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NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 325

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TOXICOLOGY AND CARCINOGENESIS STUDIES OF PENTACHLORONITROBENZENE

(CAS NO. 82-68-8)

IN B6C3F$_1$ MICE

(FEED STUDIES)

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

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NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is made up of four charter DHHS agencies: 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.

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Under the conditions of these 2-year feed studies, there was no evidence of carcinogenicity for either male or female $B6C3F_1$ mice ~~receiving~~ 2,500 or 5,000 ppm ~~of~~ pentachloronitrobenzene. Infection is considered to have decreased survival of the female mice and thus reduced the sensitivity for determining the presence or absence of a carcinogenic response.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF PENTACHLORONITROBENZENE

(CAS NO. 82-68-8)

IN B6C3F$_1$ MICE

(FEED STUDIES)

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

January 1987

NTP TR 325

NIH Publication No. 87-2581

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

NOTE TO THE READER

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 testing in the NTP Carcinogenesis Program 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. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical has the potential for hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

Five categories of interpretative conclusions were adopted for use in June 1983 in the Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice), one of the following quintet will be selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of malignant neoplasms, studies that exhibit a substantially increased incidence of benign neoplasms, or studies that exhibit an increased incidence of a combination of malignant and benign neoplasms where each increases with dose.
- **Some Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of benign neoplasms, studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.
- **Equivocal Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related marginal increase of neoplasms.
- **No Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenicity** demonstrates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

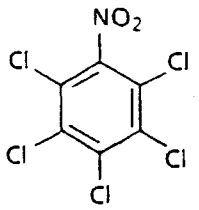
Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the earlier induction by chemicals of neoplasms that are commonly observed, or the induction by chemicals of more neoplasms than are generally found. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

This study was initiated by the National Cancer Institute's Carcinogenesis Bioassay Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

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



PENTACHLORONITROBENZENE
CAS No. 82-68-8

$C_6Cl_5NO_2$ Molecular weight 295.36

Synonyms or Trade Names: Avicol®, PCNB, quintozene, Botrilex®, Brassicol®, Folosan®, PKhNB, Tilcarex®, Terraclor®, Tritisan®

ABSTRACT

Toxicology and carcinogenesis studies of pentachloronitrobenzene (99% pure), a fungicide, were conducted by administering diets containing 0, 2,500, or 5,000 ppm pentachloronitrobenzene to groups of 50 $B6C3F_1$ mice of each sex for 103 weeks. These doses were selected because, in 13-week studies in which the chemical was administered in feed at doses up to 20,000 ppm in male mice and up to 40,000 ppm in female mice, body weight gain depression was observed at 10,000 ppm and above in males and females and deaths occurred at 40,000 ppm in females.

The National Cancer Institute had conducted 2-year studies in $B6C3F_1$ mice and Osborne-Mendel rats (Technical Report 61). Survival among male mice was low, not all livers were examined from dosed female mice, and the size of the control groups was considered to be small. For these reasons, the NCI decided to conduct additional 13-week and 2-year studies in $B6C3F_1$ mice. Under the conditions of the NCI studies (TR 61), pentachloronitrobenzene was not carcinogenic in either Osborne-Mendel rats or $B6C3F_1$ mice.

In the studies reported in this Technical Report, the survival of male mice was comparable among control and dosed groups (control, 35/50; low dose, 31/50; high dose, 32/50). Final mean body weights of low dose and high dose male mice were 96% and 90% that of the controls. All groups of female mice showed evidence of bacterial infection. At week 84, survival in dosed and control female mice was 38/50, 34/50, 30/50; after week 84, survival in dosed groups decreased, with the final survival being 30/50, 20/50, 15/50. The mean body weight of high dose female mice was more than 10% lower than that of the control group after week 20 and was 21% lower than controls at week 104. The mean body weight of low dose female mice was within 10% that of the control group until week 88 and was 18% lower than controls at week 104.

No compound-related neoplastic lesions were seen in either male or female mice. The nonneoplastic lesions observed in female mice were considered to be secondary to bacterial infection (primarily Klebsiella) and included hematopoiesis of the liver (9/50; 21/50; 23/50) and spleen (14/50; 23/48; 27/50), plasma cell hyperplasia of the mediastinal lymph nodes (1/44; 4/47; 9/45), and ovarian abscesses (12/49; 22/50; 29/50).

Pentachloronitrobenzene was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of Aroclor 1254-induced male Syrian hamster or male Sprague-Dawley rat liver S9 when tested according to the preincubation protocol. Pentachloronitrobenzene was not mutagenic at the $TK^{+/-}$ locus of L5178Y mouse lymphoma cells in the presence or absence of Aroclor 1254-induced F344 rat liver S9. In cultured Chinese hamster ovary cells, pentachloronitrobenzene did not induce sister-chromatid exchanges but did induce chromosomal aberrations both with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9.

An audit of the experimental data was conducted for the 2-year studies of pentachloronitrobenzene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenicity** for either male or female $B6C3F_1$ mice receiving 2,500 or 5,000 ppm of pentachloronitrobenzene. Infection is considered to have decreased survival of the female mice and thus reduced the sensitivity for determining the presence or absence of a carcinogenic response.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 8.

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on pentachloronitrobenzene on March 26, 1986, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors
Technical Reports Review Subcommittee

Jerry B. Hook, Ph.D. (Chair)
Vice President, Preclinical Research and Development
Smith Kline & French Laboratories
Philadelphia, Pennsylvania

Frederica Perera, Dr. P.H.
Division of Environmental Sciences
School of Public Health, Columbia University
New York, New York

James Swenberg, D.V.M., Ph.D.
Head, Department of Biochemical Toxicology and Pathobiology
Chemical Industry Institute of Toxicology
Research Triangle Park, North Carolina

Ad Hoc Subcommittee Panel of Experts

Charles C. Capen, D.V.M., Ph.D.
Department of Veterinary Pathobiology
Ohio State University
Columbus, Ohio

Vernon M. Chinchilli, Ph.D.
Department of Biostatistics
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia

John J. Crowley, Ph.D.
Division of Public Health Science
The Fred Hutchinson Cancer Research Center
Seattle, Washington

Kim Hooper, Ph.D. (Principal Reviewer)
Hazard Evaluation System and Information Services
Department of Health Services
State of California
Berkeley, California

Donald H. Hughes, Ph.D.
Scientific Coordinator, Regulatory Services Division, The Procter and Gamble Company
Cincinnati, Ohio

Franklin E. Mirer, Ph.D. (Principal Reviewer)
Director, Health and Safety Department
International Union, United Auto Workers, Detroit, Michigan

James A. Popp, D.V.M., Ph.D.
Head, Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, North Carolina

I.F.H. Purchase, B.V.Sc., Ph.D., FRC Path.*
Director, Central Toxicology Laboratory
Imperial Chemical Industries, PLC
Alderley Park, England

Robert A. Scala, Ph.D.
Senior Scientific Advisor, Medicine and Environmental Health Department
Research and Environmental Health Division, Exxon Corporation
East Millstone, New Jersey

Andrew Sivak, Ph.D. (Principal Reviewer)
Vice President, Biomedical Science
Arthur D. Little, Inc.
Cambridge, Massachusetts

*Unable to attend

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF PENTACHLORONITROBENZENE

On March 26, 1986, the draft Technical Report on the toxicology and carcinogenesis studies of pentachloronitrobenzene received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. J. Dunnick, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of pentachloronitrobenzene in mice by reviewing the experimental design, results, and proposed conclusions (no evidence of carcinogenicity for male or female mice).

Dr. Hooper, a principal reviewer, agreed with the conclusions. Noting that the widespread ovarian infection with Klebsiella reduced survival of high dose female mice, Dr. Hooper suggested that the second sentence of the conclusions be modified to read: "Poor survival among high dose females limited the sensitivity of this bioassay to detect a carcinogenic effect." Dr. Perera said that the conclusion in females should be changed to inadequate study of carcinogenicity. Dr. Swenberg noted that, under the guidelines used by the Panel for some time, a study was considered adequate if survival was greater than 50% after 78 weeks; in this case, survival in high dose females was 60% at 78 weeks. Dr. J. Huff, NTP, indicated that most organizations that use carcinogenicity findings from laboratory experiments require 50% survival at the end of a negative study. The 18-month/50% survival guideline was valid when studies were limited to this time interval in certain strains of mice that did not ordinarily survive to 2 years.

As a second principal reviewer, Dr. Mirer agreed with the conclusions but wondered whether these studies should have been conducted in view of the negative results from the previous compromised NCI study. Dr. Mirer commented on the reduced and variable number of tissues from low dose male mice which were examined histopathologically. Dr. E. McConnell, NIEHS, replied that a reduced histopathology protocol was adopted and presented to the Board of Scientific Counselors in 1982. Under this protocol, tissues from low dose animals were examined microscopically only when there was reason to suspect there might be lesions; that is, if lesions were observed at gross necropsy or microscopic lesions were seen in a particular tissue in high dose animals. Dr. S. Eustis, NTP, indicated that this would be more clearly stated in the pathology methods section. Dr. Mirer asked that more discussion be included on the nonneoplastic lesions in female mice, which might be the only compound-related effects in the study. Dr. Dunnick replied that this would be expanded in the discussion and, where appropriate, in the abstract.

As a third principal reviewer, Dr. Sivak agreed with the conclusions, stating that he believed the survival in the female high dose group to be adequate for interpretation of the study.

Dr. Hooper moved that the Technical Report on pentachloronitrobenzene be accepted with the modification of the last sentence of the conclusions as proposed by him. Dr. Swenberg seconded the motion, and after discussion the motion was defeated by 10 negative votes to 1 affirmative vote (Dr. Hooper). Dr. Hooper then moved to accept the report with the conclusions, no evidence of carcinogenicity, for mice of both sexes, including the last sentence as originally written. Dr. Mirer seconded the motion, and it was approved by 10 affirmative votes to 1 negative vote (Dr. Swenberg).

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Pentachloronitrobenzene is based on the 13-week studies that began in May 1980 and ended in August 1980 and on the 2-year studies that began in June 1981 and ended in June 1983 at EG&G Mason Research Institute.

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

June K. Dunnick, Ph.D., Chemical Manager

Jack B. Bishop, Ph.D.
Scot L. Eustis, D.V.M., Ph.D.
Joseph K. Haseman, Ph.D.
James Huff, Ph.D.
C.W. Jameson, Ph.D.
E.E. McConnell, D.V.M.
John A. Mennear, Ph.D.
G.N. Rao, D.V.M., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
James K. Selkirk, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report on 8/20/85)

Robert Kovatch, D.V.M. (Chair)
Pathology Associates, Incorporated
Roger Alison, B.V.Sc., MRCVS (NTP)
Gary A. Boorman, D.V.M., Ph.D. (NTP)
Thomas Brown, D.V.M.
University of Georgia
Bill C. Bullock, D.V.M.
Bowman Gray School of Medicine
Robert Fleischman, D.V.M.
EG&G Mason Research Institute
A.W. Macklin, D.V.M., Ph.D.
Burroughs Wellcome Laboratories
Robert Maronpot, D.V.M. (NTP)
Michael Ryan, D.V.M. (Observer)
Battelle Columbus Laboratories

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

Herman Lilja, Ph.D.
Principal Investigator
Robert Fleischman, D.V.M.
Pathologist
Miasnig Hagopian, Ph.D.
Chemist

Principal Contributors at Experimental Pathology Laboratories, Inc.
(Provided Pathology Quality Assurance)

Roger Brown, D.V.M., Pathologist
J. Gauchat, Pathology Coordinator

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

William D. Theriault, Ph.D.
Project Manager
Abigail C. Jacobs, Ph.D.
Senior Scientist
John Warner, M.S.
Chemist/Statistician

Precedin

I. INTRODUCTION

Use and Production
Environmental Occurrence and Human Exposure
Absorption, Distribution, and Metabolism
Effects in Animals
Reproductive and Teratogenic Effects
Carcinogenicity Studies
Mutagenicity
Study Rationale

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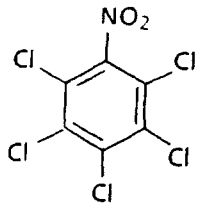
I. INTRODUCTION



PENTACHLORONITROBENZENE
CAS No. 82-68-8

$C_6Cl_5NO_2$ Molecular weight 295.36

Synonyms or Trade Names: Avicol®, PCNB, quintozene, Botrilex®, Brassicol®, Folosan®, PKhNB, Tilcarex®, Terraclor®, Tritisan®

Pentachloronitrobenzene was first synthesized in 1868; it became widely used as a fungicide both in the United States and abroad in the 1950's (IARC, 1974; WHO, 1984). A registration for technical-grade pentachloronitrobenzene as a soil fumigant and seed treatment was issued in 1959 (USEPA, 1976). The pentachloronitrobenzene used in these NTP studies was greater than 99% pure and contained less than 0.07% hexachlorobenzene (Appendix D). Some physical properties of this compound are listed in Table 1.

Use and Production

Pentachloronitrobenzene is used as a soil fumigant for crops such as cotton, peanuts, barley, corn, oats, peas, wheat, and rice; vegetables such as beans, broccoli, lettuce, brussels sprouts, and potatoes; ornamental plants such as azaleas, roses, and carnations; and fruits such as bananas. The primary uses of pentachloronitrobenzene include the control of Rhizoctonia in cotton and Sclerotium in peanuts. Approximately 2 million pounds of pentachloronitrobenzene are used annually in the United States for agricultural purposes (personal communication from J. Blasco, Uniroyal, to J. Dunnick, NTP, 1985).

Environmental Occurrence and Human Exposure

Through its use as a fungicide, pentachloronitrobenzene has the potential to contaminate food, soil, and water; the general population could be exposed via ingestion of contaminated food and water. Occupational exposure could occur in the manufacturing of the chemical or during its application to crops. The U.S. Environmental Protection Agency (USEPA, 1976) reported that pentachloronitrobenzene and its metabolites have been found in soil and vegetables. Studies have been conducted with radioactive tracers to determine the long-term environmental fate of pentachloronitrobenzene. Pentachloronitrobenzene has been reported to have the potential to concentrate in fish (Schauerte et al., 1982; Kanazawa, 1981). The EPA has established an

TABLE 1. PHYSICAL PROPERTIES OF PENTACHLORONITROBENZENE (a)

Physical state:	Pale yellow to white solid, depending on purity
Melting point:	142°-146°C
Boiling point:	328°C at 760 mm Hg (some decomposition occurs)
Vapor pressure:	1.61×10^{-3} mm Hg at 10°C
	5.0×10^{-3} mm Hg at 20°C
	11.3×10^{-3} mm Hg at 25°C
Specific gravity:	1.718 at 25°C
Solubility:	Freely soluble in carbon disulfide, benzene, chloroform, ketones, and aromatic and chlorinated hydrocarbons; slightly soluble in alkanols; water, 0.44 mg/liter at 20°C; ethanol, 2 mg/liter at 25°C

(a) USEPA, 1976; Spencer, 1973; Merck, 1983

interim tolerance for residues of pentachloronitrobenzene of 1.0 ppm on peanuts and of 0.1 ppm on other crops (Fed. Reg., 1977). Pentachloronitrobenzene is believed to be a skin irritant and sensitizer, leading to contact dermatitis (Hayes, 1982).

The EPA has asked for voluntary registration of all pentachloronitrobenzene users and for the reduction of the percentage of hexachlorobenzene to 0.1%. Available studies indicate that the impurity hexachlorobenzene is likely to be responsible for the oncogenicity of pentachloronitrobenzene reported in some of the early animal studies (Fed. Reg., 1982).

Absorption, Distribution, and Metabolism

The metabolism of pentachloronitrobenzene has been studied in micro-organisms, plants, and mammals. Micro-organisms in soil metabolize pentachloronitrobenzene to pentachloroaniline, pentachlorothioanisole, pentachlorobenzene, pentachlorophenol, pentachlorophenylmethyl sulfoxide, and pentachlorophenylmethyl sulfate (Renner, 1981).

Pentachloronitrobenzene is metabolized (1) to sulfur-containing metabolites produced by reaction with glutathione, catalyzed by glutathione *S*-transferase, (2) to non-sulfur-containing metabolites by denitration to pentachlorophenol, and (3) by reduction to pentachloroaniline (O'Grodnick et al., 1981).

Oral administration of radiolabeled pentachloronitrobenzene to rhesus monkeys showed that major metabolites in urine and feces include pentachloroaniline, pentachlorobenzene, and pentachlorophenol (Kogel et al., 1979a). Rhesus monkeys fed diets containing 2 ppm pentachloronitrobenzene for 70 days had no accumulation of pentachloronitrobenzene and no adverse effects (Kogel et al., 1979b). Metabolic products identified in the urine of rabbits administered pentachloronitrobenzene by gavage included pentachloroaniline, *N*-acetyl-*S*-pentachlorophenylcysteine, and pentachlorophenol (Betts et al., 1955). Oral administration of [^{14}C]pentachloronitrobenzene (5 mg/kg in cottonseed oil by gavage) to male and female Osborne-Mendel rats resulted in the elimination of 69%-77% of the radioactivity in 48 hours. By 144 hours, female rats had excreted 33% in urine and 54% in feces, and male rats had excreted 10% in urine and 72% in feces. The major metabolite (59%) in urine of female rats was *N*-acetyl-*S*-pentachlorophenylcysteine; small amounts of pentachloroaniline, pentachloroaniline conjugates, pentachlorophenol, and pentachlorophenyl sulfide were also detected (O'Grodnick et al., 1981).

Hexachlorobenzene and pentachloronitrobenzene are both metabolized to similar metabolites, but in contrast to hexachlorobenzene, pentachloronitrobenzene is not stored in adipose tissue (Renner, 1981; Borzelleca et al., 1971).

Effects in Animals

The oral LD_{50} value for pentachloronitrobenzene was reported to be 1,650 mg/kg in female rats, 1,710 mg/kg in male rats, and 800 mg/kg in rabbits (Finnegan et al., 1958). The LD_{50} value for pentachloronitrobenzene administered intraperitoneally to mice was 4.5 g/kg (Renner and Nguyen, 1982). Albino rats fed dietary concentrations of up to 5,000 ppm of a dust containing 20% pentachloronitrobenzene (approximately 1,000 ppm) for 3 months lost weight at 2,500 and 5,000 ppm (animals in the 5,000-ppm group were killed at 2 weeks); significant dose-related increases in liver weight to body weight ratios were found (due primarily to lower body weight); histologic examinations were not performed (Finnegan et al., 1958).

Reproductive and Teratogenic Effects

Pentachloronitrobenzene administered orally to Wistar rats at doses of 0, 50, 100, or 200 mg/kg on days 6-15 of gestation was not teratogenic and resulted in no detectable levels of the chemical in maternal or fetal tissue (Villeneuve and Khera, 1975; Khera and Villeneuve, 1975). Pentachloronitrobenzene contaminated with hexachlorobenzene was teratogenic in C57BL/6 mice, whereas purified pentachloronitrobenzene was not (Courtney et al., 1976). Pentachloronitrobenzene administered orally at 8, 20, 50, or 125 mg/kg on days 6-15 of gestation was not teratogenic in Charles River CD rats (Jordan et al., 1975).

CD rats were used in a three-generation study in which each generation was fed a diet containing 0, 5, 50, or 500 ppm of pentachloronitrobenzene. No adverse effects on reproduction, including fertility, fetus viability, or litter size, were observed. In the F_3 generation, the offspring were examined for structural or histopathologic defects; none was found. No maternal toxicity was reported (Borzelleca et al., 1971).

Carcinogenicity Studies

The EPA (Fed. Reg., 1977) reported carcinogenicity studies conducted by the Central Institute for Nutrition and Food Research in which a technical-grade pentachloronitrobenzene containing 2.7% hexachlorobenzene was fed to rats for 104 weeks and to mice for 80 weeks at 0, 100, 400, or 1,200 ppm in the diet. Histologic examinations were performed on gross lesions and on the liver and lung. An increased incidence of subcutaneous fibromas or fibrosarcomas was observed in female mice at 1,200 ppm. No compound-related tumors were reported in male and female rats or male mice.

Innes et al. (1969) conducted a carcinogenicity study of pentachloronitrobenzene in male and female C57BL/6 × C3H/Anf F_1 mice and in male and female C57BL/6 × AKR F_1 mice. The purity of the pentachloronitrobenzene was not specified, but the EPA (Fed. Reg., 1977) reported that an 11% hexachlorobenzene content was suspected. Male and female mice were administered pentachloronitrobenzene at 464 mg/kg by gavage starting at 7 days of age and continuing until 28 days of age, and then in the diet at 1,206 ppm until 78 weeks of age. A compound-related increase in liver tumors was seen in dosed C57BL/6 × AKR F_1 male mice.

Searle (1966) reported that pentachloronitrobenzene (purity unspecified) acted as a skin tumor initiator when applied to the backs of albino mice at 0.3% in acetone twice a week for 12 weeks followed by an application of croton oil as a promoter agent for 20 weeks.

The NCI (1978) reported a 2-year study with technical-grade pentachloronitrobenzene. (The supplier reported a 1.0% level of hexachlorobenzene [Fed. Reg., 1977].) Osborne-Mendel rats received time-weighted-average dietary concentrations of pentachloronitrobenzene of 0, 5,417, or 10,064 ppm (male) and 0, 7,857, or 14,635 ppm (female); B6C3F_1 mice received average dietary concentrations of 0, 2,606, or 5,213 ppm (male) and 0, 4,093, or 8,187 ppm (female). The compound was administered for 78 weeks followed by a 33- to 35-week observation period for rats and a 14- to 15-week observation period for mice. Each dose group contained 50 animals; control groups contained 20 animals. Survival in male mice was low; at the end of the study, only 4/20 control, 25/50 low dose, and 17/50 high dose male mice were alive. Survival at the end of the studies for the other groups was as follows: female mice--control, 19/20; low dose, 43/50; high dose, 39/50; male rats--control, 10/20; low dose, 21/50; high dose, 32/50; female rats--control, 16/20; low dose, 40/50; high dose, 37/50. Under the conditions of these studies, pentachloronitrobenzene was not carcinogenic in either Osborne-Mendel rats or B6C3F_1 mice. (Not all livers from the dosed female mice were examined histologically.) The World Health Organization reviewed the available carcinogenicity data for pentachloronitrobenzene and concluded that adequate carcinogenicity studies are needed (WHO, 1984).

Mutagenicity

The available literature on the genotoxicity of pentachloronitrobenzene indicates little positive mutagenic activity. Pentachloronitrobenzene was reported by Clarke (1971) to induce gene reversion in *Escherichia coli* WP2 hcr$^-$. This finding was not supported by other investigators (Simmon, 1978; Moriya et al., 1983). Host-mediated assays performed by Buselmaier et al. (1972) demonstrated no mutagenic activity for pentachloronitrobenzene tested with both *Salmonella typhimurium* G46 and *Serratia marcescens* A21 injected into the peritoneal cavity of mice. Simmon et al. (1977) and Simmon (1978) examined the mutagenic capabilities of pentachloronitrobenzene with an extensive battery of microbial assays that included *E. coli* WP2, *Bacillus subtilis*, *S. typhimurium*, and *S. cerevisiae* D3 with and without Aroclor 1254-induced rat liver S9. Pentachloronitrobenzene was not mutagenic in any of the systems studied. Moriya et al. (1983), following the protocol of Ames et al.

(1975), also demonstrated no mutagenic activity of pentachloronitrobenzene in *S. typhimurium* strains TA100, TA1535, TA1537, TA1538, or TA98.

Pentachloronitrobenzene did not induce sex-linked mutations in *Drosophila melanogaster* (Vogel and Chandler, 1974; Valencia, 1977; Paradi and Lovenyak, 1981). It was reported to cause chromosomal fragmentation in barley seedlings (George et al., 1970), but no dose-response relationship was demonstrable. However, pentachloronitrobenzene induced somatic segregation in a diploid strain of *Aspergillus nidulans* (Georgopoulos et al., 1976). Pentachloronitrobenzene was shown not to induce unscheduled DNA synthesis in human fibroblast WI-38 cells with or without metabolic activation (Simmon, 1978). Simmon (1978) also reported no mutagenic activity for pentachloronitrobenzene in a dominant lethal test with mice.

NTP mutagenicity studies of pentachloronitrobenzene indicated no mutagenic activity but clastogenic capability (Appendix C). Pentachloronitrobenzene was not mutagenic when tested according to the preincubation protocol in *S. typhimurium* strains TA100, TA1535, TA1537, or TA98 (Appendix C, Table C1). Pentachloronitrobenzene was tested with and without metabolic activation by Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 (Tables C2 and C3). Tests for cytogenetic effects in Chinese hamster ovary (CHO) cells indicated that pentachloronitrobenzene did not cause an elevated level of sister-chromatid exchanges in either the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Table C4). Pentachloronitrobenzene did cause an increase in the number of chromosomal aberrations in CHO cells both with and without metabolic activation (Table C5).

Study Rationale

These 2-year studies of pentachloronitrobenzene in $B6C3F_1$ mice were initiated because the previous NCI 2-year studies in mice had low survival in male mice, incomplete examination of livers from dosed female mice, and small control group size. The chemical was administered in feed because exposure of the general population is probably through ingestion of contaminated food and water supplies.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PENTACHLORONITROBENZENE

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

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II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PENTACHLORONITROBENZENE

Pentachloronitrobenzene was obtained in a single lot from the Olin Corporation (Little Rock, Arkansas). Chemical purity and identity analyses were conducted at Midwest Research Institute (Appendix D). The identity of the study material was confirmed by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectroscopic data were consistent with the structure of pentachloronitrobenzene. The purity of this lot was determined to be greater than 99% by elemental analysis, water analysis, thin-layer chromatography, and gas chromatography. Total impurity content by gas chromatography was approximately 0.4%. Analysis by gas chromatography/mass spectrometry indicated that this lot of pentachloronitrobenzene contained 0.07% hexachlorobenzene, which is consistent with manufacturers' specifications for this material.

Pentachloronitrobenzene was determined to be stable when stored for 2 weeks at 60° C (Appendix D). Pentachloronitrobenzene was stored at 0° ± 5° C. Periodic characterization of pentachloronitrobenzene by infrared spectroscopy and gas chromatography detected no deterioration over the course of the studies.

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diet mixtures were shown to be homogeneous (Appendix E). Pentachloronitrobenzene was stable in feed when stored for 2 weeks at room temperature. Formulated diets were prepared by adding a dry premix of feed and pentachloronitrobenzene to the appropriate amount of feed necessary to achieve the desired final concentrations (Table 2). The formulated diets were stored for no longer than 2 weeks.

Periodic analyses for pentachloronitrobenzene in feed were performed by the study and analytical chemistry laboratories to confirm that the correct concentrations were mixed (Appendix F). The method of analysis involved an acetonitrile extraction of the dosed feed and gas chromatography. All of the feed mixtures analyzed had pentachloronitrobenzene concentrations within ± 10% of the target concentrations (Table 3; Appendix G, Table G1).

TABLE 2. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF PENTACHLORONITROBENZENE

	Thirteen-Week Studies	Two-Year Studies
Preparation	Premix of chemical and feed mixed in a mortar with a pestle. Premix layered between the remaining meal in a pin-bar intensifier model Patterson-Kelly® V-blender and mixed for 15 min	Same as 13-wk studies
Maximum Storage Time	2 wk	2 wk
Storage Conditions	0° ± 5° (in the dark)	Same as 13-wk studies

TABLE 3. SUMMARY OF RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE

	Concentration of Pentachloronitrobenzene in Feed for Target Concentration (ppm)	
	2,500	5,000
Mean (ppm)	2,460	4,980
Standard deviation	86	160
Coefficient of variation (percent)	3.5	3.2
Range (ppm)	2,350-2,610	4,700-5,200
Number of samples	14	14

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of dietary administration of pentachloronitrobenzene and to determine the concentrations to be used in the 2-year studies.

Five- to six-week-old male and female $B6C3F_1$ mice were obtained from Harlan Industries, observed for 20 days, and then assigned to cages such that the average cage weights for mice of each sex were approximately equal. Diets containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm pentachloronitrobenzene were fed to groups of 10 male mice. Diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm were fed to groups of 10 female mice. These doses were selected to approximate those used in previous 8-week studies in $B6C3F_1$ mice (NCI, 1978).

Animals were checked two times per day; moribund animals were killed. Feed consumption was measured weekly by cage. Individual animal weights were recorded weekly. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 4.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 2,500, or 5,000 ppm pentachloronitrobenzene were fed to groups of 50 male and 50 female mice for 103 weeks followed by a 1-week observation period. (One animal in the male control group was removed because it was found to be female.)

Source and Specifications of Animals

The male and female $B6C3F_1$ (C57BL/6N, female, × C3H/HeN MTV^-, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding stock for the foundation colony at the production facility originated at the National Institutes of Health Repository. Mice shipped for testing were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Mice were shipped to the study laboratory at 5-6 weeks of age. The animals were quarantined at the study facility for 20 days. Thereafter, a complete necropsy was performed on five animals of each sex to assess their health status. The mice were placed on study at 60 days of age. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program (Appendix H). Bacterial cultures were taken for ovarian abscesses of six animals.

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid $B6C3F_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

TABLE 4. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF PENTACHLORONITROBENZENE

	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups	10 males and 10 females	49 or 50 males and 50 females
Doses	Male--0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm pentachloronitrobenzene in feed; female--0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm pentachloronitrobenzene in feed	0, 2,500, or 5,000 ppm pentachloronitrobenzene in feed
Date of First Dose	5/6/80	6/9/81
Date of Last Dose	8/5/80-8/7/80	5/31/83
Duration of Dosing	13 wk	103 wk
Type and Frequency of Observation	Observed 2 × d; weighed 1 × wk	Same as 13-wk studies except weighed 1 × wk for 12 wk; monthly thereafter
Necropsy and Histologic Examination	Necropsy and histologic examination performed on all animals; the following tissues examined from control and high dose groups: gross lesions and tissue masses, mandibular lymph nodes, mammary gland, skin, salivary gland, sternebrae, thyroid gland, parathyroids, small intestine, colon, liver, skin, prostate/testes or ovaries/uterus, lungs and bronchi, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, pituitary gland, spinal cord (if neurologic signs present), and eyes (if grossly abnormal).	Necropsy and histologic examination performed on all animals; the following tissues examined from all control, low dose female mice, and high dose animals, and all low dose male mice dying early: tissue masses, skin, abnormal regional lymph nodes, mammary gland, salivary gland, bone marrow, costochondral junction, thymus, larynx, trachea, lungs and bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, brain, pituitary gland, and cecum. In addition, gross lesions, livers, and nasal cavities examined for all low dose male mice.
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species	$B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source	Harlan Industries (Indianapolis, IN)	Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory	EG&G Mason Research Institute	Same as 13-wk studies
Method of Animal Identification	Ear punch	Ear punch
Time Held Before Study	20 d	20 d
Age When Placed on Study	8-9 wk	8-9 wk
Age When Killed	21-22 wk	112-113 wk
Necropsy Dates	8/5/80-8/7/80	6/7/83-6/10/83

TABLE 4. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF OF PENTACHLORONITROBENZENE (Continued)

	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Method of Animal Distribution	All animals assigned to weight classes; animals at extreme ends discarded, and remaining animals of each sex distributed such that the average body weights for each group approximately equal.	Same as 13-wk studies
Feed	NIH 07 Rat and Mouse Ration (Zeigler Bros., Gardners, PA); available ad libitum	Same as 13-wk studies
Bedding	Aspen-bed hardwood chips (American Excelsior Co., Baltimore, MD) used when available; otherwise Beta hardwood chips (Agway, Inc., Syracuse, NY) used.	Aspen-bed heat-treated hardwood chips (American Excelsior Co., Baltimore, MD)
Water	Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 13-wk studies
Cages	Polycarbonate (Lab Products, Rochelle Park, NJ)	Same as 13-wk studies
Cage Filters	Nonwoven fiber filter (Snow Filtration, Cincinnati, OH)	Same as 13-wk studies
Animals per Cage	5	5
Other Chemicals on Study in the Same Room	None	None
Animal Room Environment	Temp--17°-30° C, mean 23° C; hum--54%-80%, mean 76%; fluorescent light 12 h/d; 10-12 room air changes/h	Temp--22.9° C (mean); hum--41.6% (mean); fluorescent light 12 h/d; 15.2 room air changes/h

The C57BL/6 mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6 colony were used as parents for the hybrid $B6C3F_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

The animals were housed five per cage. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 4.

Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded once per week. Body weights by cage were recorded once per week for the first 12 weeks of the studies and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the

studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed or cannibalized, missexed, or found missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of tissues was performed according to the "inverse pyramid" design (McConnell, 1983a,b). Complete histopathologic examinations (Table 4) were performed on high dose and control animals and on all animals dying early in the studies, including those in lower dose groups. In addition, histopathologic examinations were performed on all gross lesions and tissues/organs from animals in the lower dose groups when chemically related neoplastic or nonneoplastic effects were identified in the high dose animals. If mortality in a high dose group exceeded that in the control group by 15%, complete histopathologic examinations were performed on all of the animals in the second highest dose group in addition to those in the high dose group.

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

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

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

Statistical Methods

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

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses

using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

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

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

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

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

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

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

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

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III. RESULTS

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

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THIRTEEN-WEEK STUDIES

All the female mice that received 40,000 ppm pentachloronitrobenzene died before the end of the studies (Table 5). The final mean body weight of mice that received 10,000 or 20,000 ppm was 7% or 8% lower than that of the controls for males and 5% or 7% lower for females. Data on feed consumption by the dosed groups (collected from group-housed animals) suggest that the dosed groups consumed more feed than did the control groups (Table 5), but scattering of feed (which could not be measured accurately) affects the interpretation of these data. The liver weight to body weight ratios in all dosed groups were increased compared with those of controls (Table 6). The only compound-related clinical signs reported were small body size and emaciation in 10/10 males at 20,000 ppm and 10/10 females at 20,000 and 40,000 ppm.

Lymphoid depletion of the spleen, mesenteric lymph node, or thymus was seen in 8/9 female mice examined in the 40,000-ppm group. No compound-related lesions were seen in male mice.

A spectrum of inflammatory and proliferative lesions was seen in the lung of all dosed groups and was considered consistent with that associated with Sendai virus infection. The lung lesions found in both control and dosed animals included acute inflammation of bronchioles and alveolar epithelial hyperplasia.

Dose Selection Rationale: Because of weight gain depression in males and females at 10,000 and 20,000 ppm and deaths and histopathologic effects at 40,000 ppm in the female mice, concentrations selected for mice for the 2-year studies were 2,500 and 5,000 ppm pentachloronitrobenzene in feed.

TABLE 5. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLORONITROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 12
MALE							
0	10/10	22.8 ± 0.7	29.9 ± 0.9	+ 7.1 ± 0.6	--	4.3	5.2
1,250	10/10	22.6 ± 0.4	30.8 ± 0.6	+ 8.2 ± 0.5	103.0	5.0	7.1
2,500	10/10	23.2 ± 0.5	30.2 ± 0.5	+ 7.0 ± 0.3	101.0	4.6	6.1
5,000	10/10	22.5 ± 0.4	30.0 ± 0.5	+ 7.5 ± 0.3	100.3	4.7	7.5
10,000	10/10	22.4 ± 0.6	27.8 ± 0.4	+ 5.3 ± 0.6	92.6	5.2	8.3
20,000	10/10	22.3 ± 0.6	27.5 ± 0.7	+ 5.2 ± 0.5	92.0	7.3	8.6
FEMALE							
0	10/10	18.7 ± 0.3	25.9 ± 0.5	+ 7.2 ± 0.4	--	4.4	4.7
2,500	10/10	19.0 ± 0.2	25.9 ± 0.5	+ 6.9 ± 0.4	100.0	4.9	5.7
5,000	10/10	18.8 ± 0.3	24.9 ± 0.3	+ 6.1 ± 0.2	96.1	5.3	7.8
10,000	10/10	19.0 ± 0.2	24.7 ± 0.4	+ 5.7 ± 0.3	95.4	7.3	8.0
20,000	10/10	18.8 ± 0.3	24.1 ± 0.3	+ 5.3 ± 0.3	93.1	8.3	9.2
40,000	(e) 0/10	18.8 ± 0.3	(f)	(f)	(f)	(f)	(f)

(a) Number surviving/number in group
(b) Initial mean group body weight ± standard error of the mean
(c) Mean weight change of the group ± standard error of the mean
(d) Grams of feed consumed per animal per day
(e) Week of death: all died within the first 2 weeks
(f) No data are reported due to the 100% mortality in this group.

TABLE 6. ABSOLUTE AND RELATIVE LIVER WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLORONITROBENZENE (a)

Concentration (ppm)	Liver Weight (grams)	Liver Weight/ Necropsy Body Weight Ratio (mg/g)
MALE		
0	1.52 ± 0.27	52.3 ± 7.08
1,250	(b) 1.83 ± 0.23	58.6 ± 5.09
2,500	(c) 2.14 ± 0.27	(c) 69.2 ± 6.79
5,000	(c) 2.14 ± 0.27	(c) 70.1 ± 8.08
10,000	1.74 ± 0.12	(c) 63.1 ± 4.37
20,000	1.67 ± 0.18	(c) 62.8 ± 6.34
FEMALE		
0	1.31 ± 0.13	52.2 ± 4.73
2,500	(b) 1.56 ± 0.14	60.7 ± 5.49
5,000	(c) 1.70 ± 0.23	(c) 66.6 ± 8.05
10,000	(c) 1.73 ± 0.29	(c) 68.5 ± 11.00
20,000	1.51 ± 0.20	(c) 65.0 ± 7.06

(a) Mean ± standard deviation
(b) $P<0.05$ by Dunnett's test (Dunnett, 1955)
(c) $P<0.01$ by Dunnett's test

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Final mean body weights of low dose and high dose male mice were 96% and 90% that of controls (Table 7 and Figure 1). The mean body weights of high dose female mice were more than 10% lower than those of the controls after week 20 and 21% lower by week 104. Mean body weights of low dose female mice were more than 10% lower than those of the controls after week 88. No compound-related clinical observations were reported.

The estimated average daily feed consumption by group-housed mice is listed in Appendix I. The data suggest that the dosed groups consumed more feed at certain time points than did the corresponding controls, but some feed was scattered, and it is not possible to determine if dosed groups actually ate more feed than did the controls. Based on these data, the average amount of pentachloronitrobenzene consumed per day was estimated to be 400 mg/kg or 1,000 mg/kg for low dose and high dose male mice and 600 mg/kg or 1,400 mg/kg for low dose and high dose female mice (Tables I1 and I2). The expected feed consumption in control $B6C3F_1$ mice is 4 g per mouse per day; thus, this estimation of pentachloronitrobenzene consumed is probably high due to feed scattering.

TABLE 7. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE

Weeks on Study	Control		2,500 ppm			5,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	25.2	50	25.6	102	50	25.2	100	50
1	28.2	50	28.6	101	50	27.9	99	50
2	28.4	49	28.9	102	50	28.3	100	50
3	29.8	49	30.1	101	50	29.2	98	50
4	30.6	49	31.0	101	50	30.1	98	50
5	31.0	49	31.4	101	50	30.4	98	50
6	32.0	49	32.3	101	50	31.2	98	50
7	32.1	49	32.4	101	50	31.4	98	50
8	33.7	48	33.4	99	50	31.9	95	50
9	33.5	48	35.0	104	50	32.6	97	50
10	34.8	48	34.6	99	50	33.1	95	50
11	34.4	48	33.6	98	50	32.6	95	50
12	34.6	48	34.6	100	50	33.1	96	50
16	35.8	48	35.8	100	50	34.1	95	50
20	37.3	48	37.1	99	50	35.5	95	49
24	38.6	48	38.5	100	50	36.2	94	49
28	39.5	48	38.8	98	50	36.7	93	48
32	38.0	46	38.1	100	50	36.0	95	48
36	41.6	48	40.5	97	50	37.9	91	48
40	42.7	48	40.9	96	50	38.4	90	48
44	43.5	48	42.3	97	50	38.4	88	48
48	43.2	48	41.9	97	50	38.9	90	48
52	43.1	48	41.5	96	50	38.7	90	48
56	43.0	48	41.8	97	49	38.7	90	47
61	41.6	48	41.4	100	49	38.4	92	47
64	42.4	48	42.7	101	49	38.4	91	45
68	40.9	48	41.1	100	49	38.9	95	45
72	41.1	46	40.6	99	46	38.4	93	45
76	41.8	47	42.8	102	46	38.7	93	44
80	41.3	46	41.3	100	46	38.0	92	44
84	39.9	46	39.9	100	46	37.4	94	42
88	40.2	46	40.1	100	46	38.1	95	41
92	40.5	42	39.9	99	39	36.1	89	39
96	39.1	41	38.8	99	36	37.4	96	35
100	38.7	38	39.0	101	32	37.2	96	33
104	40.6	34	39.0	96	31	36.7	90	32
FEMALE								
0	19.3	50	18.9	98	50	19.3	100	50
1	19.7	50	17.3	88	50	17.9	91	50
2	21.8	50	21.5	99	50	22.0	101	50
3	22.5	50	22.4	100	50	22.9	102	50
4	23.7	50	23.1	97	50	23.3	98	50
5	23.9	50	23.3	97	50	23.8	100	50
6	24.8	50	24.2	98	50	24.6	99	50
7	25.5	50	24.6	96	50	24.9	98	50
8	26.3	50	25.4	97	50	25.4	97	50
9	27.8	50	28.4	102	50	26.3	95	50
10	27.0	50	26.1	97	50	26.2	97	50
11	27.5	50	25.9	94	50	26.0	95	50
12	27.9	50	26.3	94	50	26.6	95	50
16	29.5	50	28.3	96	50	28.0	95	50
20	31.9	50	29.9	94	50	29.5	92	50
24	34.5	50	31.7	92	50	30.6	89	50
28	35.6	50	33.2	93	50	32.0	90	50
32	35.0	50	32.5	93	50	31.2	89	50
36	37.8	50	35.0	93	50	34.0	90	50
40	39.9	50	36.3	91	49	34.7	87	50
44	41.8	50	37.8	91	49	36.3	86	50
48	42.9	49	39.3	92	48	37.4	[illegible]	50
52	43.6	49	39.6	[illegible]	[illegible]	[illegible]	[illegible]	49
56	42.7	49	39.9	[illegible]	48	[illegible]	[illegible]	47
61	41.9	49	39.7	[illegible]	48	[illegible]	[illegible]	46
64	43.9	47	39.8	[illegible]	44	[illegible]	[illegible]	46
68	44.4	46	39.7	[illegible]	[illegible]	[illegible]	[illegible]	41
72	43.5	44	39.7	91	41	[illegible]	[illegible]	41
76	46.1	44	41.0	89	41	38.1	83	40
80	46.3	43	42.5	92	36	38.0	82	38
84	45.9	38	42.2	92	34	37.1	81	36
88	46.2	37	41.5	90	26	37.9	82	30
92	48.4	33	41.4	86	26	36.6	76	25
96	47.7	32	41.4	87	21	38.4	81	22
100	49.8	31	41.6	84	20	37.9	76	20
104	49.2	30	40.5	82	20	39.0	79	18
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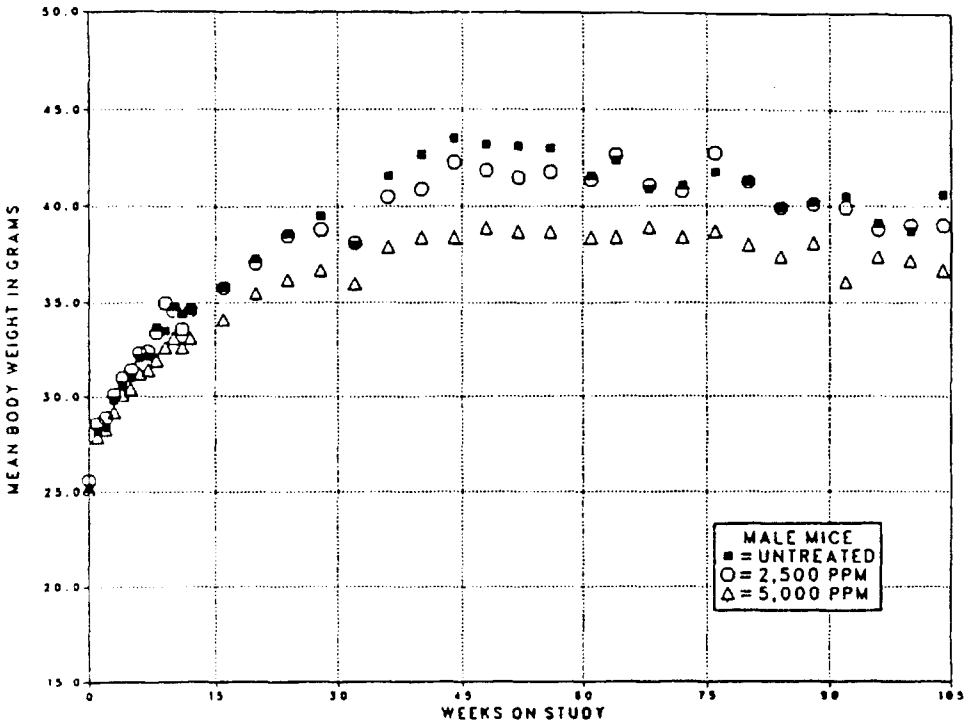


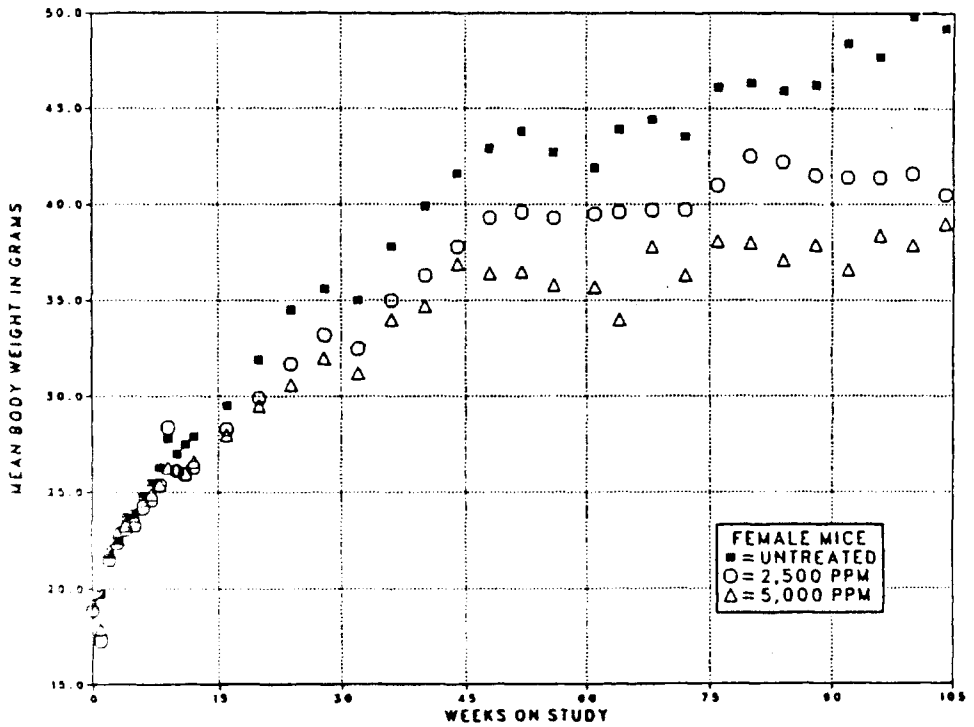


FIGURE 1. GROWTH CURVES FOR MICE FED DIETS CONTAINING PENTACHLORONITROBENZENE FOR TWO YEARS

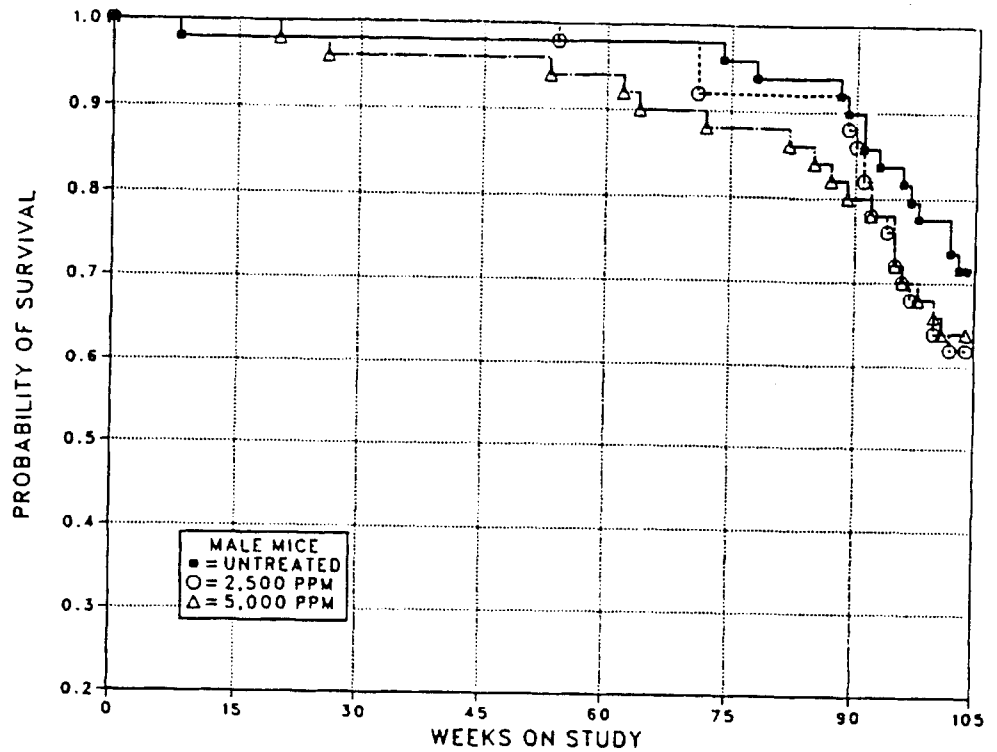
Survival

Estimates of the probabilities of survival for male and female mice fed diets containing pentachloronitrobenzene at the concentrations used in these studies and for the controls are shown in the Kaplan and Meier curves in Figure 2. The survival of the high dose group of female mice was significantly lower than that of the controls after week 86 (Table 8). No other significant differences in survival were observed between any groups of either sex.

TABLE 8. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE

	Control	2,500 ppm	5,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	14	19	18
Animals missexed	1	0	0
Killed at termination	34	31	32
Died during termination period	1	0	0
Survival P values (c)	0.401	0.388	0.451
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	20	25	35
Accidentally killed	0	(d) 5	0
Killed at termination	30	18	14
Died during termination period	0	2	1
Survival P values (c)	0.004	0.261	0.005

(a) Terminal-kill period: week 104
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.
(d) Deaths due to drowning



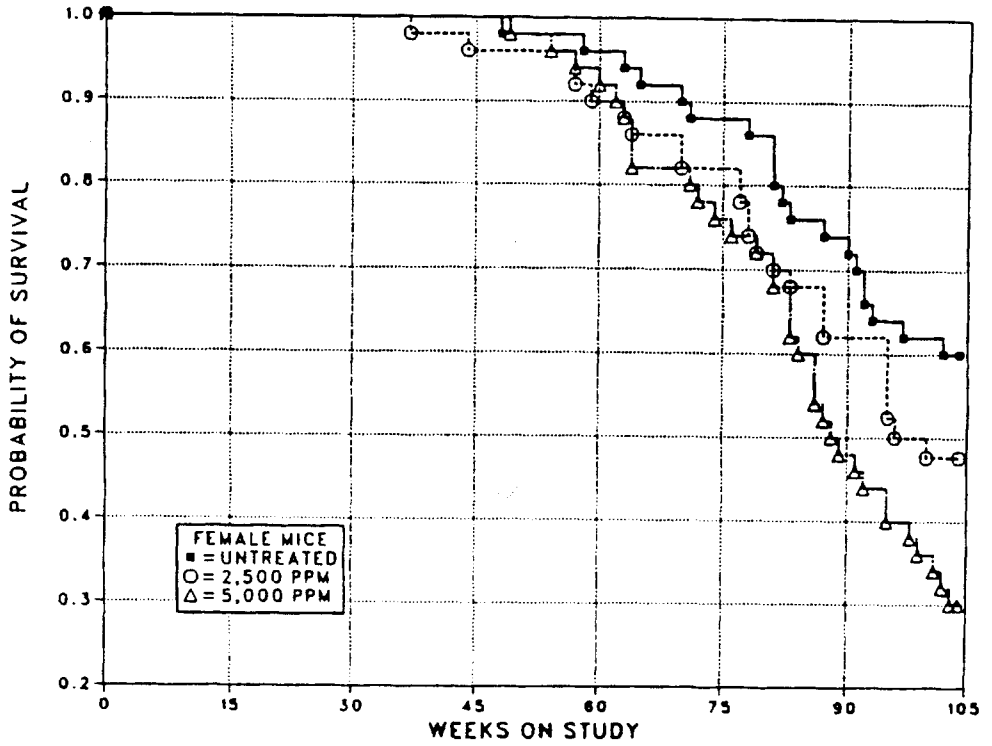


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING PENTACHLORONITROBENZENE FOR TWO YEARS

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the ovary, hematopoietic system, liver, and spleen.

Lesions in male mice are summarized in Appendix A. Histopathologic findings on neoplasms are summarized in Table A1. Table A2 gives the survival and tumor status for individual male mice. Table A3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes). Findings on nonneoplastic lesions are summarized in Table A4.

Lesions in female mice are summarized in Appendix B. Histopathologic findings on neoplasms are summarized in Table B1. Table B2 gives the survival and tumor status for individual female mice. Table B3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table B3 (footnotes). Findings on nonneoplastic lesions are summarized in Table B4.

Ovary: Ovarian abscesses, characteristic of bacterial infection, were observed in all groups of female mice (control, 12/49, 24%; low dose, 22/50, 44%; high dose, 29/50, 58%); the incidences in the dosed groups were significantly ($P<0.05$) greater than that in the controls. Six abscesses were cultured, and the bacteriologic findings indicated Klebsiella in five of the six samples (Appendix H, Table H2).

Hematopoietic System: Plasma cell hyperplasia of the mediastinal lymph node was observed at an increased ($P<0.01$) incidence in high dose female mice (control, 1/44, 2%; low dose, 4/47, 9%; high dose, 9/45, 20%).

Liver: Hematopoiesis was observed at increased ($P<0.01$) incidences in dosed female mice: control, 9/50 (18%); low dose, 21/50 (42%); high dose, 23/50 (46%). This effect was not seen in male mice (control, 0/49; low dose, 2/50, 4%; high dose, 0/50).

Spleen: Hematopoiesis was observed at increased ($P<0.05$) incidences in dosed female mice: control 14/50 (28%); low dose, 23/48 (48%); high dose, 27/50 (54%).

IV. DISCUSSION AND CONCLUSIONS

Study Design
Survival and Body Weight
Pathologic Findings
Mutagenicity

IV. DISCUSSION AND CONCLUSIONS

Study Design

Studies of the toxicology and carcinogenicity of pentachloronitrobenzene were conducted in $B6C3F_1$ mice of each sex because previous pentachloronitrobezene mouse studies (NCI, 1978) had low male mouse survival, incomplete histologic examination of livers from dosed female mice, and small control group size. In the present 2-year studies, pentachloronitrobenzene was administered in feed at concentrations of 0, 2,500, or 5,000 ppm to male and female $B6C3F_1$ mice for 103 weeks. These doses for the 2-year studies were selected because in the 13-week studies, in which the chemical was administered in feed at doses up to 20,000 ppm in male mice and up to 40,000 ppm in female mice, weight gain depression was seen at 10,000 ppm and above in males and females and deaths occurred at 40,000 ppm in females. Liver weight to body weight ratios were increased in dosed groups, although no compound-related lesions were seen in the liver. Compound-related histopathologic alterations seen in the 13-week studies were limited to lymphoid depletion in the spleen, lymph nodes, and thymus in females at 40,000 ppm; no compound-related histopathologic lesions were seen in male mice.

Survival and Body Weight

Survival of male mice in the 2-year studies was comparable in control and dosed groups (control, 35/50; low dose, 31/50; high dose, 32/50). Final mean body weights of low dose and high dose male mice were 96% and 90% that of controls.

All groups of female mice showed evidence of bacterial infection. Klebsiella was isolated from five of six ovarian abscesses cultured. Ovarian lesions, primarily associated with Klebsiella, have been seen in other female $B6C3F_1$ mice, and the incidence of this infection reaches a peak in animals 23-26 months of age (Rao et al., 1986 [draft]). At week 84, 38/50 control, 34/50 low dose, and 30/50 high dose female mice were still alive; after week 84, survival in dosed groups decreased and the deaths were attributed to the combination of chemical administration and infection (final survival: control, 30/50; low dose, 20/50; high dose, 15/50). The mean body weight of high dose female mice was greater than 10% lower than that of the controls after week 20 and by week 104 was 21% lower than that of controls. The mean body weight of low dose female mice was within 10% of the control group until week 88 and was 18% lower than the controls by week 104. Decreased survival in dosed female mice is considered to have decreased the sensitivity of the study to determine the presence or absence of a carcinogenic response.

Pathologic Findings

Gross and microscopic pathologic findings in female mice were limited to those considered to be secondary to bacterial infection, including hematopoiesis of the liver and spleen, plasma cell hyperplasia of the mediastinal lymph nodes, and ovarian abscesses (control, 12/49; low dose, 22/50; high dose, 29/50). The association between the lesions in the liver, spleen, and lymph nodes and the presence of ovarian abscess in the same animal was significant at $P \leq 0.05$ in each dosed group. The majority of animals with ovarian abscess died before study termination (control, 11/12; low dose, 18/22; high dose, 27/29). Ovarian infection was seen in all groups of female mice, but the incidence was higher in pentachloronitrobenzene-dosed animals, indicating that pentachloronitrobenzene might predispose the female mouse to infection.

No compound-related neoplastic lesions were seen in any organ system in either male or female mice. There was no indication of a carcinogenic response in the liver of mice, although it had been previously reported by Innes et al. (1969), who used pentachloronitrobenzene with a suspected hexachlorobenzene contaminant. These results support the findings of the previous NCI studies (NCI, 1978) in which pentachloronitrobenzene, administered in feed to Osborne-Mendel rats and $B6C3F_1$ mice for 2 years, showed no compound-related neoplastic lesions. The doses for male mice in the current studies and the time-weighted-average dose in the previous NCI studies were similar; the time-weighted-average doses for female mice in the NCI studies were somewhat higher (4,093 and 8,187 ppm) than those in these studies, but the female mice were initially started at doses (2,320 and 4,640 ppm) similar to those used in these studies.

Mutagenicity

The available data suggest that pentachloronitrobenzene does not induce gene mutations, but a few studies indicate that it is clastogenic. George et al. (1970) demonstrated that pentachloronitrobenzene caused chromosomal breaks and anaphase bridge formation in barley seedlings after treatment of seeds with 250 or 500 ppm pentachloronitrobenzene for 1-2 hours. However, no dose response was observed. Georgopoulos et al. (1976) reported a 3%-7% incidence of sectoring in heterozygous colonies of the mold *Aspergillus nidulans* after treatment with pentachloronitrobenzene at concentrations of 5, 10, or 17 µM, indicating that pentachloronitrobenzene induces somatic segregation in this fungal species. However, the relationship between these observations and clastogenicity has not been established. NTP studies show pentachloronitrobenzene to be an inducer of chromosomal aberrations in Chinese hamster ovary cells at doses of 7.5, 24.0, or 75.0 µg/ml both in the presence and absence of metabolic activation. Although 2% of the cells showed complex rearrangements, most of the aberrations observed were simple chromosomal and chromatid breaks. However, in the absence of S9, neither the increases in aberrations (which ranged from approximately fivefold to tenfold over baseline) nor increases in the percentage of cells with aberrations (which ranged from approximately twofold to fourfold over baseline) were correlated to dose. Evidence of genetic toxicity that is limited to induction of chromosomal aberrations and for which the response is either inversely proportional or unrelated to dose is unusual. Further cytogenetic studies, both in vivo and in vitro, would be required to understand the genetic toxicity of pentachloronitrobenzene.

The experimental and tabulated data for the NTP Technical Report on pentachloronitrobenzene were examined for accuracy, consistency, and compliance with Good Laboratory Practice requirements. As summarized in Appendix K, the audit revealed no major problems with the conduct of the studies or with the collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenicity** for either male or female $B6C3F_1$ mice receiving 2,500 or 5,000 ppm of pentachloronitrobenzene. Infection is considered to have decreased survival of the female mice and thus reduced the sensitivity for determining the presence or absence of a carcinogenic response.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 8.

V. REFERENCES

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V. REFERENCES

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

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

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

4. Betts, J.; James, S.; Thorpe, W. (1955) The metabolism of pentachloronitrobenzene and 2:3:4:6-tetrachloronitrobenzene and the formation of mercapturic acids in the rabbit. Biochem. J. 61:611-617.

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

6. Borzelleca, J.; Larson, P.; Crawford, E.; Hennigar, G., Jr.; Kuchar, E.; Klein, H. (1971) Toxicologic and metabolic studies on pentachloronitrobenzene. Toxicol. Appl. Pharmacol. 18:522-534.

7. Buselmaier, V.; Röhrborn, G.; Propping, P. (1972) Mutagenitäts-Untersuchungen mit Pestiziden im Host-mediated Assay und mit dem Dominanten Letaltest an der Maus. Biol. Zbl. 91:311-325.

8. Clarke, C. (1971) The mutagenic specificities of pentachloronitrobenzene and captan, two environmental mutagens. Mutat. Res. 11:247-248.

9. Clive, D.; Johnson, K.; Spector, J.; Batson, A.; Brown, M. (1979) Validation and characterization of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagen assay system. Mutat. Res. 59:61-108.

10. Courtney, K.; Copeland, M.; Robbins, A. (1976) The effects of pentachloronitrobenzene, hexachlorobenzene, and related compounds on fetal development. Toxicol. Appl. Pharmacol. 35:239-256.

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

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

13. Federal Register (Fed. Reg.) (1977) Environmental Protection Agency. Pesticide Programs. Rebuttable presumption against registration and continued registration of pesticide products containing pentachloronitrobenzene (PCNB). 42:56072-56098 (October 20).

14. Federal Register (Fed. Reg.) (1982) Pentachloronitrobenzene (PCNB): Notice of determination concluding the rebuttable presumption against registration. 47:18177-18179 (April 28).

15. Finnegan, J.; Larson, P.; Smith, R., Jr.; Haag, H.; Hennigar, G. (1958) Acute and chronic toxicity studies on pentachloronitrobenzene. Arch. Int. Pharmacodyn. 114:38-52.

16. Galloway, S.; Bloom, A.; Resnick, M.; Margolin, B.; Nakamura, F.; Archer, P.; Zeiger, E. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7:1-51.

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

18. George, M.; Aulakh, K.; Dhesi, J. (1970) Morphological and cytological changes induced in barley *(Hordeum vulgare)* seedlings following seed treatment with fungicides. Can. J. Genet. Cytol. 12:415-419.

19. Georgopoulos, S.; Kappas, A.; Hastie, A. (1976) Induced sectoring in diploid Aspergillus nidulans as a criterion of fungitoxicity by interference with hereditary processes. Phytopathology 66:217-220.

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

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

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

23. Hayes, W., Jr. (1982) Pesticides Studied in Man. Baltimore: Williams and Wilkins, pp. 586-587.

24. Innes, J.; Ulland, B.; Valerio, M.; Petrucelli, L.; Fishbein, L.; Hart, E.; Pallotta, A.; Bates, R.; Falk, H.; Gart, J.; Klein, M.; Mitchell, I.; Peters, J. (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. 42:1101-1114.

25. International Agency for Research on Cancer (IARC) (1974) Quintozene (Pentachloronitrobenzene). Some Organochlorine Pesticides. IARC Monographs on the Evaluation of Carcinogenic Risk on Chemicals to Man. Lyon, France: World Health Organization. 5:211-217.

26. Jordan, R.; Sperling, F.; Klein, H.; Borzelleca, J. (1975) A study of the potential teratogenic effects of pentachloronitrobenzene in rats. Toxicol. Appl. Pharmacol. 33:222-230.

27. Kanazawa, J. (1981) Measurement of the bioconcentration factors of pesticides by freshwater fish and their correlation with physicochemical properties or acute toxicities. Pestic. Sci. 12:417-424.

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

29. Khera, K.; Villeneuve, D. (1975) Teratogenicity studies on halogenated benzenes (pentachloro-, pentachloronitro-, and hexabromo-) in rats. Toxicology 5:117-122.

30. Kogel, W.; Muller, W.; Coulston, F.; Korte, F. (1979a) Biotransformation of pentachloronitrobenzene-^{14}C in Rhesus monkeys after single and chronic oral administration. Chemosphere 2:97-105.

31. Kogel, W.; Muller, W.; Coulston, F.; Korte, F. (1979b) Fate and effects of pentachloronitrobenzene in Rhesus monkeys. J. Agric. Food Chem. 27:1181-1185.

32. Linhart, M.; Cooper, J.; Martin, R.; Page, N.; Peters, J. (1974) Carcinogenesis bioassay data system. Comput. Biomed. Res. 7:230-248.

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

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

35. McConnell, E. (1983a) Pathology requirements for rodent two-year studies. I. A review of current procedures. Toxicol. Pathol. 11:60-64.

36. McConnell, E. (1983b) Pathology requirements for rodent two-year studies. II. Alternative approaches. Toxicol. Pathol. 11:65-76.

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

38. Merck Index (1983) 10th ed. Windholz, M., Ed. Rahway, NJ: Merck and Company, Inc., pp. 1168-1169.

39. Moriya, M.; Ohta, T.; Watanabe, K.; Miyazawa, T.; Kato, K.; Shirasu, Y. (1983) Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. 116:185-216.

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

41. National Cancer Institute (NCI) (1978) Bioassay of Pentachloronitrobenzene for Possible Carcinogenicity. NCI Carcinogenesis Technical Report Series No. 61. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

42. National Institutes of Health (NIH) (1978) NIH Specification, NIH-11-133f, November 1.

43. O'Grodnick, J.; Adamovics, J.; Blake, S.; Wedig, J. (1981) The metabolic fate of ^{14}C-labeled pentachloronitrobenzene in Osborne-Mendell rats. Chemosphere 10:67-72.

44. Paradi, E.; Lovenyak, M. (1981) Studies on genetical effect of pesticides in *Drosophila melanogaster*. Acta Biol. Acad. Sci. Hung. 32:119-122.

45. Rao, G.; Hickman, R.; Seilkop, S.; Boorman, G. (1986) Utero-Ovarian Infection in Aged $B6C3F_1$ Mice. Research Triangle Park, NC: National Toxicology Program, NIEHS (draft).

46. Renner, G. (1981) Biotransformation of the fungicides hexachlorobenzene and pentachloronitrobenzene. Xenobiotica 11:435-446.

47. Renner, G.; Nguyen, P.-T. (1982) Acute toxicities of the fungicide pentachloronitrobenzene and of some of its sulphur-containing metabolites administered to mice. Arch. Toxicol. 51:329-331.

48. Sadtler Standard Spectra, Sadtler Research Laboratories, Philadelphia, PA, IR No. 24813, UV No. 8786.

49. Schauerte, W.; Lay, J.; Klein, W.; Korte, F. (1982) Long-term fate of organochlorine xenobiotics in aquatic ecosystems. Distribution, residual behavior, and metabolism of hexachlorobenzene, pentachloronitrobenzene, and 4-chloroaniline in small experimental ponds. Ecotoxicol. Environ. Safety 6:560-569.

50. Searle, C. (1966) Tumor initiatory activity of some chloromononitrobenzenes and other compounds. Cancer Res. 26:12-17.

51. Simmon, V. (1978) In Vivo and in Vitro Mutagenicity--Assays of Selected Pesticides. NIH 78-1306. U.S. Department of Health, Education, and Welfare, National Institutes of Health, pp. 27-71.

52. Simmon, V.; Mitchell, A.; Jorgenson, T. (1977) Evaluation of Selected Pesticides as Chemical Mutagens--*In Vitro* and *In Vivo* Studies. EPA-600/1-77-028. U.S. Environmental Protection Agency, Office of Research and Development.

53. Spencer, E. (1973) Guide to the Chemicals Used in Crop Protection, 6th ed. Research Branch, Agriculture Canada, pp. 445-446.

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

55. U.S. Environmental Protection Agency (USEPA) (1976) Initial Scientific Review of PCNB. EPA-540/1-75-016. Washington, DC.

56. Valencia, R. (1977) Mutagenesis Screening of Pesticides Using Drosophila. U.S. Environmental Protection Agency, Washington, DC.

57. Villeneuve, D.; Khera, K. (1975) Placental transfer of halogenated benzenes (pentachloro-, pentachloronitro-, and hexabromo-) in rats. Environ. Physiol. Biochem. 328-331.

58. Vogel, E.; Chandler, J. (1974) Mutagenicity testing of cyclamate and some pesticides in *Drosophila melanogaster*. Experientia 30:621-623.

59. World Health Organization (WHO) (1984) Quintozene. Environ. Health Criteria 41:1-38.

APPENDIX A

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

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TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Squamous cell carcinoma	1 (2%)		
*Subcutaneous tissue	(49)	(50)	(50)
Sarcoma, NOS		1 (2%)	1 (2%)
Fibroma	1 (2%)	3 (6%)	1 (2%)
Fibrosarcoma	6 (12%)	8 (16%)	6 (12%)
RESPIRATORY SYSTEM			
#Lung	(49)	(14)	(50)
Carcinoma, NOS, metastatic			1 (2%)
Squamous cell carcinoma, metastatic	1 (2%)		
Hepatocellular carcinoma, metastatic	2 (4%)	1 (7%)	3 (6%)
Alveolar/bronchiolar adenoma	9 (18%)	3 (21%)	5 (10%)
Alveolar/bronchiolar carcinoma		1 (7%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Malignant lymphoma, undiffer type	4 (8%)	2 (4%)	1 (2%)
Malignant lymphoma, lymphocytic type	1 (2%)		
Malignant lymphoma, mixed type		1 (2%)	1 (2%)
#Spleen	(49)	(21)	(49)
Malignant lymphoma, undiffer type	1 (2%)	1 (5%)	
Malignant lymphoma, mixed type		1 (5%)	
#Bronchial lymph node	(45)	(25)	(49)
Alveolar/bronchiolar carcinoma, metastatic			1 (2%)
#Mediastinal lymph node	(45)	(25)	(49)
Squamous cell carcinoma, metastatic	1 (2%)		
#Mesenteric lymph node	(45)	(25)	(49)
Malignant lymphoma, undiffer type			1 (2%)
#Axillary lymph node	(45)	(25)	(49)
Fibrosarcoma, metastatic		1 (4%)	
#Liver	(49)	(50)	(50)
Malignant lymphoma, histiocytic type		2 (4%)	
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(50)
Hemangioma		1 (2%)	
#Spleen	(49)	(21)	(49)
Hemangiosarcoma, unclear primary or metasta	1 (2%)	1 (5%)	
#Heart	(49)	(10)	(50)
Alveolar/bronchiolar carcinoma, metastatic			1 (2%)
#Liver	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)
Hemangiosarcoma, unclear primary or metasta	1 (2%)	1 (2%)	
DIGESTIVE SYSTEM			
#Liver	(49)	(50)	(50)
Hepatocellular adenoma	11 (22%)	8 (16%)	11 (22%)
Hepatocellular carcinoma	8 (16%)	11 (22%)	6 (12%)
#Jejunum	(48)	(10)	(47)
Adenocarcinoma, NOS	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Pituitary intermedia	(45)	(10)	(47)
Adenoma, NOS			1 (2%)
#Adrenal medulla	(48)	(10)	(49)
Pheochromocytoma	1 (2%)		1 (2%)
#Thyroid	(44)	(11)	(46)
Follicular cell adenoma		1 (9%)	
Follicular cell carcinoma		1 (9%)	
REPRODUCTIVE SYSTEM			
None			
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(50)	(50)
Carcinoma, NOS	1 (2%)		2 (4%)
Adenoma, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Skeletal muscle	(49)	(50)	(50)
Rhabdomyosarcoma	1 (2%)		
BODY CAVITIES			
*Pleura	(49)	(50)	(50)
Mesothelioma, malignant			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(50)
Carcinoma, NOS, metastatic	1 (2%)		
Fibrosarcoma, metastatic		1 (2%)	
Carcinosarcoma, metastatic	1 (2%)		
Osteosarcoma, metastatic	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	7	10	11
Moribund sacrifice	8	9	7
Terminal sacrifice	34	31	32
Animal missexed	1		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
Total animals with primary tumors**	30	34	29
Total primary tumors	50	48	40
Total animals with benign tumors	19	15	17
Total benign tumors	23	16	19
Total animals with malignant tumors	19	25	17
Total malignant tumors	25	30	21
Total animals with secondary tumors##	6	3	5
Total secondary tumors	7	3	6
Total animals with tumors uncertain--primary or metastatic	1	1	
Total uncertain tumors	2	2	

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

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE: UNTREATED CONTROL

ANIMAL NUMBER	013	048	039	005	047	022	011	028	027	040	026	050	016	029	032	001	002	003	004	006	007	008	009	010	012
WEEKS ON STUDY	001	008	074	078	088	089	091	091	093	096	097	098	102	102	103	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma										X															
Subcutaneous tissue	S	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma															X										
Fibrosarcoma					X		X					X	X		X										
RESPIRATORY SYSTEM																									
Lungs and bronchi	S	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic										X															
Hepatocellular carcinoma, metastatic							X																		
Alveolar/bronchiolar adenoma						X				X		X										X			
Trachea	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	−	+	+	+	+	+	X
HEMATOPOIETIC SYSTEM																									
Bone marrow	S	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	−	+	+	−	+	+	−	+	+
Spleen	S	+	−	+	+	−	+	+	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+
Hemangiosarcoma, unclear primary or metastatic																X									
Malignant lymphoma, undiffer type																									
Lymph nodes	S	−	+	+	−	+	+	−	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic										X															
Thymus	S	+	−	+	−	−	−	−	−	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	S	+	+	−	+	+	+	+	+	−	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	S	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+
Liver	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Hepatocellular adenoma																								X	X
Hepatocellular carcinoma							X	X	X				X		X		X		X		X				
Hemangiosarcoma				X																		X	X	X	
Hemangiosarcoma, unclear primary or metastatic																X									
Bile duct	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	S	+	N	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+
Pancreas	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS													X												
Large intestine	S	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	S	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	S	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma							X																		
Thyroid	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	S	+	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	+	−	+	+	+	+	−	−
REPRODUCTIVE SYSTEM																									
Mammary gland	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	S	+	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	S	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−
SPECIAL SENSE ORGANS																									
Harderian gland	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS														X											
Adenoma, NOS																									
MUSCULOSKELETAL SYSTEM																									
Muscle	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rhabdomyosarcoma							X																		
ALL OTHER SYSTEMS																									
Multiple organs, NOS	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS, metastatic			X																						
Carcinosarcoma, metastatic																									
Osteosarcoma, metastatic																									
Malignant lymphoma, undiffer type								X																	
Malignant lymphoma, lymphocytic type				X																		X			

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed

: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	014	015	017	018	019	020	021	023	024	025	030	031	033	034	035	036	037	038	041	042	043	044	045	046	049	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	*49
Squamous cell carcinoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibroma																										1
Fibrosarcoma													X													6
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma, metastatic																										1
Hepatocellular carcinoma, metastatic																										2
Alveolar/bronchiolar adenoma							X				X							X	X							9
Trachea	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	49
Hemangiosarcoma, unclear prim or me																										1
Malignant lymphoma, undiffer type																X										1
Lymph nodes	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	45
Squamous cell carcinoma, metastatic																										1
Thymus	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	-	+	35
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	-	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma													X		X				X						X	11
Hepatocellular carcinoma							X						X													8
Hemangiosarcoma																										1
Hemangiosarcoma, unclear prim or me																										1
Bile duct	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	49
Gallbladder & common bile duct	+	+	-	N	+	+	+	+	+	+	+	+	-	+	+	-	N	+	+	+	N	N	+	+	+	*49
Pancreas	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	49
Esophagus	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+	-	+	46
Stomach	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
Small intestine	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	48
Adenocarcinoma, NOS																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	48
ENDOCRINE SYSTEM																										
Pituitary	+	-	-	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	45
Adrenal	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	44
Parathyroid	+	-	+	-	+	-	-	+	-	-	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	22
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Testis	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	47
Prostate	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Carcinoma, NOS																						X				1
Adenoma, NOS																										1
MUSCULOSKELETAL SYSTEM																										
Muscle	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Rhabdomyosarcoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Carcinoma, NOS, metastatic																						X				1
Carcinosarcoma, metastatic																										1
Osteosarcoma, metastatic																										1
Malignant lymphoma, undiffer type	X																	X								4
Malignant lymphoma, lymphocytic type				X																						1

* Animals necropsied

TABLE A4 INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE: LOW DOSE

ANIMAL NUMBER	046	017	033	035	009	023	030	048	049	007	014	027	008	026	040	012	020	030	042	001	002	004	005	006	010
WEEKS ON STUDY	054	071	071	071	089	089	090	091	091	092	092	094	095	095	096	097	100	100	102	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
[illegible]	+	+	+	+	+	+	+	+	+	+	N	+	N	+	+	N	N	+	N	+	N	N	+	N	N
[illegible]		X																							
[illegible]			X									X		X	X			X					X		
[illegible]							X																		
[illegible]																									
RESPIRATORY SYSTEM																									
[illegible]	+	+	−	+	+	+	−	+	+	+	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−
[illegible] metastatic				X																					
[illegible] adenoma					X										X		X								
[illegible] carcinoma																									
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HEMATOPOIETIC SYSTEM																									
[illegible]	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−
[illegible]	+	+	−	+	+	+	−	+	+	−	+	+	+	−	−	−	+	+	−	+	−	−	−	−	+
[illegible] primary or metastatic																	X								
[illegible] lymphoma, malignant type													X												
[illegible] lymphoma, mixed type	−	+	+	−	−	+	−	−	+	−	+	+	−	−	−	−	−	+	+	+	−	+	+	−	+
[illegible]			X																						
[illegible] metastatic	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]																									
CIRCULATORY SYSTEM																									
[illegible]	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DIGESTIVE SYSTEM																									
[illegible]	+	+	+	+	−	+	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−
[illegible]	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
[illegible]						X																X		X	
[illegible] adenoma			X	X							X					X	X		X						
[illegible] adenoma																									
[illegible] carcinoma																									
[illegible] primary or metastatic																	X								
[illegible] lymphoma, histiocytic type					X																				
[illegible]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
[illegible]	+	+	+	N	+	N	+	−	+	+	N	N	N	N	+	+	+	N	+	+	+	+	+	+	+
[illegible]	+	+	+	−	+	+	−	+	+	+	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−
[illegible]	+	+	+	−	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	−	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
URINARY SYSTEM																									
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−
NERVOUS SYSTEM																									
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
[illegible]									X																
[illegible]																									
[illegible]	+	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
REPRODUCTIVE SYSTEM																									
[illegible]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
[illegible]	+	+	+	+	+	+	−	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
[illegible]	+	−	−	−	+	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
[illegible]	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
URINARY TUMOR SYSTEMS																									
[illegible] NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
[illegible] metastatic						X												X							
[illegible] lymphoma, malignant type										X															
[illegible] lymphoma, mixed type																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	011	013	015	016	018	019	021	022	024	025	028	029	031	032	034	036	037	038	039	041	043	044	045	047	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	N	N	+	+	+	+	N	N	N	N	N	N	N	N	+	*50
Sarcoma, NOS													X													1
Fibroma														X	X	X										3
Fibrosarcoma																									X	8
Hemangioma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	14
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma																										3
Alveolar/bronchiolar carcinoma					X																					1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	11
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	16
Spleen	–	–	–	+	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	+	21
Hemangiosarcoma, unclear prim or met																										1
Malignant lymphoma, undiffer type				X																						1
Malignant lymphoma, mixed type																										1
Lymph nodes	–	+	–	+	–	–	–	–	–	–	–	+	+	+	+	–	–	+	+	–	–	+	–	–	+	25
Fibrosarcoma, metastatic																										1
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	12
Liver	–	+	+	+	+	+	–	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma	X		X							X				X				X								8
Hepatocellular carcinoma		X		X	X									X		X										11
Hemangiosarcoma																								X		1
Hemangiosarcoma, unclear prim or me																										1
Malignant lymphoma, histiocytic type																	X									2
Bile duct	–	+	+	+	+	–	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	N	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	10
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	13
Urinary bladder	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
ENDOCRINE SYSTEM																										
Pituitary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	11
Follicular cell adenoma																										1
Follicular cell carcinoma													X													1
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Prostate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibrosarcoma, metastatic																										1
Malignant lymphoma, undiffer type																		X								2
Malignant lymphoma, mixed type																										1

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE: HIGH DOSE

ANIMAL NUMBER	049	047	030	038	040	039	044	003	028	027	034	004	012	033	042	018	006	025	001	002	005	007	008	009	010
WEEKS ON STUDY	020	028	053	062	064	072	082	085	087	089	092	095	095	095	096	098	100	101	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	N	+	N	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+
Sarcoma, NOS									X																
Fibroma												X		X			X								
Fibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic																									
Hepatocellular carcinoma, metastatic							X				X							X							
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma								X																	X
Trachea	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Lymph nodes	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Alveolar/bronchiolar carcinoma, metastatic								X																	
Malignant lymphoma, undiffer type																									
Thymus	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+	-	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic								X																	
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Hepatocellular adenoma				X				X	X	X												X	X		
Hepatocellular carcinoma						X	X				X			X				X					X		
Hemangiosarcoma										X															
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	N	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Pancreas	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	N	N	N
Esophagus	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Stomach	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+
Small intestine	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Large intestine	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma														X											
Thyroid	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	-	-	+	-	+	-	+	-	-	+	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
BODY CAVITIES																									
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, malignant										X															
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, undiffer type										X															
Malignant lymphoma, mixed type																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	011	011	011	011	011	017	019	020	021	022	023	024	026	029	031	032	035	037	038	041	043	045	046	048	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS														X												1
Fibroma							X			X									X							1
Fibrosarcoma																										6
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic									X																	1
Hepatocellular carcinoma, metastatic																										3
Alveolar/bronchiolar adenoma		X											X							X	X					5
Alveolar/bronchiolar carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, met																										1
Malignant lymphoma, undiffer type						X																				1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	29
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metast																										1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma	X	X		X	X											X										11
Hepatocellular carcinoma																										6
Hemangiosarcoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma, NOS																				X						1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma																										1
Thyroid	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Parathyroid	−	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	−	+	+	32
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS									X			X														2
BODY CAVITIES																										
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, malignant																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, undiffer type																										1
Malignant lymphoma, mixed type							X																			1

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	Control	2,500 ppm	5,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	1/49 (2%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	2.8%	8.4%	3.1%
Terminal Rates (c)	0/35 (0%)	1/31 (3%)	1/32 (3%)
Week of First Observation	103	95	104
Life Table Tests (d)	P=0.573	P=0.268	P=0.737
Incidental Tumor Tests (d)	P=0.599	P=0.324	P=0.750
Cochran-Armitage Trend Test (d)	P=0.603N		
Fisher Exact Test (d)		P=0.316	P=0.747N
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	6/49 (12%)	8/50 (16%)	6/50 (12%)
Adjusted Rates (b)	14.3%	22.3%	16.6%
Terminal Rates (c)	1/35 (3%)	5/31 (16%)	3/32 (9%)
Week of First Observation	88	71	95
Life Table Tests (d)	P=0.486	P=0.324	P=0.548
Incidental Tumor Tests (d)	P=0.555N	P=0.482	P=0.624N
Cochran-Armitage Trend Test (d)	P=0.543N		
Fisher Exact Test (d)		P=0.403	P=0.606N
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	6/49 (12%)	9/50 (18%)	7/50 (14%)
Adjusted Rates (b)	14.3%	23.9%	18.5%
Terminal Rates (c)	1/35 (3%)	5/31 (16%)	3/32 (9%)
Week of First Observation	88	71	87
Life Table Tests (d)	P=0.378	P=0.242	P=0.430
Incidental Tumor Tests (d)	P=0.478	P=0.407	P=0.520
Cochran-Armitage Trend Test (d)	P=0.460		
Fisher Exact Test (d)		P=0.303	P=0.516
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	6/49 (12%)	12/50 (24%)	8/50 (16%)
Adjusted Rates (b)	14.3%	30.8%	21.3%
Terminal Rates (c)	1/35 (3%)	6/31 (19%)	4/32 (13%)
Week of First Observation	88	71	87
Life Table Tests (d)	P=0.287	P=0.080	P=0.325
Incidental Tumor Tests (d)	P=0.364	P=0.153	P=0.392
Cochran-Armitage Trend Test (d)	P=0.363		
Fisher Exact Test (d)		P=0.104	P=0.403
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	9/49 (18%)	(e) 3/14 (21%)	5/50 (10%)
Adjusted Rates (b)	23.0%		15.6%
Terminal Rates (c)	6/35 (17%)		5/32 (16%)
Week of First Observation	89		104
Life Table Tests (d)			P=0.252N
Incidental Tumor Tests (d)			P=0.226N
Cochran-Armitage Trend Test (d)			
Fisher Exact Test (d)			P=0.183N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	9/49 (18%)	(e) 4/14 (29%)	6/50 (12%)
Adjusted Rates (b)	23.0%		17.6%
Terminal Rates (c)	6/35 (17%)		5/32 (16%)
Week of First Observation	89		85
Life Table Test (d)			P=0.360N
Incidental Tumor Test (d)			P=0.315N
Cochran-Armitage Trend Test (d)			
Fisher Exact Test (d)			P=0.274N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	Control	2,500 ppm	5,000 ppm
Hematopoietic System: Malignant Lymphoma, Undifferentiated Type			
Overall Rates (a)	5/49 (10%)	(f) 3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	13.3%	8.5%	5.5%
Terminal Rates (c)	4/35 (11%)	2/31 (6%)	1/32 (3%)
Week of First Observation	78	89	89
Life Table Tests (d)	P=0.193N	P=0.408N	P=0.256N
Incidental Tumor Tests (d)	P=0.145N	P=0.323N	P=0.193N
Cochran-Armitage Trend Test (d)	P=0.151N		
Fisher Exact Test (d)		P=0.346N	P=0.210N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	6/49 (12%)	(f) 7/50 (14%)	3/50 (6%)
Adjusted Rates (b)	16.1%	17.9%	8.5%
Terminal Rates (c)	5/35 (14%)	3/31 (10%)	2/32 (6%)
Week of First Observation	78	89	89
Life Table Tests (d)	P=0.260N	P=0.440	P=0.289N
Incidental Tumor Tests (d)	P=0.191N	P=0.587	P=0.229N
Cochran-Armitage Trend Test (d)	P=0.199N		
Fisher Exact Test (d)		P=0.516	P=0.233N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	11/49 (22%)	8/50 (16%)	11/50 (22%)
Adjusted Rates (b)	29.7%	24.3%	28.9%
Terminal Rates (c)	9/35 (26%)	7/31 (23%)	7/32 (22%)
Week of First Observation	102	89	62
Life Table Tests (d)	P=0.459	P=0.411N	P=0.500
Incidental Tumor Tests (d)	P=0.525	P=0.356N	P=0.590
Cochran-Armitage Trend Test (d)	P=0.530N		
Fisher Exact Test (d)		P=0.288N	P=0.574N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	8/49 (16%)	11/50 (22%)	6/50 (12%)
Adjusted Rates (b)	20.1%	28.3%	14.7%
Terminal Rates (c)	5/35 (14%)	5/31 (16%)	1/32 (3%)
Week of First Observation	91	71	72
Life Table Tests (d)	P=0.425N	P=0.247	P=0.454N
Incidental Tumor Tests (d)	P=0.282N	P=0.414	P=0.328N
Cochran-Armitage Trend Test (d)	P=0.327N		
Fisher Exact Test (d)		P=0.323	P=0.371N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	17/49 (35%)	18/50 (36%)	16/50 (32%)
Adjusted Rates (b)	42.0%	46.1%	37.4%
Terminal Rates (c)	12/35 (34%)	11/31 (35%)	7/32 (22%)
Week of First Observation	91	71	62
Life Table Tests (d)	P=0.497	P=0.364	P=0.542
Incidental Tumor Tests (d)	P=0.419N	P=0.563	P=0.448N
Cochran-Armitage Trend Test (d)	P=0.429N		
Fisher Exact Test (d)		P=0.530	P=0.472N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Not all tissues were examined microscopically
(f) Not all spleens were examined microscopically

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Inflammation, acute necrotizing	4 (8%)	7 (14%)	4 (8%)
Inflammation, active chronic		1 (2%)	1 (2%)
Inflammation, chronic necrotizing		2 (4%)	
Fibrosis, focal			1 (2%)
Hyperplasia, basal cell	1 (2%)		
Acanthosis	1 (2%)		
*Subcutaneous tissue	(49)	(50)	(50)
Edema, NOS	1 (2%)		
Hemorrhage	1 (2%)		
Inflammation, acute	1 (2%)		
Abscess, NOS	1 (2%)	2 (4%)	
Inflammation, active chronic			3 (6%)
Inflammation, chronic			2 (4%)
Fibrosis	1 (2%)	1 (2%)	1 (2%)
Necrosis, NOS		1 (2%)	
RESPIRATORY SYSTEM			
*Nasal cavity	(49)	(50)	(50)
Inflammation, acute		4 (8%)	2 (4%)
Inflammation, active chronic			1 (2%)
Polyp, NOS		1 (2%)	
*Nasal mucosa	(49)	(50)	(50)
Inflammation, active chronic			1 (2%)
#Lung/bronchiole	(49)	(14)	(50)
Hyperplasia, epithelial	2 (4%)		
#Lung	(49)	(14)	(50)
Congestion, NOS	2 (4%)	1 (7%)	2 (4%)
Edema, NOS			3 (6%)
Hemorrhage	5 (10%)		2 (4%)
Inflammation, interstitial		1 (7%)	
Inflammation, acute	5 (10%)	1 (7%)	4 (8%)
Inflammation, active chronic	7 (14%)		9 (18%)
Inflammation, chronic	1 (2%)	1 (7%)	3 (6%)
Fibrosis, focal			1 (2%)
Hyperplasia, alveolar epithelium	15 (31%)	1 (7%)	18 (36%)
Histiocytosis	7 (14%)	3 (21%)	13 (26%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Depletion, lymphoid		1 (2%)	1 (2%)
#Bone marrow	(49)	(16)	(48)
Hyperplasia, hematopoietic	1 (2%)		1 (2%)
#Spleen	(49)	(21)	(49)
Necrosis, NOS			1 (2%)
Infarct, NOS	1 (2%)		
Depletion, lymphoid	5 (10%)	3 (14%)	3 (6%)
Angiectasis	1 (2%)		1 (2%)
Hyperplasia, lymphoid	3 (6%)	1 (5%)	2 (4%)
Hematopoiesis	7 (14%)	9 (43%)	10 (20%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#Mandibular lymph node	(45)	(25)	(49)
Pigmentation, NOS			1 (2%)
Depletion, lymphoid			1 (2%)
Hyperplasia, NOS	2 (4%)	1 (4%)	1 (2%)
Angiectasis		1 (4%)	
#Bronchial lymph node	(45)	(25)	(49)
Hemorrhage			1 (2%)
#Mediastinal lymph node	(45)	(25)	(49)
Hyperplasia, NOS	2 (4%)		
#Pancreatic lymph node	(45)	(25)	(49)
Inflammation, acute		1 (4%)	
Depletion, lymphoid	1 (2%)	1 (4%)	
Angiectasis	2 (4%)	2 (8%)	
#Lumbar lymph node	(45)	(25)	(49)
Hemorrhage			1 (2%)
Inflammation, acute			1 (2%)
Necrosis, NOS			1 (2%)
Hyperplasia, lymphoid		1 (4%)	
#Mesenteric lymph node	(45)	(25)	(49)
Hemorrhage		4 (16%)	4 (8%)
Inflammation, acute	1 (2%)	1 (4%)	
Necrosis, NOS		1 (4%)	1 (2%)
Depletion, lymphoid	1 (2%)	2 (8%)	3 (6%)
Hyperplasia, NOS		1 (4%)	1 (2%)
Angiectasis	31 (69%)	16 (64%)	19 (39%)
#Inguinal lymph node	(45)	(25)	(49)
Pigmentation, NOS	1 (2%)		
Hyperplasia, NOS		1 (4%)	3 (6%)
#Liver	(49)	(50)	(50)
Hematopoiesis		2 (4%)	
#Peyer's patch	(48)	(10)	(47)
Hyperplasia, lymphoid	2 (4%)		1 (2%)
#Thymus	(35)	(5)	(29)
Cyst, NOS	7 (20%)	1 (20%)	4 (14%)
Necrosis, NOS			1 (3%)
Depletion, lymphoid	2 (6%)	3 (60%)	1 (3%)
CIRCULATORY SYSTEM			
#Spleen	(49)	(21)	(49)
Thrombus, organized	1 (2%)	1 (5%)	
#Mesenteric lymph node	(45)	(25)	(49)
Lymphangiectasis		2 (8%)	
#Renal lymph node	(45)	(25)	(49)
Thrombus, organized		1 (4%)	
#Lung	(49)	(14)	(50)
Periarteritis	3 (6%)		1 (2%)
#Heart	(49)	(10)	(50)
Inflammation, chronic focal			2 (4%)
Fibrosis, focal			2 (4%)
Periarteritis	1 (2%)		
Degeneration, NOS			3 (6%)
*Aorta	(49)	(50)	(50)
Mineralization			1 (2%)
*Central veins/liver	(49)	(50)	(50)
Inflammation, active chronic	2 (4%)		
#Liver	(49)	(50)	(50)
Thrombus, organized		1 (2%)	

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
*Tooth	(49)	(50)	(50)
Deformity, NOS	25 (51%)	25 (50%)	31 (62%)
Inflammation, NOS	14 (29%)	15 (30%)	25 (50%)
Fibrosis			4 (8%)
#Salivary gland	(48)	(12)	(50)
Dilatation/ducts	1 (2%)		
Inflammation, chronic	25 (52%)	4 (33%)	24 (48%)
Fibrosis	1 (2%)		
Atrophy, NOS	2 (4%)		
#Salivary gland capsule	(48)	(12)	(50)
Mineralization			1 (2%)
#Parotid gland	(48)	(12)	(50)
Mineralization		1 (8%)	
#Liver	(49)	(50)	(50)
Congestion, NOS			1 (2%)
Hemorrhage			1 (2%)
Inflammation, active chronic	1 (2%)	2 (4%)	2 (4%)
Inflammation, chronic	1 (2%)	2 (4%)	
Necrosis, coagulative	4 (8%)	9 (18%)	10 (20%)
Infarct, NOS	1 (2%)		
Nuclear alteration			2 (4%)
Basophilic cyto change			1 (2%)
Eosinophilic cyto change	1 (2%)		
Clear cell change	2 (4%)		1 (2%)
Angiectasis		2 (4%)	
#Liver/Kupffer cell	(49)	(50)	(50)
Hyperplasia, NOS	2 (4%)		
#Liver/hepatocytes	(49)	(50)	(50)
Nuclear alteration			2 (4%)
*Gallbladder	(49)	(50)	(50)
Hyperplasia, epithelial		1 (2%)	
#Pancreas	(49)	(13)	(49)
Dilatation/ducts	1 (2%)		
Inflammation, chronic	3 (6%)	2 (15%)	
Cytoplasmic change, NOS	1 (2%)		1 (2%)
Atrophy, NOS	3 (6%)	2 (15%)	
#Stomach	(49)	(10)	(47)
Mineralization	1 (2%)		
Dilatation/ducts			1 (2%)
Cyst, NOS			2 (4%)
Inflammation, active chronic	4 (8%)		1 (2%)
Necrosis, coagulative			1 (2%)
Hyperkeratosis	2 (4%)		
Acanthosis	2 (4%)		
#Forestomach	(49)	(10)	(47)
Ulcer, acute	1 (2%)		
Inflammation, acute focal			1 (2%)
Inflammation, active chronic			1 (2%)
Atrophy, focal			1 (2%)
Hyperkeratosis			1 (2%)
Acanthosis			2 (4%)
#Peyer's patch	(48)	(10)	(47)
Inflammation, active chronic	1 (2%)		
#Duodenum	(48)	(10)	(47)
Congestion, NOS			1 (2%)
#Jejunum	(48)	(10)	(47)
Abscess, NOS	1 (2%)		
Inflammation, active chronic	1 (2%)		

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#Cecum	(48)	(10)	(48)
Necrosis, focal	1 (2%)		
*Rectum	(49)	(50)	(50)
Inflammation, acute focal			1 (2%)
Metaplasia, squamous			1 (2%)
URINARY SYSTEM			
*Urethral lumen	(49)	(50)	(50)
Inflammation, acute			1 (2%)
#Kidney	(49)	(13)	(49)
Cyst, NOS			1 (2%)
Glomerulonephritis, NOS	3 (6%)	2 (15%)	
Pyelonephritis, acute	1 (2%)		
Inflammation, active chronic	1 (2%)		
Pyelonephritis, acute/chronic			1 (2%)
Inflammation, chronic	31 (63%)	4 (31%)	38 (78%)
Nephropathy		1 (8%)	2 (4%)
Infection, bacterial	1 (2%)		
Glomerulosclerosis, NOS	3 (6%)		1 (2%)
Infarct, healed			1 (2%)
Metaplasia, osseous	2 (4%)		
#Renal papilla	(49)	(13)	(49)
Necrosis, coagulative	1 (2%)		1 (2%)
#Kidney/tubule	(49)	(13)	(49)
Mineralization	2 (4%)		2 (4%)
Inflammation, acute focal			1 (2%)
Necrosis, focal			1 (2%)
Regeneration, NOS	23 (47%)		21 (43%)
#Kidney/pelvis	(49)	(13)	(49)
Inflammation, acute	1 (2%)		
Inflammation, chronic		2 (15%)	
#Urinary bladder	(48)	(13)	(50)
Calculus, gross observation only		3 (23%)	1 (2%)
Calculus, microscopic examination	1 (2%)	1 (8%)	4 (8%)
Inflammation, active chronic	1 (2%)		2 (4%)
Inflammation, chronic	19 (40%)	6 (46%)	21 (42%)
Fibrosis		1 (8%)	
#Perivesical tissue	(48)	(13)	(50)
Abscess, NOS		1 (8%)	
ENDOCRINE SYSTEM			
#Anterior pituitary	(45)	(10)	(47)
Cyst, NOS	7 (16%)		4 (9%)
Congestion, NOS		1 (10%)	
Hyperplasia, NOS	7 (16%)	1 (10%)	11 (23%)
Angiectasis			1 (2%)
#Adrenal	(48)	(10)	(49)
Angiectasis	1 (2%)		1 (2%)
#Adrenal cortex	(48)	(10)	(49)
Hyperplasia, NOS	3 (6%)		1 (2%)
#Adrenal medulla	(48)	(10)	(49)
Hyperplasia, focal			1 (2%)
#Thyroid	(44)	(11)	(46)
Inflammation, chronic focal			1 (2%)
Hyperplasia, follicular cell	1 (2%)		1 (2%)
#Parathyroid	(22)	(5)	(32)
Cyst, NOS	1 (5%)		1 (3%)
#Pancreatic islets	(49)	(13)	(49)
Hyperplasia, NOS	1 (2%)		

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*Preputial gland	(49)	(50)	(50)
Mineralization	1 (2%)		
Dilatation/ducts	3 (6%)		
Cyst, NOS		1 (2%)	1 (2%)
Hemorrhage		1 (2%)	1 (2%)
Inflammation, acute			
Inflammation, acute necrotizing	2 (4%)		1 (2%)
Abscess, NOS	1 (2%)	4 (8%)	4 (8%)
Inflammation, active chronic	19 (39%)	3 (6%)	9 (18%)
Inflammation, chronic	2 (4%)	6 (12%)	5 (10%)
Fibrosis	1 (2%)		
Atrophy, NOS	1 (2%)		
#Prostate	(47)	(7)	(49)
Inflammation, acute			3 (6%)
Abscess, NOS			1 (2%)
Inflammation, active chronic	1 (2%)		3 (6%)
Inflammation, chronic	9 (19%)		4 (8%)
Fibrosis, focal	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
*Seminal vesicle	(49)	(50)	(50)
Hemorrhage		1 (2%)	
Inflammation, acute			
#Testis	(47)	(11)	2 (4%)
Hemorrhage	1 (2%)		(50)
Atrophy, NOS	5 (11%)	4 (36%)	6 (12%)
#Testis/tubule	(47)	(11)	(50)
Mineralization	5 (11%)		2 (4%)
#Spermatogonia	(47)	(11)	(50)
Cytomegaly			1 (2%)
*Epididymis	(49)	(50)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute	1 (2%)		
Inflammation, chronic			
Inflammation, chronic focal		1 (2%)	1 (2%)
NERVOUS SYSTEM			
#Brain/meninges	(49)	(10)	(50)
Inflammation, chronic			1 (2%)
#Brain	(49)	(10)	(50)
Mineralization			1 (2%)
Inflammation, chronic focal			1 (2%)
Malacia			1 (2%)
#Brain/thalamus	(49)	(10)	(50)
Mineralization	32 (65%)	2 (20%)	29 (58%)
SPECIAL SENSE ORGANS			
*Eye/cornea	(49)	(50)	(50)
Inflammation, active chronic			1 (2%)
Necrosis, coagulative			1 (2%)
*Nasolacrimal duct	(49)	(50)	(50)
Inflammation, acute	1 (2%)	3 (6%)	1 (2%)
Inflammation, active chronic			2 (4%)
Inflammation, chronic	1 (2%)		3 (6%)

TABLE A4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*Maxilla	(49)	(50)	(50)
Osteoarthritis	1 (2%)		
*Sternum	(49)	(50)	(50)
Hyperostosis		1 (2%)	
*Carpometacarpal joint	(49)	(50)	(50)
Ankylosis	1 (2%)		
*Tarsal joint	(49)	(50)	(50)
Ankylosis	36 (73%)	38 (76%)	37 (74%)
Osteoarthritis	36 (73%)	37 (74%)	35 (70%)
BODY CAVITIES			
*Thorax	(49)	(50)	(50)
Hemothorax			1 (2%)
*Peritoneal cavity	(49)	(50)	(50)
Hemoperitoneum	1 (2%)	3 (6%)	2 (4%)
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(50)
Inflammation, active chronic			1 (2%)
Inflammation, chronic	8 (16%)	1 (2%)	2 (4%)
Omentum			
Mineralization			1
Necrosis, coagulative			1
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		2	
Animal missexed/no necropsy	1		

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

APPENDIX B

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(50)
Fibrosarcoma	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(49)
Adenocarcinoma, NOS, metastatic			1 (2%)
Hepatocellular carcinoma, metastatic		1 (2%)	
Alveolar/bronchiolar adenoma	1 (2%)	4 (8%)	4 (8%)
Alveolar/bronchiolar carcinoma	2 (4%)		
Osteosarcoma, metastatic		1 (2%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, NOS			1 (2%)
Malignant lymphoma, undiffer type	4 (8%)	3 (6%)	2 (4%)
Malignant lymphoma, lymphocytic type		1 (2%)	
Malignant lymphoma, histiocytic type	1 (2%)		1 (2%)
Malignant lymphoma, mixed type		2 (4%)	2 (4%)
Lymphocytic leukemia	1 (2%)		
#Spleen	(50)	(48)	(50)
Malignant lymphoma, undiffer type	2 (4%)		1 (2%)
#Mesenteric lymph node	(44)	(47)	(45)
Malignant lymphoma, mixed type		1 (2%)	
#Kidney	(50)	(50)	(50)
Malignant lymphoma, undiffer type	1 (2%)		
#Thymus	(25)	(30)	(23)
Malignant lymphoma, lymphocytic type	1 (4%)		
CIRCULATORY SYSTEM			
#Lung	(50)	(50)	(49)
Hemangiosarcoma, metastatic		1 (2%)	
#Heart	(50)	(49)	(49)
Hemangiosarcoma		1 (2%)	
#Urinary bladder	(48)	(46)	(47)
Hemangioma	1 (2%)		
#Uterus	(50)	(49)	(49)
Hemangioma			1 (2%)
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Hepatocellular adenoma	2 (4%)	3 (6%)	4 (8%)
Hepatocellular carcinoma	1 (2%)	1 (2%)	
#Forestomach	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	2 (4%)	
URINARY SYSTEM			
None			

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#Anterior pituitary	(36)	(43)	(36)
Adenoma, NOS	9 (25%)	7 (16%)	6 (17%)
#Adrenal medulla	(48)	(50)	(49)
Pheochromocytoma			1 (2%)
#Thyroid	(48)	(47)	(48)
Follicular cell adenoma			1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS		1 (2%)	1 (2%)
#Uterus	(50)	(49)	(49)
Adenocarcinoma, NOS	1 (2%)		
Leiomyoma		1 (2%)	
Endometrial stromal polyp		1 (2%)	1 (2%)
#Ovary	(49)	(50)	(50)
Papillary adenoma	1 (2%)		
Cystadenoma, NOS		1 (2%)	1 (2%)
Granulosa cell tumor		1 (2%)	
Teratoma, NOS		1 (2%)	1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenoma, NOS	3 (6%)		
Adenocarcinoma, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*Pelvic bones	(50)	(50)	(50)
Osteosarcoma		1 (2%)	
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Adenocarcinoma, NOS, metastatic	1 (2%)		
Mesothelioma, metastatic	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	16	21	30
Moribund sacrifice	4	6	6
Terminal sacrifice	30	18	14
Accidentally killed, nda		5	

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
Total animals with primary tumors**	26	24	21
Total primary tumors	33	32	29
Total animals with benign tumors	16	17	14
Total benign tumors	18	19	19
Total animals with malignant tumors	15	11	9
Total malignant tumors	15	11	9
Total animals with secondary tumors##	2	3	1
Total secondary tumors	2	3	1
Total animals with tumors uncertain--benign or malignant		2	1
Total uncertain tumors		2	1

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

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE: UNTREATED CONTROL

ANIMAL NUMBER	016	025	049	014	011	003	010	008	036	038	033	026	012	042	022	028	031	006	034	023	001	002	004	005	007
WEEKS ON STUDY	048	058	063	065	070	071	078	081	081	081	082	083	087	090	091	092	092	093	097	102	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Fibrosarcoma																		X							
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X												
Alveolar/bronchiolar carcinoma																									
Trachea	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, undiffer type																						X			
Lymph nodes	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Thymus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	−	+	−	+	+	−
Malignant lymphoma, lymphocytic type																									
CIRCULATORY SYSTEM																									
Heart	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	−	−	+	+	−	−	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular carcinoma																							X		
Bile duct	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	N	N	+	N	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	−	−	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, undiffer type																									
Urinary bladder	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma															X										
ENDOCRINE SYSTEM																									
Pituitary	+	−	+	−	+	+	+	+	+	−	−	+	+	−	−	+	+	−	−	+	+	+	+	−	+
Adenoma, NOS																				X	X				
Adrenal	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	−	+	−	+	+	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	−
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	+	N	N	N	N	+	+	+	+	N	+	N	+	+	+	+	+	+	+	N	N	N	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS														X											
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Papillary adenoma																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																								X	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS, metastatic														X											
Mesothelioma, metastatic							X																		
Malignant lymphoma, undiffer type																									
Malignant lymphoma, histiocytic type																									X
Lymphocytic leukemia																			X						

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed

: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	009	013	015	017	018	019	020	021	024	027	029	030	032	035	037	039	040	041	043	044	045	046	047	048	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	*50
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X																							1
Alveolar/bronchiolar carcinoma														X												2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, undiffer type						X																				2
Lymph nodes	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	44
Thymus	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	25
Malignant lymphoma, lymphocytic type			X																							1
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																	X						X			2
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	N	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma								X																		1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, undiffer type	X																									1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma																										1
ENDOCRINE SYSTEM																										
Pituitary	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	38
Adenoma, NOS			X						X	X		X	X		X				X							9
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	48
Parathyroid	+	-	-	+	+	-	-	-	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	26
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	-	N	+	+	N	N	+	N	+	N	N	N	N	+	N	N	N	N	+	N	*50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Papillary adenoma			X																							1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS				X										X												3
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenocarcinoma, NOS, metastatic																										1
Mesothelioma, metastatic																										1
Malignant lymphoma, undiffer type				X									X										X			4
Malignant lymphoma, histiocytic type																						X				1
Lymphocytic leukemia																										1

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE: LOW DOSE

ANIMAL NUMBER	040	026	016	033	019	027	024	021	050	028	046	004	023	013	015	041	011	022	031	032	034	035	043	048	010
WEEKS ON STUDY	037	044	057	057	059	063	064	070	070	077	077	088	088	079	081	083	087	087	087	087	087	087	087	087	095
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																									
Alveolar/bronchiolar adenoma											X														
Hemangiosarcoma, metastatic																									
Osteosarcoma, metastatic	X																								
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	+	+	+	+	+	+	N	+	+	+	N	N	+	N	+	+	N	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	+	N	+	+	+	N	N	+	N	N	N	+	N	+
Adenocarcinoma, NOS															X										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Endometrial stromal polyp																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma, NOS																					X				
Granulosa cell tumor																									
Teratoma, NOS		X																							
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Osteosarcoma	X																								
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, undiffer type																									
Malignant lymphoma, lymphocytic type																									
Malignant lymphoma, mixed type																									X

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	012	029	030	045	017	001	002	003	005	006	007	008	009	014	018	020	025	036	037	038	039	042	044	047	049	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	095	095	095	096	100	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																				X						1
Alveolar/bronchiolar adenoma	X								X														X			4
Hemangiosarcoma, metastatic																							X			1
Osteosarcoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Malignant lymphoma, mixed type												X														1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	30
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																							X			1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma		X															X	X								3
Hepatocellular carcinoma																				X						1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gall bladder & common bile duct	+	N	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	N	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma						X	X																			2
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Adenoma, NOS			X	X						X				X					X		X	X				7
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	31
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	N	+	+	+	+	N	+	+	+	N	N	+	N	+	+	+	+	+	+	+	N	N	*50
Adenocarcinoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyoma														X												1
Endometrial stromal polyp																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma, NOS														X												1
Granulosa cell tumor							X																			1
Teratoma, NOS																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Osteosarcoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, undiffer type			X															X	X							3
Malignant lymphoma, lymphocytic type																X										1
Malignant lymphoma, mixed type																					X					2

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE: HIGH DOSE

ANIMAL NUMBER	008	042	034	021	040	013	003	011	038	018	006	036	024	044	002	048	030	043	050	028	023	029	046	027	033
WEEKS ON STUDY	049	054	057	060	062	063	064	064	064	071	072	074	076	079	081	081	083	083	083	084	086	086	086	087	088
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS, metastatic																								X	
Alveolar/bronchiolar adenoma																									X
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, undiffer type																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	N	+	+	+	N	N	+	+	N	+	+	+	+	+	+	+	N	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	N	N	+	N	N	N	N	N	N	N	+	N	+	N	N	+	N	N	N	N	N	N	N
Adenocarcinoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp																				X					
Hemangioma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma, NOS																									
Teratoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS																								X	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS	X																								
Malignant lymphoma, undiffer type																									
Malignant lymphoma, histiocytic type												X													
Malignant lymphoma, mixed type																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	005	039	025	022	026	041	004	049	012	010	001	007	009	014	015	017	018	019	020	031	032	035	037	045	047	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	089	091	092	095	095	098	099	101	102	103	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, NOS, metastatic																										1
Alveolar/bronchiolar adenoma						X		X								X										4
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, undiffer type												X														1
Lymph nodes	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	45
Thymus	−	+	−	+	+	+	−	−	−	+	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	23
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma									X					X								X		X		4
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	N	+	+	+	+	+	N	+	+	+	+	+	+	N	+	N	+	+	+	+	N	+	N	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Esophagus	+	+	+	+	−	+	+	+	+	−	+	+	−	+	+	−	−	−	−	+	−	−	+	+	+	38
Stomach	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	48
Large intestine	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	−	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	+	−	+	−	+	+	+	+	+	36
Adenoma, NOS										X				X			X		X				X	X		6
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma							X																			1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma							X																			1
Parathyroid	+	+	−	+	−	+	−	−	+	−	+	+	−	+	−	−	−	+	+	+	−	+	+	−	+	24
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	+	−	+	N	N	N	N	N	N	+	N	+	N	N	+	N	+	N	N	+	*50
Adenocarcinoma, NOS															X											1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endometrial stromal polyp																										1
Hemangioma							X																			1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma, NOS																X										1
Teratoma, NOS																					X					1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenocarcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																										1
Malignant lymphoma, undiffer type				X				X																		2
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type							X																	X		2

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	Control	2,500 ppm	5,000 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	1/50 (2%)	4/50 (8%)	4/49 (8%)
Adjusted Rates (b)	3.3%	15.6%	19.5%
Terminal Rates (c)	1/30 (3%)	2/20 (10%)	1/15 (7%)
Week of First Observation	104	77	88
Life Table Tests (d)	P=0.045	P=0.104	P=0.060
Incidental Tumor Tests (d)	P=0.137	P=0.178	P=0.191
Cochran-Armitage Trend Test (d)	P=0.140		
Fisher Exact Test (d)		P=0.181	P=0.175
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	4/49 (8%)
Adjusted Rates (b)	9.1%	15.6%	19.5%
Terminal Rates (c)	2/30 (7%)	2/20 (10%)	1/15 (7%)
Week of First Observation	87	77	88
Life Table Tests (d)	P=0.180	P=0.349	P=0.235
Incidental Tumor Tests (d)	P=0.410	P=0.480	P=0.511
Cochran-Armitage Trend Test (d)	P=0.413		
Fisher Exact Test (d)		P=0.500	P=0.489
Hematopoietic System: Malignant Lymphoma, Undifferentiated Type			
Overall Rates (a)	7/50 (14%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	23.3%	13.5%	15.9%
Terminal Rates (c)	7/30 (23%)	2/20 (10%)	1/15 (7%)
Week of First Observation	104	95	95
Life Table Tests (d)	P=0.391N	P=0.351N	P=0.517N
Incidental Tumor Tests (d)	P=0.231N	P=0.295N	P=0.339N
Cochran-Armitage Trend Test (d)	P=0.107N		
Fisher Exact Test (d)		P=0.159N	P=0.159N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	0.0%	13.5%	11.6%
Terminal Rates (c)	0/30 (0%)	2/20 (10%)	1/15 (7%)
Week of First Observation		95	99
Life Table Tests (d)	P=0.082	P=0.070	P=0.122
Incidental Tumor Tests (d)	P=0.160	P=0.107	P=0.239
Cochran-Armitage Trend Test (d)	P=0.202		
Fisher Exact Test (d)		P=0.121	P=0.247
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	9/50 (18%)	7/50 (14%)	7/50 (14%)
Adjusted Rates (b)	30.0%	30.8%	29.3%
Terminal Rates (c)	9/30 (30%)	5/20 (25%)	2/15 (13%)
Week of First Observation	104	95	49
Life Table Tests (d)	P=0.289	P=0.499	P=0.345
Incidental Tumor Tests (d)	P=0.532	P=0.601	P=0.601
Cochran-Armitage Trend Test (d)	P=0.339N		
Fisher Exact Test (d)		P=0.393N	P=0.393N
Hematopoietic System: Lymphoma or Leukemia			
Overall Rates (a)	10/50 (20%)	7/50 (14%)	7/50 (14%)
Adjusted Rates (b)	32.2%	30.8%	29.3%
Terminal Rates (c)	9/30 (30%)	5/20 (25%)	2/15 (13%)
Week of First Observation	97	95	49
Life Table Tests (d)	P=0.378	P=0.587	P=0.431
Incidental Tumor Tests (d)	P=0.439N	P=0.477N	P=0.469N
Cochran-Armitage Trend Test (d)	P=0.248N		
Fisher Exact Test (d)		P=0.298N	P=0.298N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	Control	2,500 ppm	5,000 ppm
Liver: Hepatocellular Adenoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (b)	6.7%	13.5%	24.7%
Terminal Rates (c)	2/30 (7%)	2/20 (10%)	3/15 (20%)
Week of First Observation	104	95	102
Life Table Tests (d)	P=0.069	P=0.337	P=0.092
Incidental Tumor Tests (d)	P=0.124	P=0.413	P=0.152
Cochran-Armitage Trend Test (d)	P=0.264		
Fisher Exact Test (d)		P=0.500	P=0.339
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	10.0%	18.3%	24.7%
Terminal Rates (c)	3/30 (10%)	3/20 (15%)	3/15 (20%)
Week of First Observation	104	95	102
Life Table Tests (d)	P=0.124	P=0.300	P=0.167
Incidental Tumor Tests (d)	P=0.200	P=0.362	P=0.249
Cochran-Armitage Trend Test (d)	P=0.424		
Fisher Exact Test (d)		P=0.500	P=0.500
Pituitary Gland: Adenoma			
Overall Rates (a)	9/36 (25%)	7/43 (16%)	6/36 (17%)
Adjusted Rates (b)	35.5%	31.2%	45.3%
Terminal Rates (c)	8/24 (33%)	5/20 (25%)	5/12 (42%)
Week of First Observation	102	95	103
Life Table Tests (d)	P=0.367	P=0.578N	P=0.381
Incidental Tumor Tests (d)	P=0.536N	P=0.270N	P=0.617
Cochran-Armitage Trend Test (d)	P=0.227N		
Fisher Exact Test (d)		P=0.248N	P=0.281N
Harderian Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	10.0%	0.0%	0.0%
Terminal Rates (c)	3/30 (10%)	0/20 (0%)	0/15 (0%)
Week of First Observation	104		
Life Table Tests (d)	P=0.092N	P=0.200N	P=0.265N
Incidental Tumor Tests (d)	P=0.092N	P=0.200N	P=0.265N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.121N	P=0.121N
Harderian Gland: Adenoma or Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	10.0%	0.0%	3.7%
Terminal Rates (c)	3/30 (10%)	0/20 (0%)	0/15 (0%)
Week of First Observation	104		87
Life Table Tests (d)	P=0.330N	P=0.200N	P=0.536N
Incidental Tumor Tests (d)	P=0.276N	P=0.200N	P=0.457N
Cochran-Armitage Trend Test (d)	P=0.176N		
Fisher Exact Test (d)		P=0.121N	P=0.309N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst		1 (2%)	
Inflammation, acute		1 (2%)	
Inflammation, acute necrotizing		1 (2%)	
Inflammation, acute/chronic		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Inflammation, acute			1 (2%)
Abscess, NOS	1 (2%)		
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Inflammation, acute		1 (2%)	2 (4%)
*Nose	(50)	(50)	(50)
Abscess, NOS			1 (2%)
*Nasal gland	(50)	(50)	(50)
Inflammation, acute	9 (18%)	12 (24%)	6 (12%)
*Nasopharynx	(50)	(50)	(50)
Congenital malformation, NOS		1 (2%)	
#Lung/bronchiole	(50)	(50)	(49)
Inflammation, acute necrotizing		1 (2%)	
#Lung	(50)	(50)	(49)
Aspiration, foreign body		2 (4%)	
Mineralization			1 (2%)
Atelectasis			1 (2%)
Congestion, NOS	3 (6%)	6 (12%)	4 (8%)
Hemorrhage	5 (10%)	3 (6%)	1 (2%)
Inflammation, interstitial		1 (2%)	
Inflammation, acute	1 (2%)	2 (4%)	1 (2%)
Abscess, NOS		2 (4%)	
Inflammation, active chronic	5 (10%)	4 (8%)	4 (8%)
Inflammation, chronic	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic focal			1 (2%)
Bacterial septicemia			1 (2%)
Hyperplasia, alveolar epithelium	8 (16%)	4 (8%)	6 (12%)
Histiocytosis	7 (14%)	2 (4%)	5 (10%)
#Lung/alveoli	(50)	(50)	(49)
Metaplasia, osseous	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Depletion, lymphoid		1 (2%)	1 (2%)
Leukemoid reaction			1 (2%)
Hyperplasia, plasma cell			1 (2%)
#Bone marrow	(49)	(50)	(48)
Myelofibrosis	25 (51%)	14 (28%)	11 (23%)
Hyperplasia, hematopoietic	7 (14%)	11 (22%)	10 (21%)
#Spleen	(50)	(48)	(50)
Necrosis, coagulative	1 (2%)		
Depletion, lymphoid	8 (16%)	8 (17%)	6 (12%)
Hyperplasia, lymphoid	6 (12%)	6 (13%)	5 (10%)
Hematopoiesis	14 (28%)	23 (48%)	27 (54%)
#Splenic capsule	(50)	(48)	(50)
Fibrosis, focal		1 (2%)	

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#Splenic follicles	(50)	(48)	(50)
Necrosis, NOS		1 (2%)	
#Lymph node	(44)	(47)	(45)
Inflammation, acute		1 (2%)	
Abscess, NOS	1 (2%)		2 (4%)
Depletion, lymphoid	1 (2%)		1 (2%)
Angiectasis		1 (2%)	
Hyperplasia, lymphoid		1 (2%)	
#Mandibular lymph node	(44)	(47)	(45)
Depletion, lymphoid		1 (2%)	
Hyperplasia, NOS		1 (2%)	1 (2%)
#Tracheal lymph node	(44)	(47)	(45)
Inflammation, acute	1 (2%)		
Abscess, NOS	1 (2%)	1 (2%)	
Depletion, lymphoid	1 (2%)		
Hyperplasia, plasma cell	1 (2%)	4 (9%)	1 (2%)
#Mediastinal lymph node	(44)	(47)	(45)
Hemorrhage		1 (2%)	
Abscess, NOS		4 (9%)	3 (7%)
Inflammation, acute/chronic	1 (2%)		
Depletion, lymphoid	1 (2%)		5 (11%)
Hyperplasia, plasma cell	1 (2%)	4 (9%)	9 (20%)
Hyperplasia, lymphoid		1 (2%)	1 (2%)
#Pancreatic lymph node	(44)	(47)	(45)
Inflammation, active chronic			1 (2%)
Depletion, lymphoid		1 (2%)	
Angiectasis		1 (2%)	
Hyperplasia, plasma cell		1 (2%)	
#Lumbar lymph node	(44)	(47)	(45)
Abscess, NOS			2 (4%)
Inflammation, active chronic			1 (2%)
Hyperplasia, plasma cell			2 (4%)
Hyperplasia, lymphoid		1 (2%)	
#Mesenteric lymph node	(44)	(47)	(45)
Hemorrhage	1 (2%)		3 (7%)
Abscess, NOS		1 (2%)	
Adhesion, NOS			2 (4%)
Necrosis, NOS		1 (2%)	
Depletion, lymphoid	1 (2%)	2 (4%)	
Hyperplasia, NOS	4 (9%)	4 (9%)	3 (7%)
Angiectasis	5 (11%)	10 (21%)	4 (9%)
#Renal lymph node	(44)	(47)	(45)
Hemorrhage			1 (2%)
Adhesion, NOS		1 (2%)	
Depletion, lymphoid			1 (2%)
Hyperplasia, NOS		2 (4%)	2 (4%)
Angiectasis		2 (4%)	
#Inguinal lymph node	(44)	(47)	(45)
Hyperplasia, lymphoid			1 (2%)
*Intercostal muscle	(50)	(50)	(50)
Hyperplasia, hematopoietic	1 (2%)		
#Liver	(50)	(50)	(50)
Hematopoiesis	9 (18%)	21 (42%)	23 (46%)
#Peyer's patch	(50)	(48)	(48)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#Thymus	(25)	(30)	(23)
Cyst, NOS	6 (24%)	1 (3%)	3 (13%)
Hemorrhage	1 (4%)		
Abscess, NOS			1 (4%)
Inflammation, chronic	1 (4%)		
Necrosis, NOS		2 (7%)	
Depletion, lymphoid	3 (12%)	5 (17%)	7 (30%)
Hyperplasia, lymphoid	1 (4%)		
#Thymic lymphocytes	(25)	(30)	(23)
Necrosis, NOS			1 (4%)
CIRCULATORY SYSTEM			
#Mesenteric lymph node	(44)	(47)	(45)
Thrombosis, NOS	1 (2%)		
#Lung	(50)	(50)	(49)
Perivasculitis	4 (8%)	5 (10%)	2 (4%)
#Heart	(50)	(49)	(49)
Mineralization	1 (2%)	1 (2%)	1 (2%)
Thrombus, organized		1 (2%)	
Inflammation, acute			2 (4%)
Abscess, NOS		1 (2%)	
Inflammation, active chronic		2 (4%)	5 (10%)
Inflammation, chronic	3 (6%)	4 (8%)	1 (2%)
Fibrosis, focal	1 (2%)		2 (4%)
Periarteritis	1 (2%)		
Degeneration, NOS	1 (2%)		4 (8%)
Necrosis, coagulative	1 (2%)		
#Myocardium	(50)	(49)	(49)
Degeneration, NOS	1 (2%)		1 (2%)
*Artery	(50)	(50)	(50)
Inflammation, chronic			1 (2%)
Hyperplasia, focal			1 (2%)
*Aorta	(50)	(50)	(50)
Mineralization		1 (2%)	
*Mesenteric artery	(50)	(50)	(50)
Thrombus, mural			1 (2%)
*Renal artery	(50)	(50)	(50)
Thrombus, organized			1 (2%)
#Uterus/endometrium	(50)	(49)	(49)
Thrombus, organized	1 (2%)		1 (2%)
DIGESTIVE SYSTEM			
*Tooth	(50)	(50)	(50)
Deformity, NOS	1 (2%)	1 (2%)	2 (4%)
Abscess, NOS		1 (2%)	4 (8%)
Inflammation, active chronic			3 (6%)
#Salivary gland	(44)	(47)	(46)
Inflammation, chronic	21 (48%)	9 (19%)	13 (28%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#Liver	(50)	(50)	(50)
Congenital malformation, NOS			1 (2%)
Congestion, NOS		2 (4%)	2 (4%)
Abscess, NOS			1 (2%)
Inflammation, active chronic	5 (10%)	6 (12%)	5 (10%)
Inflammation, chronic	2 (4%)	1 (2%)	
Adhesion, fibrous	1 (2%)	1 (2%)	
Necrosis, coagulative	8 (16%)	11 (22%)	6 (12%)
Metamorphosis, fatty	2 (4%)	1 (2%)	
Pigmentation, NOS	1 (2%)		
Nuclear enlargement		1 (2%)	
Basophilic cyto change	1 (2%)		
Eosinophilic cyto change			1 (2%)
Clear cell change	1 (2%)		
Hepatocytomegaly		2 (4%)	
#Pancreas	(47)	(44)	(42)
Dilatation/ducts			2 (5%)
Inflammation, active chronic	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic	8 (17%)	3 (7%)	2 (5%)
Adhesion, NOS	1 (2%)	1 (2%)	2 (5%)
Necrosis, coagulative	1 (2%)		
Atrophy, NOS	2 (4%)	1 (2%)	1 (2%)
Histiocytosis	1 (2%)		
#Esophagus	(46)	(45)	(38)
Inflammation, acute			1 (3%)
#Stomach	(50)	(50)	(50)
Hyperplasia, epithelial			1 (2%)
#Glandular stomach	(50)	(50)	(50)
Mineralization		2 (4%)	
Inflammation, chronic		1 (2%)	
#Gastric muscularis	(50)	(50)	(50)
Mineralization			1 (2%)
#Stomach wall	(50)	(50)	(50)
Mineralization		1 (2%)	
#Forestomach	(50)	(50)	(50)
Foreign body, NOS		2 (4%)	
Mineralization	1 (2%)		1 (2%)
Cyst, NOS	1 (2%)		
Ulcer, NOS	4 (8%)		3 (6%)
Inflammation, acute	1 (2%)	1 (2%)	
Inflammation, acute focal	2 (4%)		
Inflammation, acute necrotizing	1 (2%)		3 (6%)
Inflammation, active chronic	9 (18%)	4 (8%)	1 (2%)
Inflammation, chronic			4 (8%)
Adhesion, NOS		1 (2%)	
Hyperplasia, pseudoepitheliomatous	1 (2%)		
Hyperplasia, basal cell	1 (2%)	1 (2%)	1 (2%)
Hyperkeratosis	7 (14%)	4 (8%)	6 (12%)
Acanthosis	8 (16%)	4 (8%)	8 (16%)
#Colon	(50)	(48)	(47)
Adhesion, fibrous		1 (2%)	
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Mineralization	1 (2%)		
Hydronephrosis	2 (4%)	1 (2%)	
Cyst, NOS			1 (2%)
Glomerulonephritis, NOS	3 (6%)	8 (16%)	4 (8%)
Pyelonephritis, NOS	1 (2%)		5 (10%)
Inflammation, NOS			1 (2%)
Abscess, NOS	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#Kidney (Continued)	(50)	(50)	(50)
Inflammation, active chronic	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic	28 (56%)	22 (44%)	30 (60%)
Glomerulonephritis, chronic		1 (2%)	
Adhesion, NOS	2 (4%)		
Nephropathy	1 (2%)		
Glomerulosclerosis, NOS	3 (6%)	3 (6%)	5 (10%)
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)
#Kidney/cortex	(50)	(50)	(50)
Metaplasia, osseous		1 (2%)	
#Renal papilla	(50)	(50)	(50)
Necrosis, coagulative			3 (6%)
#Kidney/glomerulus	(50)	(50)	(50)
Adhesion, NOS		1 (2%)	1 (2%)
#Kidney/tubule	(50)	(50)	(50)
Dilatation, NOS	1 (2%)		1 (2%)
Cast, NOS	1 (2%)		
Degeneration, NOS		1 (2%)	
Atrophy, focal			2 (4%)
Regeneration, NOS	9 (18%)	5 (10%)	1 (2%)
#Kidney/pelvis	(50)	(50)	(50)
Inflammation, chronic focal	1 (2%)		
#Urinary bladder	(48)	(46)	(47)
Inflammation, acute focal	1 (2%)		
Inflammation, chronic	23 (48%)	13 (28%)	24 (51%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(36)	(43)	(36)
Cyst, NOS	1 (3%)	2 (5%)	1 (3%)
Hyperplasia, NOS	9 (25%)	16 (37%)	10 (28%)
Angiectasis		1 (2%)	1 (3%)
#Adrenal	(48)	(50)	(49)
Congestion, NOS			1 (2%)
Hemorrhage		1 (2%)	
Inflammation, acute/chronic	1 (2%)		
Inflammation, chronic		1 (2%)	
Adhesion, NOS			1 (2%)
Angiectasis	1 (2%)		2 (4%)
#Adrenal/capsule	(48)	(50)	(49)
Inflammation, active chronic			1 (2%)
Adhesion, NOS			1 (2%)
#Adrenal cortex	(48)	(50)	(49)
Cyst, NOS			1 (2%)
Hyperplasia, NOS			1 (2%)
#Periadrenal tissue	(48)	(50)	(49)
Abscess, NOS	1 (2%)		
Inflammation, active chronic	1 (2%)		
#Thyroid	(48)	(47)	(48)
Follicular cyst, NOS	1 (2%)	1 (2%)	
Inflammation, acute	1 (2%)		
Inflammation, chronic focal	1 (2%)		
Hyperplasia, follicular cell	3 (6%)	1 (2%)	
#Pancreatic islets	(47)	(44)	(42)
Hyperplasia, NOS	2 (4%)		
Angiectasis	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Galactocele	1 (2%)	1 (2%)	
Cyst, NOS	1 (2%)		
Inflammation, active chronic	2 (4%)		
Inflammation, chronic			3 (6%)
Hyperplasia, NOS	3 (6%)		1 (2%)
*Fallopian tube lumen	(50)	(50)	(50)
Dilatation, NOS		1 (2%)	
Inflammation, acute		1 (2%)	1 (2%)
#Uterus	(50)	(49)	(49)
Hydrometra	5 (10%)	2 (4%)	6 (12%)
Inflammation, acute	3 (6%)	11 (22%)	7 (14%)
Abscess, NOS	1 (2%)	1 (2%)	1 (2%)
Inflammation, active chronic	2 (4%)	1 (2%)	
Necrosis, coagulative			1 (2%)
Hyperplasia, epithelial	3 (6%)	7 (14%)	2 (4%)
Hyperplasia, stromal		1 (2%)	
Angiectasis			2 (4%)
Metaplasia, squamous		1 (2%)	
#Uterus/endometrium	(50)	(49)	(49)
Hyperplasia, NOS	33 (66%)	33 (67%)	28 (57%)
#Fallopian tube	(50)	(49)	(49)
Dilatation, NOS		1 (2%)	
#Ovary/parovarian	(49)	(50)	(50)
Mineralization	1 (2%)		
Abscess, NOS	12 (24%)	20 (40%)	29 (58%)
Inflammation, active chronic	1 (2%)	10 (20%)	3 (6%)
Inflammation, chronic	5 (10%)	2 (4%)	
Fibrosis	1 (2%)		
Adhesion, NOS	2 (4%)	7 (14%)	9 (18%)
Necrosis, NOS	1 (2%)		
#Ovary	(49)	(50)	(50)
Mineralization		1 (2%)	1 (2%)
Cyst, NOS	9 (18%)	8 (16%)	7 (14%)
Parovarian cyst	10 (20%)	7 (14%)	7 (14%)
Hemorrhage	1 (2%)		1 (2%)
Hemorrhagic cyst	2 (4%)	2 (4%)	2 (4%)
Inflammation, acute		1 (2%)	
Abscess, NOS		2 (4%)	
Necrosis, NOS		1 (2%)	
Angiectasis	2 (4%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
#Cerebrum	(49)	(50)	(50)
Mineralization			1 (2%)
#Brain	(49)	(50)	(50)
Hemorrhage			1 (2%)
Inflammation, acute focal			1 (2%)
#Brain/thalamus	(49)	(50)	(50)
Mineralization	27 (55%)	28 (56%)	21 (42%)
SPECIAL SENSE ORGANS			
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, NOS	1 (2%)		
Inflammation, acute			1 (2%)
Inflammation, active chronic	3 (6%)		
Inflammation, chronic	2 (4%)	1 (2%)	3 (6%)
*Ear canal	(50)	(50)	(50)
Necrosis, NOS			1 (2%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE (Continued)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*Intercostal muscle	(50)	(50)	(50)
Inflammation, acute	5 (10%)	3 (6%)	8 (16%)
Abscess, NOS			1 (2%)
Inflammation, active chronic	1 (2%)		3 (6%)
*Abdominal muscle	(50)	(50)	(50)
Abscess, NOS			1 (2%)
Inflammation, chronic		1 (2%)	
BODY CAVITIES			
*Thorax	(50)	(50)	(50)
Hemothorax		1 (2%)	1 (2%)
Empyema	2 (4%)		2 (4%)
*Mediastinum	(50)	(50)	(50)
Inflammation, acute			1 (2%)
Abscess, NOS			4 (8%)
*Peritoneum	(50)	(50)	(50)
Inflammation, acute	8 (16%)	8 (16%)	17 (34%)
Inflammation, acute necrotizing		2 (4%)	
Inflammation, active chronic	3 (6%)	3 (6%)	3 (6%)
Inflammation, chronic			1 (2%)
Adhesion, fibrous			1 (2%)
*Peritoneal cavity	(50)	(50)	(50)
Hemoperitoneum	1 (2%)	1 (2%)	
*Pleura	(50)	(50)	(50)
Inflammation, acute	5 (10%)	6 (12%)	7 (14%)
Inflammation, acute/chronic			1 (2%)
*Epicardium	(50)	(50)	(50)
Inflammation, acute	1 (2%)		1 (2%)
*Mesentery	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Inflammation, acute	1 (2%)	4 (8%)	6 (12%)
Inflammation, active chronic	2 (4%)		3 (6%)
Inflammation, chronic	8 (16%)	17 (34%)	6 (12%)
Adhesion, NOS		1 (2%)	
Bacterial septicemia	1 (2%)	2 (4%)	2 (4%)
Omentum			
Mineralization		1	
Abscess, NOS	1		
Inflammation, active chronic	3	1	
Inflammation, chronic		2	
Fibrosis		1	
Necrosis, NOS	1	2	
SPECIAL MORPHOLOGY SUMMARY			
None			

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

APPENDIX C

GENETIC TOXICOLOGY OF PENTACHLORONITROBENZENE

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TABLE C1. MUTAGENICITY OF PENTACHLORONITROBENZENE IN *SALMONELLA TYPHIMURIUM*

Strain	Dose (µg/plate)	Revertants/plate (a,b)		
		−S9	+S9 (rat)	+S9 (hamster)
TA100	0	124 ± 15.0	176 ± 1.2	102 ± 2.8
	100	101 ± 5.4	166 ± 15.4	107 ± 5.4
	333	118 ± 11.9	149 ± 9.5	98 ± 14.1
	1,000	120 ± 8.1	147 ± 6.4	110 ± 10.3
	3,333	122 ± 4.6	149 ± 10.7	126 ± 12.4
	6,667	113 ± 6.1	139 ± 6.0	122 ± 11.7
TA1535	0	15 ± 2.4	18 ± 1.3	15 ± 1.7
	100	19 ± 2.6	13 ± 3.3	15 ± 2.2
	333	21 ± 2.4	9 ± 2.0	13 ± 2.5
	1,000	19 ± 1.2	17 ± 2.7	12 ± 0.3
	3,333	19 ± 2.3	13 ± 1.5	14 ± 2.1
	6,667	21 ± 2.6	14 ± 2.5	14 ± 2.3
TA1537	0	24 ± 2.3	35 ± 5.2	26 ± 4.8
	100	9 ± 3.0	24 ± 4.2	15 ± 3.1
	333	11 ± 3.3	31 ± 10.1	21 ± 5.2
	1,000	15 ± 0.0	29 ± 1.8	20 ± 5.6
	3,333	16 ± 1.2	26 ± 1.0	33 ± 2.0
	6,667	19 ± 2.1	22 ± 3.3	26 ± 4.5
TA98	0	25 ± 4.8	43 ± 1.2	37 ± 5.8
	100	36 ± 3.7	45 ± 3.1	36 ± 1.8
	333	32 ± 6.3	42 ± 5.5	41 ± 0.7
	1,000	29 ± 4.4	43 ± 3.9	36 ± 3.8
	3,333	35 ± 2.6	37 ± 3.2	36 ± 3.0
	6,667	32 ± 2.9	37 ± 3.6	35 ± 2.3

(a) The S9 fractions were prepared from the liver of Aroclor 1254-induced male Sprague-Dawley rats and male Syrian hamsters. Cells and study compound or solvent (DMSO) were incubated for 20 minutes at 37° C in the presence of either S9 or buffer. After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 hours (Haworth et al., 1983). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.
(b) Mean ± standard error

TABLE C2. MUTAGENICITY OF PENTACHLORONITROBENZENE IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS IN THE ABSENCE OF S9 (a)

Compound	Dose (µg/ml)	Total Mutant Clones	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutation Frequency (mutants/10^6 clonable cells)
Acetone					
	1%	102	99.7	84.0	34
		107	112.3	106.0	32
		92	116.8	97.0	(b) 26 (33)
Methyl methanesulfonate					
	5.0	494	72.0	40.5	229
		391	56.7	36.0	230
		439	89.0	49.7	164 (208)
Pentachloronitrobenzene					
	1.25	80	93.7	68.4	28
		74	86.3	78.5	29
		96	94.7	84.4	34 (30)
	2.50	83	89.3	83.4	31
		70	103.0	85.9	23
		86	93.3	80.6	31 (28)
	5.00	108	106.3	78.0	34
		116	115.0	76.9	34
		88	85.3	63.6	34 (34)
	7.50	75	96.8	85.6	26
		88	115.2	96.9	25
		63	107.5	95.6	20 (24)
	10.00	69	81.2	76.4	28
		85	110.8	67.9	26
		63	111.8	95.5	19 (24)

(a) Experiments were performed twice; all doses were tested in duplicate or triplicate. Because the results were similar, data from only one experiment are shown. The protocol was basically that of Clive et al. (1979). Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium to determine the percentage of viable cells.
(b) The mean value is in parentheses.

TABLE C3. MUTAGENICITY OF PENTACHLORONITROBENZENE IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS IN THE PRESENCE OF S9 (a)

Compound	Dose (µg/ml)	Total Mutant Clones	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutation Frequency (mutants/10^6 clonable cells)
Acetone					
	1%	70	86.3	87.0	27
		77	75.2	93.0	34
		74	101.8	115.0	24
		59	82.5	103.0	(b) 24 (27)
3-Methylcholanthrene					
	2.5	775	50.2	33.1	515
		625	57.8	45.6	360
		778	78.3	38.1	331 (402)
Pentachloronitrobenzene					
	1.25	71	82.3	89.4	29
		74	82.0	81.5	30
		68	85.8	78.6	26 (28)
	2.50	80	80.5	88.7	33
		72	78.8	79.3	30
		107	100.5	90.3	35 (33)
	5.00	71	86.2	90.7	27
		85	108.7	98.3	26
		78	85.5	79.0	30 (28)
	7.50	97	109.0	85.0	30
		89	98.2	89.1	30
		87	100.7	101.5	29 (30)
	10.00	92	90.0	82.7	34
		84	98.8	80.0	28
		80	88.0	91.6	30 (31)

(a) Experiments were performed twice; all doses were tested in duplicate. Because the results were similar, data from only one experiment are shown. The protocol was basically that of Clive et al. (1979). Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium to determine the percentage of viable cells. S9 was prepared from the liver of Aroclor 1254-induced male F344 rats.
(b) The mean value is in parentheses.

TABLE C4. INDUCTION OF SISTER-CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY PENTACHLORONITROBENZENE (a)

−S9 (b)		+S9 (c)	
Dose (µg/ml)	SCE/Cell (d)	Dose (µg/ml)	SCE/Cell (d)
DMSO (10 µl)	8.4	DMSO (10 µl)	10.0
Pentachloronitrobenzene		Pentachloronitrobenzene	
0.75	8.5	7.5	8.7
2.40	9.3	24.0	11.0
7.50	9.3	75.0	9.3
Mitomycin C		Cyclophosphamide	
0.005	16.7	1.0	18.3

(a) SCE = sister-chromatid exchange
(b) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C; 2 hours after initiation of treatment, 10 µM BrdU was added, and incubation was continued for 22-24 hours. Cells were washed, fresh medium containing BrdU (10 µM) and colcemid (0.1 µg/ml) was added, and incubation was continued for 2-3 hours (Galloway et al., 1985).
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Then cells were washed, and medium containing 10 µM BrdU was added. Cells were incubated for a further 26 hours, with colcemid (0.1 µg/ml) present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague-Dawley rats.
(d) Cells were collected by mitotic shake-off, treated for 3 minutes with potassium chloride (75mM), washed twice with fixative, and dropped onto slides and air-dried (Galloway et al., 1985).

TABLE C5. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY PENTACHLORONITROBENZENE (a)

−S9 (b)		+S9 (c)	
Dose (µg/ml)	Abs/100 Cells (percent cells w/abs)	Dose (µg/ml)	Abs/100 Cells (percent cells w/abs)
DMSO (10 µl)	3 (3)	DMSO (10 µl)	3 (3)
Pentachloronitrobenzene		Pentachloronitrobenzene	
2.4	5 (5)	2.4	8 (8)
7.5	28 (16)	7.5	10 (9)
24.0	22 (19)	24.0	6 (6)
75.0	17 (10)	75.0	19 (13)
Mitomycin C		Cyclophosphamide	
0.150	44 (24)	15.0	22 (18)

(a) Abs = aberrations
(b) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid (0.1 µg/ml) was added. After a further 2-3 hours of incubation, cells were harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation continued for 8-10 hours. Colcemid (0.1 µg/ml) was added for the last 2-3 hours of incubation; then cells were harvested and fixed as above. S9 was from the liver of Aroclor 1254-induced male Sprague-Dawley rats.

APPENDIX D

CHEMICAL CHARACTERIZATION OF PENTACHLORONITROBENZENE

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APPENDIX D. CHEMICAL CHARACTERIZATION

I. Identity and Purity Determinations of Pentachloronitrobenzene Lot No. 0120679 Performed by the Analytical Chemistry Laboratory

A. Physical properties	Determined	Literature Values
1. Appearance:	Yellow, microcrystalline solid	Colorless solid (Merck Index, 1983)
2. Melting point:	143.1°-145.1° C (visual, Büchi 510)	144° C (Merck Index, 1983)

B. Spectral data

1. Infrared

Instrument:	Beckman IR-12	
Phase:	1.2% potassium bromide pellet	
Results:	See Figure 3	Consistent with literature spectrum (Sadtler Standard Spectra)

2. Ultraviolet/visible

Instrument:	Cary 118	
Solvent:	Methanol	Methanol

Results:

λ_{max} (nm)	$\varepsilon \times 10^{-2}$	λ_{max} (nm)	$\varepsilon \times 10^{-2}$
302	7.19 ± 0.06(6)	301	6.35
212	No ε calculated for this peak because the region in which it appears is unsuitable for good quantitation	212.5	729

(Sadtler Standard Spectra)

3. Nuclear magnetic resonance

Instrument:	Varian EM-360A	
Solvent:	Carbon tetrachloride with tetramethylsilane internal standard	
Results:	No peaks detected	Consistent with structure

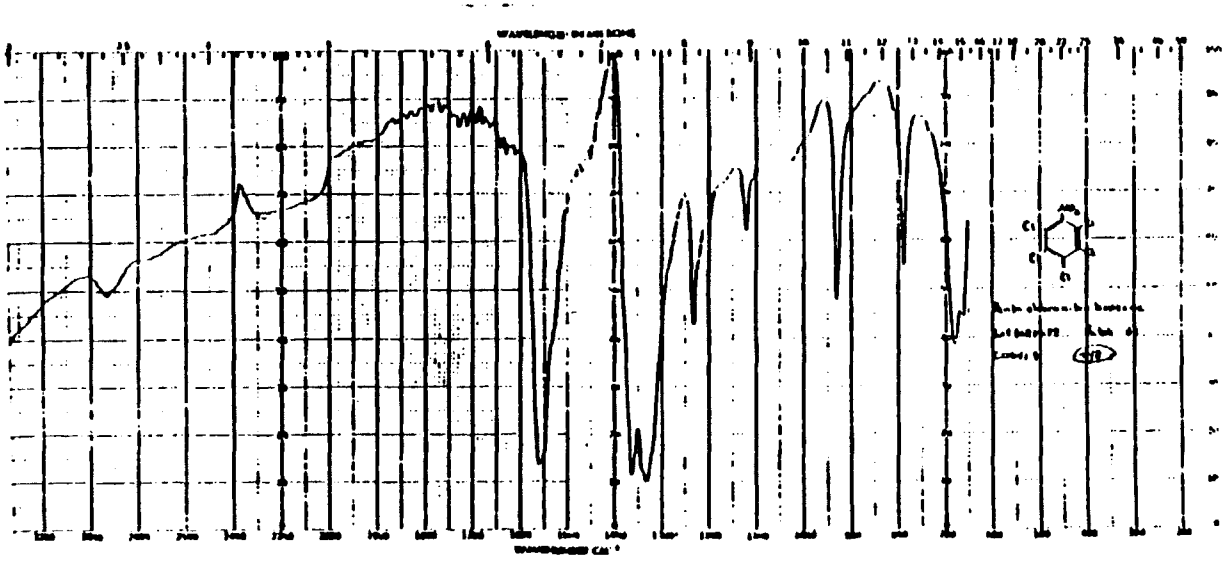

FIGURE 3. INFRARED ABSORPTION SPECTRUM OF PENTACHLORONITROBENZENE (LOT NO. 0120679)

C. **Water analysis (Karl Fischer):** <0.02%

D. **Elemental analysis**

Element	C	Cl	N
Theory (T)	24.40	60.02	4.74
Determined (D)	24.25 24.58	61.05 60.85	4.39 4.68
Percent D/T	99.9	101.5	95.8

E. **Chromatographic analysis**

1. **Thin-layer chromatography**

Reference standard: 1-Chloro-3-nitrobenzene
Amount spotted: 1, 10, and 30 µl of a 10 µg/µl chloroform solution of the compound, 10 µl of chloroform and 10 µg of the reference standard
Visualization: (1) Ultraviolet 254 nm, (2) 0.2% Fluorescein in absolute ethanol followed by a 1% silver nitrate in 0.5 N ammonium hydroxide spray

System 1

Solvent: *n*-Pentane
Plates: Silica Gel 60 F-254, 25 mm thick

System 2

Solvent: Acetone:acetonitrile (1:1)
Plates: Whatman $KC_{18}F$ reverse phase, 20 mm thick

Results	R_f	R_{st}
System 1		
Major spot	0.51	2.2
Reference standard	0.23	1.0
System 2		
Major spot	0.59	0.87
Reference standard	0.68	1.00

2. **Gas chromatography**

Instrument: Varian 3700
Detector: Flame ionization
Inlet temperature: 200° C
Detector temperature: 250° C
Carrier gas: Nitrogen, 70 ml/min

System 1

Column: 1.8 m × 4 mm ID glass, silylated and packed with 3% SP2250 on 100/120 Supelcoport
Oven temperature program: 5 min at 50° C followed by a 10° C/min increase to 250° C
Samples injected: Approximately 3 µl of 1.0% and 0.5% (w/v) chloroform solutions to detect and quantitate impurities and to establish linearity of detector response

Results: Two peaks were detected preceding the major peak.

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	18.7	0.84	0.10
2	21.2	0.95	0.28
3	22.3	1.00	100.0

System 2

Column: 1.8 m × 4 mm ID glass, silylated and packed with 10% Carbowax 20M-TPA on 80/100 Chromosorb W(AW)
Oven temperature program: 50° C for 5 min followed by a 10° C/min increase to 200° C
Samples injected: Approximately 3 µl of 0.5% and 1% (w/v) chloroform solutions to detect and quantitate impurities and to establish linearity of detector response

Results: Three impurities with relative areas greater than 0.1% (peaks 2 and 3 were not completely resolved and were combined for calculation purposes) were detected preceding the major peak. One additional impurity, occurring as a shoulder on the front of the major peak, with a relative area of <0.1%, was also detected.

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak		Area (percent of major peak)
1	21.9	0.76		0.15
2	24.2	0.84	Unresolved	0.24
3	24.5	0.85		
4	28.7	1.00		100

F. **Conclusions:** The results of the elemental analysis for carbon agreed with theoretical values. The determined value was slightly higher than theoretical for chlorine and slightly lower for nitrogen. The water content by Karl Fischer titrimetry was less than 0.02%. Two thin-layer chromatographic systems detected only a single component. One gas chromatographic system detected a major peak preceded by two impurities with relative areas of 0.10% and 0.28%. A second gas chromatographic system detected a major peak and three impurities preceding it with relative areas of 0.15% and 0.24% (impurities 2 and 3 combined). An additional impurity with a relative area of <0.10%, occurring as a shoulder on the front of the major peak, was also seen in the second system. The infrared and nuclear magnetic resonance spectra were consistent with the structure and did not indicate the presence of any notable impurities. The ultraviolet/visible spectrum was consistent with a literature spectrum in λ_{max}. However, the determined ε_{302} value was higher than the reported literature value.

II. Quantitation of Hexachlorobenzene Present in Pentachloronitrobenzene Lot No. 0120679 Performed by the Analytical Chemistry Laboratory

A. **Analytical method:** Gas chromatography/mass spectrometry

Instrument: Varian MAT 311-A mass spectrometer coupled via a single-stage glass jet helium separator to a Varian 2700 gas chromatograph. Data handled by an Incos 2300 data system
Column: 1% SP2250 on 100/120 Supelcoport, 1.8 m × 2 mm ID, glass
Oven temperature: 175° C
Carrier gas: Helium, 30 ml/min
Temperatures
Inlet, 180° C
Helium separator, 280° C
Transfer line, 290° C
Ion source, 250° C
Electron energy: 70 eV
Scan range: 40-350 AMU
Accelerator voltage: 3,000 V
Electron multiplier voltage: −2,000 V
Emission current: 1 mA
Resolution: 1,000

Standard solutions of hexachlorobenzene (103.41 and 51.72 µg/ml in chloroform), when injected on the system given above, gave a peak with a retention time of 2.3 min, which was confirmed to be hexachlorobenzene by its mass spectrum. Injection of solutions of pentachloronitrobenzene (100.21 and 100.10 mg/ml in chloroform) gave a peak at this retention time which was shown to be a mixture of hexachlorobenzene and an isomer of tetrachloronitrobenzene. Comparison of the abundance of m/e 284 (largest ion in the molecular ion cluster of hexachlorobenzene) in the sample injections with the abundance of this ion provided by standard injections was used to determine the amount of hexachlorobenzene present in the pentachloronitrobenzene sample.

B. **Results:** Hexachlorobenzene was found to be present in pentachloronitrobenzene at a level of 0.07% ± 0.01% (w/w).

III. Stability Study of Pentachloronitrobenzene Lot No. 0120679 Performed by the Analytical Chemistry Laboratory

A. **Sample storage:** Samples were stored protected from light in glass vials with Teflon®-lined lids for 2 weeks at temperatures of −20°, 5°, 25°, or 60° C.

B. **Analysis:** Triplicate samples were prepared from each storage temperature by diluting approximately 150 mg of the compound, accurately weighed, and approximately 50 mg of 1-nitronaphthalene, the internal standard, accurately weighed, to 50 ml with chloroform. Samples were analyzed by the following gas chromatographic system.

Instrument: Varian 3700
Detector: Flame ionization
Inlet temperature: 220° C
Detector temperature: 250° C
Carrier gas: Nitrogen, 70 ml/min
Column: 1.8 m × 4 mm ID glass, silylated and packed with 3% SP2250 on 100/120 Supelcoport
Oven temperature: 210° C, isothermal

C. **Results**

Storage Temperature	Percent Compound (normalized to −20° C sample)
−20° C	100.0
5° C	100.6 ± 0.7(δ)
25° C	100.5 ± 0.7(δ)
60° C	101.0 ± 0.7(δ)

D. **Conclusions:** Pentachloronitrobenzene was stable within the limits of error of the analysis at temperatures of up to 60° C when stored for 2 weeks in glass vials protected from light.

IV. Stability Study of Pentachloronitrobenzene Lot No. 0120679 Performed by the Study Laboratory

A. Storage conditions

Bulk: 0° C
Reference: −18° C or lower

B. Analytical methods

1. Infrared spectroscopy

Instrument: Perkin-Elmer Infracord #137
Phase: Potassium bromide pellet

2. Gas chromatography

Instrument: Varian 1400 with HP3390A integrator or Varian 3700 with CDS-111
Detection: Flame ionization
Column: 3% SP2250 on 100/120 Supelcoport, 6 ft × 2 mm ID, glass
Inlet temperature: 200° or 210° C
Oven temperature program: 100°-230° C at 10° C/min
Detector temperature: 240° or 250° C

C. Results

1. **Infrared spectroscopy:** All bulk spectra were consistent with the reference spectra and with the spectrum supplied by the analytical chemistry laboratory.

2. **Gas chromatography**

Date of Analysis	Percent Pentachloronitrobenzene Bulk	Percent Pentachloronitrobenzene Reference
05/29/80	99.40	--
08/26/80	99.58	99.54
12/23/80	99.50	99.47
04/16/81	99.50	99.49
08/12/81	99.58	99.56
12/21/81	99.59	99.59
04/05/82	99.58	99.55
08/06/82	99.58	99.59
12/17/82	99.60	99.62
04/25/83	99.53	99.53
06/23/83	99.52	99.55

D. Conclusions:

Results show no notable degradation of the study material occurred during the studies.

APPENDIX E

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

I. Studies Conducted by the Analytical Chemistry Laboratory

A. Analysis of formulated diets for mixing homogeneity

1. **Study parameters**

   Concentration: 20,000 ppm
   Vehicle: NIH 07 Rat and Mouse Ration
   Blender: Patterson-Kelly® twin-shell, 4-quart, stainless steel, with intensifier bar

2. **Premix preparation:** Pentachloronitrobenzene (30.00 g) was transferred to a 600-ml beaker and thoroughly mixed by spatula with approximately 30 g of feed. More feed was added in 30- to 40-g portions with mixing between additions until the weight of the premix was 200 g.

3. **Bulk-mixing and sampling:** A 600-g portion of feed was layered evenly into the blender; then the 200-g premix was added in equal amounts to both sides of the blender. The fine material adhering to the beaker walls was taken up by briefly stirring 100 g of feed in the beaker and then adding it to the blender. An additional 600 g of feed was layered over the premix, and the blender ports were sealed. The mix was blended with the intensifier bar on for the first 5 minutes and off for 10 minutes. Periodically, the outside of the blender was given a firm tap with a block of wood to dislodge any feed packed in the corners. At the end of the 15-minute mixing period, approximately 50 g of the blend was sampled from the upper left- and right-hand shells and from the bottom discharge port. Triplicate 10-g samples from each location (± 0.01 g) were transferred to 200-ml foil-wrapped centrifuge bottles for the analysis of pentachloronitrobenzene. The target concentration of the chemical in the feed blend was 20,000 ppm.

4. **Analysis procedure:** Samples (10.00 g in 200-ml foil-wrapped centrifuge bottles) were extracted with 100 ml of reagent-grade acetonitrile by shaking for 30 minutes on a Burrell Wrist-Action® Shaker. Aliquots (5 ml) of the extracts, clarified by centrifugation, were pipetted into 10-ml amber septum vials containing 5 ml of internal standard solution (carbazole, 0.7 mg/ml in acetonitrile). The vials were sealed and mixed; then the pentachloronitrobenzene concentration was determined by the gas chromatographic system described below.

   Instrument: Varian 3700 Gas Chromatograph with CDS-111 integrator and autosampler
   Column: 3% SP2100 on 100/120 mesh Supelcoport; 1.8 m × 2 mm; glass, silanized
   Detector: Flame ionization
   Temperature
   Oven, 170° C, isothermal
   Dectector, 260° C
   Injector, 200° C
   Carrier gas: Nitrogen, 30 ml/min
   Volume injected: 3 µl
   Retention times: Pentachloronitrobenzene--4.7 min
   Internal standard--5.6 min

5. **Quality assurance measures:** Analyses were performed in random order with duplicate injections of sample solutions prepared in triplicate. Recovery of the chemical from feed was determined in triplicate with undosed feed spiked at the same level as the samples and carried through the sample analysis procedure. All determinations were related to an internal standard incorporated into the sample solutions. Results were calculated from electronically integrated peak areas of the calibration standards.

   The linearity of the gas chromatographic detector system was evaluated with standard solutions of pentachloronitrobenzene at concentrations of 0.6, 1.0, and 1.2 mg/ml. The correlation coefficient was 0.99999.

6. **Results**

15-Min Blend Sampling Location	Pentachloronitrobenzene in Feed (ppm) (a,b)	Percent Recovery (c)
Left	20,200	101.1 ± 0.9
Right	20,110	100.6 ± 0.4
Bottom	20,230	101.2 ± 1.9

(a) Results are the mean of three determinations corrected for a zero-time spiked recovery yield of 98.1% ± 0.2%.
(b) Target concentration of pentachloronitrobenzene in feed was 20,000 ppm.
(c) Error values are average deviations from the mean and include both analytical precision and feed blend variability.

7. **Conclusions:** Pentachloronitrobenzene was blended into animal feed at a concentration of 20,000 ppm and showed <1% variation in the mean concentration determined at three sampling points in the blender. The mean of the nine analyses was 100.9% of the target concentration.

B. Analysis of formulated diets for stability of pentachloronitrobenzene

1. Study parameters

Concentration: 20,000 ppm
Vehicle: NIH 07 Rat and Mouse Ration
Duration: 2 weeks
Temperatures: 5°, 25°, and 45° C

2. **Sample preparation and storage:** From the dosed feed blend prepared as described in Section I.A.3. of this appendix, four 12-oz screw-cap jars were filled with about 250 g of mix and tightly sealed. Individual jars were stored in the dark at 5°, 25°, or 45° C for the 2-week stability study.

3. **Analysis procedure:** Same as in Section I.A.4. of this appendix

4. **Quality assurance measures:** Same as in Section I.A.5. of this appendix

5. **Results**

Storage Temperature	Pentachloronitrobenzene in Feed (ppm) (a,b)	Percent Recovery (c)
5° C	20,330	101.7 ± 0.3
25° C	20,170	100.8 ± 1.2
45° C	19,340	96.7 ± 0.4

(a) Results are the mean of three determinations corrected for a zero-time spiked recovery yield of 97.7% ± 0.3%.
(b) Target concentration of pentachloronitrobenzene in feed was 20,000 ppm.
(c) Error values are average deviations from the mean and include both analytical precision errors plus feed blend variability.

6. **Conclusions:** Pentachloronitrobenzene blended into animal feed at 20,000 ppm was stable after 2 weeks' storage in the dark at temperatures of up to 25° C. There was a 3.3% loss after 2 weeks' storage at 45° C.

II. Analysis of Formulated Diets for Mixing Homogeneity Conducted by the Study Laboratory

A. Preparation of formulated diets: The appropriate amount of pentachloronitrobenzene was weighed, added to an aliquot of NIH 07 Rat and Mouse Ration in a mortar, and mixed with a pestle to form a visually uniform premix. The premix was sandwiched between the remaining meal in a Patterson-Kelly® Intensifier Bar model V-blender and mixed for 15 minutes.

B. Sampling and analysis: Duplicate 2-g samples were taken from the top right, top left, and bottom positions of the blender for each of the 40,000- and 1,250-ppm mixtures. Samples were extracted with 50 ml of absolute ethanol. The supernatant solutions for the 40,000-ppm concentration were analyzed by ultraviolet spectrophotometry at 302 nm after a 1:10 dilution with ethanol. The 1,250-ppm concentration supernatant solutions were analyzed by flame ionization detection/gas chromatography at 200° C on a 6 ft × 2 mm ID glass column packed with 3% SP2250 on 100/120 mesh Supelcoport.

C. Results

Sample Location	Target Concentration (ppm)	Measured Concentration (ppm) (a)	Percent of Target
Top left	40,000	39,300	98.3
Top right	40,000	36,500	91.3
Bottom	40,000	36,800	92.0
Top left	1,250	1,210	96.8
Top right	1,250	1,130	90.4
Bottom	1,250	1,130	90.4

(a) Results of duplicate analysis

D. Conclusions: Concentrations found were all within ± 10% of the target values.

APPENDIX F

METHODS OF ANALYSIS OF FORMULATED DIETS

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APPENDIX F. METHODS OF ANALYSIS

I. Study Laboratory

Procedure: Duplicate 2-g samples were extracted with acetonitrile containing 0.09 mg/ml carbazole as the internal standard. Spiked reference samples were prepared in the same manner to provide calibration data. The supernatant solutions were analyzed by flame ionization detection/gas chromatography at 200° C on a 6 ft × 2 mm ID glass column packed with 3% SP2250 on 100/120 mesh Supelcoport.

II. Analytical Chemistry Laboratory

Pentachloronitrobenzene is light sensitive. All operations were therefore performed in subdued light with foil-covered or amber glassware.

A. **Preparation of spiked feed standards:** Two standard solutions of pentachloronitrobenzene were prepared independently in acetonitrile. These solutions were diluted with acetonitrile to make four additional standards. Aliquots (20 ml) of the six standard solutions were pipetted into individual 200-ml centrifuge bottles containing 10 g of undosed feed to make spiked feed standards bracketing the specified concentration range of the referee sample. One 200-ml centrifuge bottle containing 10 g of undosed feed was treated with 20 ml of acetonitrile for use as a blank. The spiked feed standards and the feed blank were sealed and allowed to stand overnight at room temperature before being analyzed.

B. **Preparation of the referee sample:** Triplicate amounts of the referee feed sample (approximately 10 g weighed to the nearest 0.001 g) were transferred to individual 200-ml centrifuge bottles. Acetonitrile (20 ml) was pipetted into each sample; the bottles were sealed and allowed to stand overnight at room temperature before analysis.

C. **Analysis:** Acetonitrile (80 ml) was pipetted into each blank, standard, and referee sample bottle, and the bottles were shaken at maximum stroke for 30 minutes on a wrist-action shaker. After being centrifuged for 5 minutes, a 5-ml aliquot of each extract was mixed with 5 ml of internal standard solution (carbazole in acetonitrile). The pentachloronitrobenzene content of these solutions was determined by the gas chromatographic system described below.

Instrumental system

Instrument: Varian 3700 Gas Chromatograph with Autosampler and Varian CDS 111-C integrator
Column: 3% SP2250 on 100/120 mesh Supelcoport, 1.8 m × 4 mm ID, glass, silanized
Detection: Flame ionization
Temperatures
Inlet, 220° C
Oven, 200° C, isothermal
Detector, 250° C
Carrier gas: Nitrogen, 30 ml/min
Volume of solution injected: 3 µl

The total amount of pentachloronitrobenzene in the referee feed samples was determined from a linear regression equation relating each spiked feed standard to the internal standard.

D. **Quality assurance measures:** The referee feed sample was analyzed in triplicate, and the undosed feed sample was analyzed once. Individually spiked portions of undosed feed (six levels bracketing the specified concentration range of the referee sample) were prepared from two independently weighed standards and were treated like the referee feed samples to obtain standard data. Triplicate injections of each standard and sample were made into the gas chromatograph in a random order. All determinations were related to an internal standard incorporated into the sample solutions.

APPENDIX G

RESULTS OF ANALYSIS OF FORMULATED DIETS

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TABLE G1. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLORONITROBENZENE

Date Mixed	Concentration (a) of Pentachloronitrobenzene in Feed (ppm)		Determined as a Percent of Target
	Target	Determined	
05/05/80	2,500	2,300	92
	5,000	4,600	92
	10,000	10,300	103
	20,000	19,800	99
	40,000	37,500	94

(a) Results of duplicate analysis

TABLE G2. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE

Date Mixed	Concentration (a) of Pentachloronitrobenzene in Feed for Target Concentration (ppm)	
	2,500	5,000
06/03/81	2,500	4,900
07/20/81	2,450	5,100
08/24/81	2,400	5,100
10/05/81	2,600	4,900
01/04/82	2,400	4,900
03/01/82	2,500	5,200
04/26/82	2,540	4,700
05/24/82	2,370	4,930
07/19/82	2,370	5,160
09/27/82	2,610	4,990
10/25/82	2,350	5,100
01/24/83	2,500	4,700
02/14/83	2,400	5,100
04/25/83	2,400	5,000
Mean (ppm)	2,460	4,980
Standard deviation	86	160
Coefficient of variation (percent)	3.5	3.2
Range (ppm)	2,350-2,610	4,700-5,200
Number of samples	14	14

(a) Results of duplicate analysis

TABLE G3. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
06/03/81	5,000	4,900	5,000
10/05/81	2,500	2,600	2,600
04/26/82	2,500	2,540	2,500
10/25/82	5,000	5,100	4,900
04/25/83	2,500	2,400	2,500

(a) Results of duplicate analysis
(b) Results of triplicate analysis

APPENDIX H

RESULTS OF SEROLOGIC ANALYSIS AND CULTURE OF OVARIAN ABSCESSES

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APPENDIX H. SEROLOGY AND CULTURE OF ABSCESSES

I. Sentinel Animal Program

A. 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 viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both 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.

Fifteen $B6C3F_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected control animals of each sex. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the antibody titers. The following tests are performed:

Hemagglutination Inhibition	Complement Fixation	ELISA
PVM (pneumonia virus of mice)	M.Ad. (mouse adenovirus)	MHV (mouse hepatitis virus)
Reo 3 (reovirus type 3)	LCM (lymphocytic choriomeningitis virus)	*M. pul. (Mycoplasma pulmonis)* (24 mo)
GDVII (Theiler's encephalomyelitis virus)	Sendai (12 mo)	
Poly (polyoma virus)		
MVM (minute virus of mice)		
Ectro (infectious ectromelia)		
Sendai (6, 18, 24 mo)		

B. Results

TABLE H1. MURINE ANTIBODY DETERMINATIONS IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE (a)

Interval (months)	No. of Animals	Positive Serologic Reaction for
6	--	None positive
12	--	None positive
18	3/8	MHV
24	7/9	Sendai
	1/9	*M. pul.*
	5/10	MHV

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates, Inc. (Bethesda, MD) for the Animal Disease Screening Program.

II. Results of Culture of Ovarian Abscesses

TABLE H2. SUMMARY OF BACTERIOLOGIC FINDINGS IN OVARIAN ABSCESSES IN MICE IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE (a)

Date of Sampling	Animal Identification	Bacteriologic Finding
2/04/83	56HF #23	*Klebsiella pneumoniae*
3/11/83	56UF #22	*Staphylococcus aureus*
6/10/83	56UF #24	*K. oxytoca*
6/10/83	56UF #21	*K. pneumoniae*
6/09/83	56LF #49	*K. oxytoca*
6/09/83	56LF #7	*K. oxytoca*

(a) Identification of enteric organisms was by the API 20E bacterical identification system (Analytab Products, Inc., Plainview, NY 11803).

APPENDIX I

FEED AND COMPOUND CONSUMPTION BY MICE IN THE TWO-YEAR FEED STUDIES OF PENTACHLORONITROBENZENE

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TABLE I1. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
4	7	30.6	6	31.0	0.9	484	6	30.1	0.9	997
8	7	33.7	7	33.4	1.0	524	8	31.9	1.1	1,254
12	6	34.6	6	34.6	1.0	434	7	33.1	1.2	1,057
16	6	35.8	6	35.8	1.0	419	7	34.1	1.2	1,026
20	6	37.3	6	37.1	1.0	404	7	35.5	1.2	986
24	6	38.6	6	38.5	1.0	390	7	36.2	1.2	967
28	6	39.5	5	38.8	0.8	322	6	36.7	1.0	817
32	6	38.0	6	38.1	1.0	394	7	36.0	1.2	972
36	6	41.6	7	40.5	1.2	432	8	37.9	1.3	1,055
40	5	42.7	6	40.9	1.2	367	8	38.4	1.6	1,042
44	6	43.5	7	42.3	1.2	414	8	38.4	1.3	1,042
48	5	43.2	6	41.9	1.2	358	7	38.9	1.4	900
52	5	43.1	5	41.5	1.0	301	6	38.7	1.2	775
56	5	43.0	5	41.8	1.0	299	7	38.7	1.4	904
61	7	41.6	6	41.4	0.9	362	7	38.4	1.0	911
64	4	42.4	5	42.7	1.3	293	6	38.4	1.5	781
68	5	40.9	6	41.4	1.2	362	6	38.9	1.2	771
72	5	41.1	5	40.8	1.0	306	6	38.4	1.2	781
76	5	41.8	6	42.8	1.2	350	7	38.7	1.4	904
80	6	41.3	7	41.3	1.2	424	8	38.0	1.3	1,053
84	5	39.9	5	39.9	1.0	313	6	37.4	1.2	802
88	5	40.2	6	40.1	1.2	374	7	38.1	1.4	919
92	5	40.5	7	39.9	1.4	439	7	36.1	1.4	970
96	4	39.1	6	38.8	1.5	387	8	37.4	2.0	1,070
100	8	38.7	8	39.0	1.0	513	8	37.2	1.0	1,075
Mean	5.6	39.7	6.0	39.4	1.1	387	7.0	36.9	1.3	953
SD (d)	1.0		0.8		0.2	64	0.8		0.2	121
CV (e)	17.9		13.3		18.2	16.5	11.4		15.4	12.7

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of pentachloronitrobenzene consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE I2. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF PENTACHLORONITROBENZENE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
4	7	23.7	7	23.1	1.0	758	7	23.3	1.0	1,502
8	7	26.3	8	25.4	1.1	787	10	25.4	1.4	1,969
12	6	27.9	6	26.3	1.0	570	8	26.6	1.3	1,504
16	6	29.5	7	28.3	1.2	618	8	28.0	1.3	1,429
20	7	31.9	8	29.9	1.1	669	8	29.5	1.1	1,356
24	7	34.5	9	31.7	1.3	710	9	30.6	1.3	1,471
28	6	35.6	7	33.2	1.2	527	7	32.0	1.2	1,094
32	7	35.0	8	32.5	1.1	615	8	31.2	1.1	1,282
36	8	37.8	9	35.0	1.1	643	8	34.0	1.0	1,176
40	7	39.9	8	36.3	1.1	551	9	34.7	1.3	1,297
44	7	41.6	9	37.8	1.3	595	10	36.9	1.4	1,355
48	6	42.9	8	39.3	1.3	509	9	36.4	1.5	1,236
52	6	43.8	7	39.6	1.2	442	8	36.5	1.3	1,096
56	6	42.7	7	39.3	1.2	445	8	35.8	1.3	1,117
61	5	41.9	8	39.5	1.6	506	8	35.7	1.6	1,120
64	6	43.9	7	39.6	1.2	442	7	34.0	1.2	1,029
68	6	44.4	8	39.7	1.3	504	10	37.8	1.7	1,323
72	7	43.5	7	39.7	1.0	441	10	36.3	1.4	1,377
76	7	46.1	10	41.0	1.4	610	10	38.1	1.4	1,312
80	8	46.3	9	42.5	1.1	529	10	38.0	1.3	1,316
84	7	45.9	9	42.2	1.3	533	11	37.1	1.6	1,482
88	8	46.2	9	41.5	1.1	542	13	37.9	1.6	1,715
92	8	48.4	10	41.4	1.3	604	11	36.6	1.4	1,503
96	8	47.7	11	41.4	1.4	664	12	38.4	1.5	1,563
100	8	49.8	13	41.6	1.6	781	10	37.9	1.3	1,319
Mean	6.8	39.9	8.4	36.3	1.2	584	9.2	33.9	1.3	1,358
SD (d)	0.9		1.5		0.2	103	1.6		0.2	210
CV (e)	13.2		17.9		16.7	17.6	17.4		15.4	15.5

(a) Grams of feed removed from the feeder; not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of pentachloronitrobenzene consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX J

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

Meal Diet: April 1981 to April 1983

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

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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
Brewer's dried 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) NIH, 1978; NCI, 1976
(b) Ingredients 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 activity
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

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

TABLE J3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrient	Mean ± Standard Deviation	Range	No. of Samples
Crude protein (percent by weight)	24.19 ± 1.07	22.4-26.3	25
Crude fat (percent by weight)	5.02 ± 0.47	4.2-6.0	25
Crude fiber (percent by weight)	3.37 ± 0.37	2.4-4.2	25
Ash (percent by weight)	6.54 ± 0.26	5.97-7.03	25
Essential Amino Acids (percent of total diet) (a)			
Arginine	1.300	1.21-1.38	3
Cystine	0.340	0.23-0.40	3
Glycine	1.137	1.06-1.20	3
Histidine	0.561	0.530-0.578	3
Isoleucine	0.899	0.881-0.934	3
Leucine	1.930	1.85-1.98	3
Lysine	1.243	1.20-1.30	3
Methionine	0.329	0.306-0.368	3
Phenylalanine	0.991	0.960-1.04	3
Threonine	0.851	0.827-0.886	3
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.647	0.566-0.769	3
Valine	1.090	1.05-1.12	3
Essential Fatty Acids (percent of total diet) (a)			
Linoleic	2.40	2.37-2.44	2
Linolenic	0.284	0.259-0.308	2
Vitamins (a)			
Vitamin A (IU/kg)	11,936 ± 2,547	8,900-22,000	25
Vitamin D (IU/kg)	5,220	4,140-6,300	2
α-Tocopherol (ppm)	39.1	31.1-44.0	3
Thiamine (ppm)	18.7 ± 3.20	14.0-26.0	24 (b)
Riboflavin (ppm)	7.3	6.1-8.1	3
Niacin (ppm)	82	65-97	3
Pantothenic acid (ppm)	30.2	23.0-30.5	3
Pyridoxine (ppm)	7.7	5.6-8.8	3
Folic acid (ppm)	2.5	1.8-3.4	3
Biotin (ppm)	0.27	0.21-0.32	3
Vitamin B_{12} (ppb)	21.2	10.6-38.0	3
Choline (ppm)	3,337	3,200-3,430	3
Minerals (a)			
Calcium (percent)	1.22 ± 0.10	1.10-1.45	25
Phosphorus (percent)	0.96 ± 0.05	0.84-1.10	25
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.581	0.479-0.635	3
Sodium (percent)	0.307	0.258-0.349	3
Magnesium (percent)	0.165	0.151-0.177	3
Sulfur (percent)	0.292	0.270-0.290	3
Iron (ppm)	420	409-431	3
Manganese (ppm)	87.7	81.7-95.5	3
Zinc (ppm)	52.1	46.1-56.0	3
Copper (ppm)	11.15	8.09-15.70	3
Iodine (ppm)	2.66	1.52-3.64	3
Chromium (ppm)	1.72	1.44-1.93	3
Cobalt (ppm)	0.64	0.49-0.78	3

(a) Two or three batches of feed analyzed for nutrients reported in this table were manufactured in 1983 and 1984.
(b) One batch (7/22/81) was not analyzed for thiamine.

TABLE J4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminant	Mean ± Standard Deviation	Range	No. of Samples
Arsenic (ppm)	0.45 ± 0.11	0.21-0.65	25
Cadmium (ppm) (a)	<0.1		25
Lead (ppm)	0.95 ± 0.78	0.27-2.93	25
Mercury (ppm) (a)	< 0.05		25
Selenium (ppm)	0.28 ± 0.06	0.16-0.40	25
Aflatoxins (ppb) (b)	<10	<5.0-10.0	25
Nitrate nitrogen (ppm) (c)	9.85 ± 4.55	0.6-19.0	25
Nitrite nitrogen (ppm) (c)	1.92 ± 1.28	0.4-5.3	25
BHA (ppm) (d)	5.67 ± 5.07	1.5-20.0	25
BHT (ppm) (d)	3.35 ± 2.55	<1.0-13.0	25
Aerobic plate count (CFU/g) (e)	121,420 ± 94,844	7,000-420,000	25
Coliform (MPN/g) (f)	965 ± 991	<3-2,400	25
E. coli (MPN/g) (f,g)	6.76 ± 7.06	<3-23	24
E. coli (MPN/g) (f,h)	12.64 ± 29.46	<3-150	25
Total nitrosamines (ppb) (i, j)	4.40 ± 3.16	<1.2-12.9	24
Total nitrosamines (ppb) (i,k)	8.29 ± 19.41	1.2-100.3	25
N-Nitrosodimethylamine (ppb) (i,l)	3.05 ± 3.05	0.6-12.0	24
N-Nitrosodimethylamine (ppb) (i,m)	6.89 ± 19.42	0.6-99.0	25
N-Nitrosopyrrolidine (ppb)	1.20 ± 0.62	<0.3-2.4	25
Pesticides (ppm)			
α-BHC (a,n)	<0.01		25
β-BHC (a)	<0.02		25
γ-BHC-Lindane (a)	<0.01		25
δ-BHC (a)	<0.01		25
Heptachlor (a)	<0.01		25
Aldrin (a)	<0.01		25
Heptachlor epoxide (a)	<0.01		25
DDE (o)	<0.01	0.05 (7/14/81)	25
DDD (a)	<0.01		25
DDT (a)	<0.01		25
HCB (a)	<0.01		25
Mirex (a)	<0.01		25
Methoxychlor (p)	<0.05	0.13 (8/25/81); 0.6 (6/29/82)	25
Dieldrin (a)	<0.01		25
Endrin (a)	<0.01		25
Telodrin (a)	<0.01		25
Chlordane (a)	<0.05		25
Toxaphene (a)	<0.1		25
Estimated PCB's (a)	<0.2		25
Ronnel (a)	<0.01		25
Ethion (a)	<0.02		25
Trithion (a)	<0.05		25
Diazinon (a)	<0.1		25
Methyl parathion (a)	<0.02		25
Ethyl parathion (a)	<0.02		25
Malathion (q)	0.08 ± 0.05	<0.05-0.25	25
Endosulfan I (a)	<0.01		25
Endosulfan II (a)	<0.01		25
Endosulfan sulfate (a)	<0.03		25

TABLE J4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) CFU = colony-forming unit
(f) MPN = most probable number
(g) Mean, standard deviation, and range exclude one value of 150 for batch produced on 8/26/82.
(h) Mean, standard deviation, and range include the high value given in footnote f.
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude one value of 100.3 ppb for batch produced on 4/27/81.
(k) Mean, standard deviation, and range include the high value given in footnote i.
(l) Mean, standard deviation, and range exclude one value of 99 for batch produced on 4/27/81.
(m) Mean, standard deviation, and range include the high value listed in footnote k.
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(p) Two observations were above the detection limit. The values and the dates they were obtained are listed under the range.
(q) Ten batches contained more than 0.05 ppm.

APPENDIX K

DATA AUDIT SUMMARY

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APPENDIX K. DATA AUDIT SUMMARY

The experimental data, records, and pathology materials for the 2-year toxicology and carcinogenesis studies of pentachloronitrobenzene in $B6C3F_1$ mice were audited for accuracy, consistency, completeness, and compliance with Good Laboratory Practice (GLP) regulations of the Food and Drug Administration (implemented by the NTP beginning October 1, 1981). The studies were conducted for the NTP, National Institute of Environmental Health Sciences (NIEHS), by EG&G Mason Research Institute, Worcester, Massachusetts, under a subcontract with Tracor Jitco, Inc. Animal dosing with pentachloronitrobenzene began in June 1981 and ended June 1983. The retrospective audit was conducted at the NTP Archives, Research Triangle Park, North Carolina, in October 1985 and September and October 1986 by Program Resources, Inc. The individuals involved in the audit are listed in the full audit report, which is on file at the NIEHS. The audit included a review of the following:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.

(2) All chemistry records.

(3) Body weight and clinical observation data for a random 10% sample of the study animals.

(4) Food consumption data for each group of animals.

(5) All inlife records concerning environmental conditions, masses, mortality, and animal identification.

(6) All postmortem records for individual animals concerning identification, disposition codes, condition codes, and correlation between gross observations and microscopic diagnoses.

(7) Wet tissues from a random 10% sample of the study animals to verify animal identification and to examine for untrimmed lesions.

(8) Slides and blocks of tissues from all control and high dose animals to examine for proper match and inventory.

(9) Tabulated pathology diagnoses for a random 10% of study animals to verify computer data entry.

The review of the chemistry records indicated that the formulated diets were properly prepared throughout the course of the studies. The inlife observations as reported in the Technical Report accurately reflect the recorded findings. Procedures early in the course of the studies (before GLP procedures went into effect) which were not completely documented included quarantine observations, randomization of animals into dose groups, and animal housing conditions. These procedures were described in the final laboratory report, and their absence from the study records had no adverse impact on the interpretation of the studies.

The review of the pathology data indicated good correlation between slides and blocks. There were some miscellaneous observations noted at necropsy for which there were no microscopic diagnoses; these do not affect the conclusions of the Technical Report. The wet tissues of 52 mice were examined for untrimmed potential lesions; four were found. These were not examined further. A review of the wet tissues for animal identification showed that 3 of 144 animals examined (1 control male and 2 high dose females) had identification markings that did not correspond to the numbers on the bags. Further review of the inlife data, including body weight records and identification markings for animals with corresponding numbers in other dose groups, indicated that animal mixup had not occurred.

The NIEHS/NTP conclude that the data reported were adequate to support the conclusions presented in this Technical Report.

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