | 47.9 | 5.2 | 47.5 | 138 | 5.3 | 46.2 | 285 | 5.7 | 43.5 | 650 |
| 69 | 4.8 | 47.8 | 5.0 | 48.0 | 130 | 5.0 | 46.3 | 269 | 5.1 | 42.8 | 592 |
| 73 | 4.6 | 49.5 | 5.1 | 48.9 | 131 | 5.2 | 48.2 | 268 | 5.0 | 44.4 | 560 |
| 77 | 4.8 | 49.2 | 5.0 | 48.8 | 128 | 5.1 | 48.2 | 265 | 5.1 | 44.7 | 571 |
| 81 | 5.0 | 49.2 | 4.8 | 48.1 | 125 | 4.9 | 47.1 | 260 | 5.0 | 43.5 | 570 |
| 85 | 4.7 | 48.0 | 4.6 | 48.4 | 120 | 4.7 | 47.3 | 247 | 4.7 | 43.6 | 543 |
| 89 | 4.8 | 48.9 | 4.9 | 48.8 | 125 | 4.8 | 47.4 | 256 | 5.2 | 43.3 | 597 |
| 93 | 5.0 | 48.0 | 5.3 | 49.0 | 135 | 5.2 | 47.5 | 272 | 5.3 | 43.1 | 619 |
| 97 | 4.9 | 46.5 | 5.1 | 47.5 | 134 | 5.1 | 46.6 | 275 | 5.6 | 41.8 | 671 |
| 101 | 5.0 | 45.9 | 5.3 | 47.7 | 139 | 5.4 | 46.4 | 289 | 6.0 | 41.4 | 722 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 5.4 | 29.3 | 5.3 | 28.8 | 230 | 5.1 | 29.1 | 442 | 5.5 | 28.1 | 983 |
| 14-52 | 4.7 | 41.8 | 4.8 | 41.4 | 145 | 4.7 | 41.2 | 289 | 5.0 | 38.6 | 657 |
| 53-101 | 4.9 | 47.9 | 5.0 | 47.9 | 131 | 5.0 | 46.9 | 268 | 5.2 | 43.2 | 607 |

^a Grams of feed consumed per animal per day

^b Milligrams of *p*-nitrobenzoic acid consumed per day per kilogram body weight

TABLE I4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of *p*-Nitrobenzoic Acid

| Week | 0 ppm | | 1,250 ppm | | | 2,500 ppm | | | 5,000 ppm | | |
|-----------------------|------------------------------|-----------------------|-----------------|-----------------------|--|-----------------|-----------------------|-----------------------------|-----------------|-----------------------|-----------------------------|
| | Feed (g/day) ^a | Body Weight (g) | Feed (g/day) | Body Weight (g) | Dose/ Day ^b (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) | Feed (g/day) | Body Weight (g) | Dose/ Day (mg/kg/day) |
| 2 | 5.2 | 20.4 | 4.8 | 20.2 | 299 | 4.6 | 20.1 | 571 | 4.7 | 19.8 | 1,198 |
| 6 | 5.6 | 24.3 | 5.9 | 24.5 | 301 | 6.2 | 24.2 | 637 | 6.8 | 23.3 | 1,457 |
| 10 | 5.6 | 27.1 | 5.5 | 27.0 | 253 | 5.4 | 26.1 | 518 | 6.2 | 25.3 | 1,217 |
| 12 | 6.7 | 28.8 | 6.8 | 28.5 | 298 | 6.6 | 27.6 | 595 | 7.5 | 26.4 | 1,417 |
| 16 | 5.4 | 31.2 | 5.7 | 30.2 | 234 | 5.9 | 29.7 | 495 | 7.2 | 27.4 | 1,315 |
| 20 | 4.8 | 33.4 | 4.8 | 32.3 | 186 | 4.9 | 31.5 | 390 | 6.0 | 28.9 | 1,037 |
| 24 | 4.3 | 34.3 | 5.0 | 33.7 | 185 | 5.1 | 32.1 | 400 | 5.3 | 29.7 | 896 |
| 28 | 4.8 | 36.8 | 4.7 | 35.7 | 166 | 5.4 | 34.8 | 385 | 5.7 | 31.1 | 922 |
| 32 | 4.9 | 39.2 | 5.4 | 38.1 | 178 | 5.3 | 37.2 | 359 | 5.7 | 32.9 | 864 |
| 36 | 5.9 | 40.4 | 5.9 | 39.7 | 187 | 6.0 | 38.6 | 390 | 6.2 | 34.1 | 916 |
| 40 | 5.0 | 42.6 | 5.4 | 42.6 | 157 | 5.3 | 41.0 | 321 | 6.0 | 35.6 | 843 |
| 44 | 5.2 | 44.6 | 5.4 | 44.1 | 154 | 5.6 | 42.5 | 330 | 5.6 | 36.4 | 769 |
| 48 | 5.6 | 46.0 | 5.6 | 45.7 | 153 | 5.6 | 44.0 | 316 | 5.8 | 38.1 | 764 |
| 52 | 5.1 | 47.0 | 5.2 | 46.3 | 142 | 5.2 | 45.0 | 287 | 5.5 | 38.5 | 708 |
| 56 | 5.6 | 46.3 | 5.6 | 45.8 | 153 | 5.7 | 43.8 | 328 | 7.1 | 37.0 | 957 |
| 60 | 5.3 | 47.0 | 5.1 | 46.9 | 136 | 5.4 | 44.5 | 305 | 5.6 | 37.2 | 750 |
| 64 | 5.5 | 48.8 | 5.6 | 48.0 | 146 | 5.7 | 44.7 | 321 | 6.5 | 37.6 | 859 |
| 68 | 5.2 | 48.5 | 5.5 | 48.7 | 140 | 5.2 | 45.9 | 283 | 5.8 | 38.0 | 766 |
| 72 | 5.2 | 49.7 | 5.4 | 49.5 | 137 | 5.6 | 47.2 | 299 | 5.7 | 39.0 | 728 |
| 76 | 5.4 | 51.2 | 5.5 | 51.4 | 135 | 5.6 | 49.0 | 287 | 6.0 | 40.0 | 748 |
| 80 | 5.6 | 52.1 | 5.3 | 51.0 | 130 | 5.6 | 48.2 | 289 | 6.1 | 39.9 | 758 |
| 84 | 5.1 | 51.2 | 5.2 | 51.0 | 128 | 5.4 | 48.2 | 278 | 5.4 | 40.5 | 669 |
| 88 | 5.3 | 52.0 | 5.4 | 50.6 | 133 | 5.3 | 48.1 | 277 | 6.2 | 39.6 | 789 |
| 93 | 5.5 | 50.6 | 5.8 | 49.5 | 147 | 5.6 | 47.4 | 295 | 6.5 | 38.5 | 838 |
| 96 | 5.4 | 49.9 | 5.5 | 48.5 | 142 | 6.0 | 46.5 | 322 | 6.5 | 39.3 | 828 |
| 100 | 5.4 | 49.8 | 5.9 | 48.6 | 152 | 6.2 | 46.2 | 335 | 6.9 | 38.8 | 887 |
| 104 | 5.4 | 48.2 | 5.9 | 47.7 | 155 | 6.2 | 45.2 | 343 | 6.9 | 38.8 | 887 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 5.8 | 25.2 | 5.8 | 25.1 | 288 | 5.7 | 24.5 | 580 | 6.3 | 23.7 | 1,322 |
| 14-52 | 5.1 | 39.6 | 5.3 | 38.8 | 174 | 5.4 | 37.6 | 367 | 5.9 | 33.3 | 903 |
| 53-104 | 5.4 | 49.6 | 5.5 | 49.0 | 141 | 5.7 | 46.5 | 305 | 6.2 | 38.8 | 805 |

^a Grams of feed consumed per animal per day

^b Milligrams of *p*-nitrobenzoic acid consumed per day per kilogram body weight

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

| | | |
|-----------------|---|------------|
| TABLE J1 | Ingredients of NIH-07 Rat and Mouse Ration | 294 |
| TABLE J2 | Vitamins and Minerals in NIH-07 Rat and Mouse Ration | 294 |
| TABLE J3 | Nutrient Composition of NIH-07 Rat and Mouse Ration | 295 |
| TABLE J4 | Contaminant Levels in NIH-07 Rat and Mouse Ration | 296 |

TABLE J1
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 J2
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 |
| <i>d</i> - α -Tocopheryl acetate | 20,000 IU | |
| Choline | 560.0 g | Choline chloride |
| Folic acid | 2.2 g | |
| Niacin | 30.0 g | |
| <i>d</i> -Pantothenic acid | 18.0 g | <i>d</i> -Calcium pantothenate |
| Riboflavin | 3.4 g | |
| Thiamine | 10.0 g | Thiamine mononitrate |
| B ₁₂ | 4,000 μ g | |
| Pyridoxine | 1.7 g | Pyridoxine hydrochloride |
| Biotin | 140.0 mg | <i>d</i> -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 J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

| Nutrient | Mean \pm Standard Deviation | Range | Number of Samples |
|--|-------------------------------|---------------|-------------------|
| Protein (% by weight) | 22.90 \pm 1.03 | 21.30 – 24.60 | 26 |
| Crude fat (% by weight) | 5.31 \pm 0.26 | 4.80 – 5.90 | 26 |
| Crude fiber (% by weight) | 3.64 \pm 0.55 | 2.80 – 4.80 | 26 |
| Ash (% by weight) | 6.73 \pm 0.31 | 6.12 – 7.27 | 26 |
| Amino Acids (% of total diet) | | | |
| Arginine | 1.287 \pm 0.084 | 1.100 – 1.390 | 10 |
| Cystine | 0.306 \pm 0.075 | 0.181 – 0.400 | 10 |
| Glycine | 1.160 \pm 0.050 | 1.060 – 1.220 | 10 |
| Histidine | 0.580 \pm 0.024 | 0.531 – 0.608 | 10 |
| Isoleucine | 0.917 \pm 0.034 | 0.867 – 0.965 | 10 |
| Leucine | 1.972 \pm 0.052 | 1.850 – 2.040 | 10 |
| Lysine | 1.273 \pm 0.051 | 1.200 – 1.370 | 10 |
| Methionine | 0.437 \pm 0.115 | 0.306 – 0.699 | 10 |
| Phenylalanine | 0.994 \pm 0.125 | 0.665 – 1.110 | 10 |
| Threonine | 0.896 \pm 0.055 | 0.824 – 0.985 | 10 |
| Tryptophan | 0.223 \pm 0.160 | 0.107 – 0.671 | 10 |
| Tyrosine | 0.677 \pm 0.105 | 0.564 – 0.794 | 10 |
| Valine | 1.089 \pm 0.057 | 0.962 – 1.170 | 10 |
| Essential Fatty Acids (% of total diet) | | | |
| Linoleic | 2.389 \pm 0.233 | 1.830 – 2.570 | 9 |
| Linolenic | 0.277 \pm 0.036 | 0.210 – 0.320 | 9 |
| Vitamins | | | |
| Vitamin A (IU/kg) | 6,554 \pm 1,288 | 4,100 – 9,190 | 26 |
| Vitamin D (IU/kg) | 4,450 \pm 1,382 | 3,000 – 6,300 | 4 |
| α -Tocopherol (ppm) | 36.92 \pm 9.32 | 22.5 – 48.9 | 9 |
| Thiamine (ppm) | 19.96 \pm 2.88 | 15.0 – 28.0 | 26 |
| Riboflavin (ppm) | 7.92 \pm 0.93 | 6.10 – 9.00 | 10 |
| Niacin (ppm) | 100.95 \pm 25.92 | 65.0 – 150.0 | 9 |
| Pantothenic acid (ppm) | 30.30 \pm 3.60 | 23.0 – 34.6 | 10 |
| Pyridoxine (ppm) | 9.25 \pm 2.62 | 5.60 – 14.0 | 10 |
| Folic acid (ppm) | 2.51 \pm 0.64 | 1.80 – 3.70 | 10 |
| Biotin (ppm) | 0.267 \pm 0.049 | 0.19 – 0.35 | 10 |
| Vitamin B ₁₂ (ppb) | 40.14 \pm 20.04 | 10.6 – 65.0 | 10 |
| Choline (ppm) | 3,068 \pm 314 | 2,400 – 3,430 | 9 |
| Minerals | | | |
| Calcium (%) | 1.26 \pm 0.13 | 0.90 – 1.55 | 26 |
| Phosphorus (%) | 0.96 \pm 0.05 | 0.88 – 1.10 | 26 |
| Potassium (%) | 0.887 \pm 0.067 | 0.772 – 0.971 | 8 |
| Chloride (%) | 0.526 \pm 0.092 | 0.380 – 0.635 | 8 |
| Sodium (%) | 0.315 \pm 0.344 | 0.258 – 0.370 | 10 |
| Magnesium (%) | 0.168 \pm 0.008 | 0.151 – 0.180 | 10 |
| Sulfur (%) | 0.274 \pm 0.063 | 0.208 – 0.420 | 10 |
| Iron (ppm) | 356.2 \pm 90.0 | 255.0 – 523.0 | 10 |
| Manganese (ppm) | 92.24 \pm 5.35 | 81.70 – 99.40 | 10 |
| Zinc (ppm) | 58.14 \pm 9.91 | 46.10 – 81.60 | 10 |
| Copper (ppm) | 11.50 \pm 2.40 | 8.090 – 15.39 | 10 |
| Iodine (ppm) | 3.70 \pm 1.14 | 1.52 – 5.83 | 10 |
| Chromium (ppm) | 1.71 \pm 0.45 | 0.85 – 2.09 | 9 |
| Cobalt (ppm) | 0.797 \pm 0.23 | 0.490 – 1.150 | 6 |

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration

| | Mean \pm Standard Deviation ^a | Range | Number of Samples |
|---|---|------------------|-------------------|
| Contaminants | | | |
| Arsenic (ppm) | 0.25 \pm 0.17 | 0.05 – 0.60 | 26 |
| Cadmium (ppm) | <0.10 | | 26 |
| Lead (ppm) | 0.26 \pm 0.18 | 0.10 – 0.90 | 26 |
| Mercury (ppm) ^b | 0.04 \pm 0.01 | 0.02 – 0.08 | 26 |
| Selenium (ppm) | 0.34 \pm 0.10 | 0.15 – 0.55 | 26 |
| Aflatoxins (ppb) | <5.0 | | 26 |
| Nitrate nitrogen (ppm) ^c | 15.21 \pm 5.00 | 0.30 – 22.0 | 26 |
| Nitrite nitrogen (ppm) ^c | 0.19 \pm 0.14 | <0.10 – 0.60 | 26 |
| BHA (ppm) ^d | 1.49 \pm 0.70 | <2.00 – 3.00 | 26 |
| BHT (ppm) ^d | 1.25 \pm 0.64 | <1.00 – 3.00 | 26 |
| Aerobic plate count (CFU/g) ^e | 115,769 \pm 92,211 | 25,000 – 380,000 | 26 |
| Coliform (MPN/g) ^f | 31.96 \pm 29.95 | <3.00 – 93 | 26 |
| <i>E. coli</i> (MPN/g) | 3.35 \pm 1.20 | <3.00 – 9.00 | 26 |
| Total nitrosoamines (ppb) ^g | 7.63 \pm 2.90 | 2.00 – 13.70 | 26 |
| N-Nitrosodimethylamine (ppb) ^g | 5.33 \pm 2.38 | 1.00 – 11.00 | 26 |
| N-Nitrosopyrrolidine (ppb) ^g | 2.30 \pm 1.23 | 1.00 – 4.70 | 26 |
| Pesticides (ppm) | | | |
| α -BHC ^h | <0.01 | | 26 |
| β -BHC | <0.02 | | 26 |
| γ -BHC | <0.01 | | 26 |
| δ -BHC | <0.01 | | 26 |
| Heptachlor | <0.01 | | 26 |
| Aldrin | <0.01 | | 26 |
| Heptachlor epoxide | <0.01 | | 26 |
| DDE | <0.01 | | 26 |
| DDD | <0.01 | | 26 |
| DDT | <0.01 | | 26 |
| HCB | <0.01 | | 26 |
| Mirex | <0.01 | | 26 |
| Methoxychlor | <0.05 | | 26 |
| Dieldrin | <0.01 | | 26 |
| Endrin | <0.01 | | 26 |
| Telodrin | <0.01 | | 26 |
| Chlordane | <0.05 | | 26 |
| Toxaphene | <0.1 | | 26 |
| Estimated PCBs | <0.2 | | 26 |
| Ronnel | <0.01 | | 26 |
| Ethion | <0.02 | | 26 |
| Trithion | <0.05 | | 26 |
| Diazinon | <0.1 | | 26 |
| Methyl parathion | <0.02 | | 26 |
| Ethyl parathion | <0.02 | | 26 |
| Malathion | 0.22 \pm 0.28 | 0.05 – 1.29 | 26 |
| Endosulfan I | <0.01 | | 26 |
| Endosulfan II | <0.01 | | 26 |
| Endosulfan sulfate | <0.03 | | 26 |

TABLE J4

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

- ^a For values less than the limit of detection, the detection limit is given as the mean.
- ^b The lot milled 03 September 1986 contained 0.08 ppm; all other lots were less than or equal to the detection limit.
- ^c Sources of contamination: alfalfa, grains, and fish meal
- ^d Sources of contamination: soy oil and fish meal
- ^e CFU = colony forming units
- ^f MPN = most probable number
- ^g All values were corrected for percent recovery.
- ^h BHC is hexachlorocyclohexane or benzene hexachloride

APPENDIX K
SENTINEL ANIMAL PROGRAM

METHODS **300**

SENTINEL ANIMAL PROGRAM

METHODS

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

Rats

For the 2-year study, 15 male and 15 female rats were selected at the time of randomization and allocation of the animals to the various study groups. Sera were obtained from five male and five female sentinel rats at 6, 12, and 18 months into the study. Serum for the 24-month screening was obtained from five high-dose males and five mid-dose females. Blood from each collection was processed appropriately, shipped to Microbiological Associates (Bethesda, MD), and screened for the following:

| <u>Method of Analysis</u> | <u>Time of Analysis</u> |
|---|--------------------------|
| ELISA | |
| PVM (pneumonia virus of mice) | 6, 12, 18, and 24 months |
| RCV/SDA (rat coronavirus/ sialodacryoadenitis virus) | 6, 12, 18, and 24 months |
| Sendai | 6, 12, 18, and 24 months |
| Hemagglutination Inhibition | |
| H-1 (Toolan's H-1 virus) | 6, 12, 18, and 24 months |
| KRV (Kilham rat virus) | 6, 12, 18, and 24 months |

Mice

For the 13-week study, samples were obtained from five male and five female controls at the end of the study. These samples were processed appropriately and were submitted to Microbiological Associates for viral titer screening. The following tests were performed:

| <u>Method of Analysis</u> | <u>Time of Analysis</u> |
|--|-------------------------|
| Complement Fixation | |
| LCM (lymphocytic choriomeningitis virus) | Study termination |
| ELISA | |
| CARB (cilia-associated respiratory bacillus) | Study termination |
| Ectromelia virus | Study termination |
| GDVII (mouse encephalomyelitis virus) | Study termination |
| MHV (mouse hepatitis virus) | Study termination |
| Mouse adenoma virus | Study termination |
| <i>Mycoplasma arthritidis</i> | Study termination |
| <i>Mycoplasma pulmonis</i> | Study termination |
| PVM | Study termination |
| Reovirus 3 | Study termination |
| Sendai | Study termination |

Mice (continued)

| <u>Method of Analysis</u> | <u>Time of Analysis</u> |
|--|-------------------------|
| Hemagglutination Inhibition | |
| K (papovavirus) | Study termination |
| MVM (minute virus of mice) | Study termination |
| Polyoma virus | Study termination |
| Immunofluorescence Assay | |
| EDIM (epizootic diarrhea of infant mice) | Study termination |

For the 2-year study, 15 male and 15 female mice were selected at the time of randomization and allocation of the animals to the various study groups. Sera were obtained from as many as five male and five female sentinel mice at 6, 12, and 18 months into the study. Serum for the 24-month screening was obtained from five high-dose males and five high-dose females. Blood from each collection was processed appropriately, shipped to Microbiological Associates, and screened for the following:

| <u>Method of Analysis</u> | <u>Time of Analysis</u> |
|-----------------------------|--------------------------|
| ELISA | |
| Ectromelia virus | 6, 12, 18, and 24 months |
| GDVII | 6, 12, 18, and 24 months |
| LCM | 18 and 24 months |
| MHV | 6, 12, 18, and 24 months |
| MVM | 6 and 12 months |
| Mouse adenoma virus | 6, 12, 18, and 24 months |
| PVM | 6, 12, 18, and 24 months |
| Reovirus 3 | 6, 12, 18, and 24 months |
| Sendai | 6, 12, 18, and 24 months |
| Hemagglutination Inhibition | |
| K | 6, 12, 18, and 24 months |
| Polyoma virus | 6, 12, 18, and 24 months |
| Immunofluorescence Assay | |
| EDIM | 6, 12, 18, and 24 months |
| LCM | 6 and 12 months |
| MVM | 18 and 24 months |

All test results were negative.

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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 Trichloroethylene (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

TR No. CHEMICAL

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)phosphonium Chloride
 298 Dimethyl Morpholinophosphoramidate
 299 C.I. Disperse Blue 1
 300 3-Chloro-2-methylpropene
 301 *o*-Phenylphenol
 303 4-Vinylcyclohexene
 304 Chlorendic Acid
 305 Chlorinated Paraffins (C₂₃, 43% chlorine)
 306 Dichloromethane (Methylene Chloride)
 307 Ephedrine Sulfate
 308 Chlorinated Paraffins (C₁₂, 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
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| TR No. | CHEMICAL | TR No. | CHEMICAL |
|--------|--|--------|---|
| 336 | Penicillin VK | 387 | Amphetamine Sulfate |
| 337 | Nitrofurazone | 388 | Ethylene Thiourea |
| 338 | Erythromycin Stearate | 389 | Sodium Azide |
| 339 | 2-Amino-4-nitrophenol | 390 | 3,3'-Dimethylbenzidine Dihydrochloride |
| 340 | Iodinated Glycerol | 391 | Tris(2-chloroethyl) Phosphate |
| 341 | Nitrofurantoin | 392 | Chlorinated Water and Chloraminated Water |
| 342 | Dichlorvos | 393 | Sodium Fluoride |
| 343 | Benzyl Alcohol | 394 | Acetaminophen |
| 344 | Tetracycline Hydrochloride | 395 | Probenecid |
| 345 | Roxarsone | 396 | Monochloroacetic Acid |
| 346 | Chloroethane | 397 | C.I. Direct Blue 15 |
| 347 | D-Limonene | 398 | Polybrominated Biphenyls |
| 348 | α -Methyldopa Sesquihydrate | 399 | Titanocene Dichloride |
| 349 | Pentachlorophenol | 400 | 2,3-Dibromo-1-propanol |
| 350 | Tribromomethane | 401 | 2,4-Diaminophenol Dihydrochloride |
| 351 | <i>p</i> -Chloroaniline Hydrochloride | 402 | Furan |
| 352 | <i>N</i> -Methylolacrylamide | 403 | Resorcinol |
| 353 | 2,4-Dichlorophenol | 404 | 5,5-Diphenylhydantoin |
| 354 | Dimethoxane | 405 | C.I. Acid Red 114 |
| 355 | Diphenhydramine Hydrochloride | 406 | γ -Butyrolactone |
| 356 | Furosemide | 407 | C.I. Pigment Red 3 |
| 357 | Hydrochlorothiazide | 408 | Mercuric Chloride |
| 358 | Ochratoxin A | 409 | Quercetin |
| 359 | 8-Methoxypsoralen | 410 | Naphthalene |
| 360 | <i>N,N</i> -Dimethylaniline | 411 | C.I. Pigment Red 23 |
| 361 | Hexachloroethane | 412 | 4,4-Diamino-2,2-stilbenedisulfonic Acid |
| 362 | 4-Vinyl-1-cyclohexene Diepoxide | 413 | Ethylene Glycol |
| 363 | Bromoethane (Ethyl Bromide) | 414 | Pentachloroanisole |
| 364 | Rhodamine 6G (C.I. Basic Red 1) | 415 | Polysorbate 80 |
| 365 | Pentaerythritol Tetranitrate | 416 | <i>o</i> -Nitroanisole |
| 366 | Hydroquinone | 417 | <i>p</i> -Nitrophenol |
| 367 | Phenylbutazone | 418 | <i>p</i> -Nitroaniline |
| 368 | Nalidixic Acid | 419 | HC Yellow 4 |
| 369 | α -Methylbenzyl Alcohol | 420 | Triamterene |
| 370 | Benzofuran | 421 | Talc |
| 371 | Toluene | 422 | Coumarin |
| 372 | 3,3-Dimethoxybenzidine Dihydrochloride | 423 | Dihydrocoumarin |
| 373 | Succinic Anhydride | 424 | <i>o</i> -Benzyl- <i>p</i> -chlorophenol |
| 374 | Glycidol | 425 | Promethazine Hydrochloride |
| 375 | Vinyl Toluene | 426 | Corn Oil, Safflower Oil, and Tricaprylin |
| 376 | Allyl Glycidyl Ether | 427 | Turmeric Oleoresin |
| 377 | <i>o</i> -Chlorobenzalmononitrile | 428 | Manganese (II) Sulfate Monohydrate |
| 378 | Benzaldehyde | 430 | C.I. Direct Blue 218 |
| 379 | 2-Chloroacetophenone | 431 | Benzyl Acetate |
| 380 | Epinephrine Hydrochloride | 432 | Barium Chloride Dihydrate |
| 381 | <i>d</i> -Carvone | 433 | Tricresyl Phosphate |
| 382 | Furfural | 434 | 1,3-Butadiene |
| 384 | 1,2,3-Trichloropropane | 437 | Hexachlorocyclopentadiene |
| 385 | Methyl Bromide | 440 | Ozone and Ozone/NNK |
| 386 | Tetranitromethane | 443 | Oxazepam |

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**NIH Publication No. 95-3358
December 1994**