NTP REPORT ON THE

TOXICITY STUDIES OF 1,2,4,5-TETRACHLOROBENZENE IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

January 1991

NTP TOX 7
NIH Publication No. 91-3126

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

FOREWORD

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

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

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals. Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure.

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report, is encouraged to make this information known to the NTP. Comments and questions should be directed to Dr. J.R Bucher, NIEHS, P O Box 12333, Research Triangle Park, NC 27709(919-541-4532).

These NTP Toxicity Study 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 Toxicity Study Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

TOXICITY STUDIES OF

1,2,4,5-TETRACHLOROBENZENE

(CAS NO. 95-94-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

Margarita M. McDonald, D.V.M., Ph.D.

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

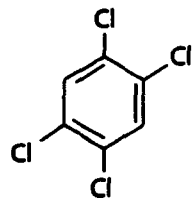
January 1991

NTP TOX 7
NIH Publication No. 91-3126

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

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund by interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

CONTENTS



1,2,4,5-TETRACHLOROBENZENE

CAS No. 95-94-3

$C_6H_2Cl_4$ Molecular weight 215.9

Synonyms: *s*-tetrachlorobenzene; benzene tetrachloride

ABSTRACT

Toxicology studies were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice of each sex to 1,2,4,5-tetrachlorobenzene (greater than 99% pure) at various concentrations in formulated diets for 14 days or 13 weeks.

Dietary concentrations were 0, 30, 100, 300, 1,000, or 3,000 ppm 1,2,4,5-tetrachlorobenzene in the 14-day studies. All rats survived to the end of the studies, but all mice in the 3,000-ppm groups died (five animals per group). Histologically, exposed male rats had an accumulation of abnormal hyaline droplets in the renal cortical epithelium. Significant histologic lesions were not seen in female rats or in mice of either sex.

Dietary concentrations were 0, 30, 100, 300, 1,000, or 2,000 ppm 1,2,4,5-tetrachlorobenzene in the 13-week studies (10 animals per group). All rats survived to the end of the studies; two female mice in the 2,000-ppm group were killed in a moribund condition. Body weight gains in the higher dose groups of rats and mice were less than those of controls. In exposed male rats, lesions included renal cortical tubular epithelial hyaline droplet formation, cortical tubular regeneration, and medullary granular casts and mineralization. This spectrum of renal lesions in male rats is consistent with the entity described as "hydrocarbon or hyaline droplet nephropathy." In some exposed female rats (30- to 2,000-ppm groups), there was renal cortical tubular cell regeneration plus accumulation of an unidentified yellow-brown pigment in the renal cortical epithelium.

Centrilobular hepatocellular hypertrophy was observed in the livers of exposed male and female rats. In mice, minimal-to-mild centrilobular hepatocellular hypertrophy was present in males in the 1,000- and 2,000-ppm groups and in females in the 2,000-ppm group. Minimal-to-mild individual hepatocyte degeneration occurred in mice of each sex in the 2,000-ppm groups. Increased serum sorbitol dehydrogenase and alanine aminotransferase activity was observed in the two highest dose groups of male and female mice and indicated hepatocellular injury. Thyroid follicular cell hypertrophy was present in male rats in the 300- to 2,000-ppm groups and in female rats in the 100- to 2,000-ppm groups. Decreased free thyroxin and total thyroxin concentrations in male rats in the 300- to 2,000-ppm groups and female rats in the 30- to 2,000-ppm groups indicated a primary hypothyroid state. Hematologic findings for rats that received 1,000 or 2,000 ppm included significantly decreased hematocrit values, hemoglobin concentration, and erythrocyte counts for males and decreased mean cell volume for females; for mice, decreased hemoglobin concentrations, mean corpuscular hemoglobin,

hematocrit, and mean cell volume were observed in males in the 2,000-ppm group and in females in the 1,000- and 2,000-ppm groups. These findings suggest a poorly regenerative anemia in both species.

The no-observed-effect level (NOEL) for histologic lesions was 30 ppm for male and female rats. The NOEL for histologic lesions in male and female mice was 300 ppm.

CONTRIBUTORS

The NTP Report on the Toxicity Studies of 1,2,4,5-Tetrachlorobenzene is based on the 14-day and 13-week studies that began in November 1986 at Microbiological Associates, Inc. (Bethesda, MD).

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

Margarita M. McDonald, D.V.M., Ph.D.

John Bucher, Ph.D.
Michael Elwell, D.V.M., Ph.D.
Richard Griesemer, D.V.M., Ph.D.
C.W. Jameson, Ph.D.
Joel Leininger, D.V.M., Ph.D.
Bernard Schwetz, D.V.M., Ph.D.
James Selkirk, Ph.D.
Raymond S.H. Yang, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report for Rats and Mice on 1/20/89)

Paul Hildebrandt, D.V.M. (Chair) (NTP)
Michael Elwell, D.V.M., Ph.D. (NTP)
Katharina Heider, D.V.M. (Ciba-Geigy)
A.W. Macklin, D.V.M., Ph.D.
Burroughs Wellcome Laboratories
Margarita M. McDonald, D.V.M., Ph.D. (NTP)
John Peckham, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.

Principal Contributors at Microbiological Associates Inc.
(Conducted Studies and Evaluated Tissues)

Louis T. Mulligan, Ph.D.
R. Kovatch, D.V.M.

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

John Peckham, D.V.M., Ph.D.

Principal Contributors at Environmental Health Research and Testing
(Contractor for Sperm and Vaginal Analysis)

Dushyant Gulati, Ph.D.
Theresa Cocanaugher, B.A.
Susan Z. Russell, B.A.

Principal Contributors at Analytical Sciences, Inc.
(Contractor for Statistical Analysis)

Steven Seilkop, M.S.
Janet Teague, M.S.

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

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

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of 1,2,4,5-tetrachlorobenzene on November 20, 1989, 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 four 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, and (d) to judge the significance of the experimental results by scientific criteria.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D. (Chair)
Senior Scientific Advisor, Medicine and
Environmental Health Department
Research and Environmental Health Division
Exxon Biomedical Sciences, East Millstone, NJ

Daniel S. Longnecker, M.D. (Principal Reviewer)
Professor, Department of Pathology
Dartmouth Medical School
Hanover, NH

Ellen K. Silbergeld, Ph.D.
Senior Scientist
Environmental Defense Fund
Washington, DC

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Imperial Chemical Industries, PLC
Central Toxicology Laboratory
Alderley Park, England

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

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

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

Lois Swirsky Gold, Ph.D.
University of California
Lawrence Berkeley Laboratory
Berkeley, CA

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

Curtis D. Klaassen, Ph.D. (Principal Reviewer) Professor, Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

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

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

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

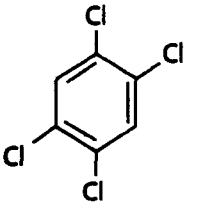
On November 20, 1989, the draft report on the toxicity studies of 1,2,4,5-tetrachlorobenzene received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.M. McDonald, NIEHS, introduced the short-term toxicity studies by reviewing the rationale, experimental design, and results.

Dr. Klaassen, a principal reviewer, stated that some of the more important data should be available in the text; in particular, he thought that the thyroxin data should be provided in a table (see Table 6, page 20).

Dr. Longnecker, a second principal reviewer, said that it might be helpful to include a concise, integrative summary of previous studies at the front of the report. He said that the ratio of corn oil to diet should be stated (see Table 2, page 14).

The Panel recommended completion of the report with consideration of the points discussed.



1,2,4,5-TETRACHLOROBENZENE

CAS No. 95-94-3

$C_6H_2Cl_4$ Molecular weight 215.9

Synonyms: *s*-tetrachlorobenzene; benzene tetrachloride

I. INTRODUCTION

Chemical and Physical Properties

1,2,4,5-Tetrachlorobenzene occurs as white flakes or as needles of monoclinic prisms from ether, ethanol, or benzene. It has a density of 1.853 at 22° C, a melting point of 139.5° C, a boiling point of 243°-246° C, a flash point of 155° C, and a vapor pressure of less than 1 mm mercury at 25° C. It is insoluble in water, slightly soluble in hot ethanol, and soluble in ether, benzene, chloroform, and carbon disulfide (USEPA, 1980; Condensed Chemical Dictionary, 1981).

Production, Use, and Environmental Occurrence

In 1980, the estimated U.S. production of 1,2,4,5-tetrachlorobenzene was 5.4×10^6 kg (USEPA, 1980). Commercial production of 1,2,4,5-tetrachlorobenzene in the United States ceased in 1983 (communication from Dr. Victor Fung to Dr. R. Yang, NTP, September 5, 1985). It has been used primarily as an intermediate in the manufacture of various industrial and commercial chemicals, including antifungal agents (e.g., 2,4,5-trichlorophenol), herbicides (e.g., 2,4,5-T), insecticides (e.g., Ronnel), and mordant dye intermediates (e.g., 2-amino-3,4,6-trichlorophenol). It has also been used as a dielectric fluid in electrical transformers and to impart moisture resistance to various substances (USEPA, 1980).

Environmental contamination by 1,2,4,5-tetrachlorobenzene can occur via leaching from chemical dump sites (TIRC/ORNL, 1979; USEPA, 1979) or from the use of pesticides containing 1,2,4,5-tetrachlorobenzene (Jan and Malnersic, 1980; Strik, 1986). 1,2,4,5-Tetrachlorobenzene has been identified as an industrial effluent (USEPA, 1976) and in settling particulates in lakes and estuaries (Oliver and Nicol, 1982; Oliver and Charlton, 1984; Onuska and Terry, 1985; Pereira et al., 1988). It has been detected in fly-ash samples from municipal waste incinerators (Olie et al., 1980; Viau et al., 1984) and in ambient air near industrial complexes (Bruckmann et al., 1988).

Ecotoxicology

The metabolism and toxicity of 1,2,4,5-tetrachlorobenzene have been investigated in plants and in invertebrate and vertebrate animals. In the field, 1,2,4,5-tetrachlorobenzene residues have been found in herring gull eggs (Hallett et al., 1982; Ellenton et al., 1985) and in freshwater fish and mussels (Jan and Malnersic, 1980; Oliver and Niimi, 1983; Jaffe and Hites, 1986). Bioaccumulation of 1,2,4,5-tetrachlorobenzene by oligochaete worms was demonstrated under laboratory conditions and in the field (Oliver, 1987). Uptake of 1,2,4,5-tetrachlorobenzene by fingerling (Melancon and Lech, 1985) and adult (Oliver and Niimi, 1983) rainbow trout was also demonstrated experimentally. In laboratory studies with rainbow trout, early fry were shown to be the most susceptible to 1,2,4,5-tetrachlorobenzene toxicity (Van Leeuwen et al., 1985).

Exposure to 1,2,4,5-tetrachlorobenzene inhibited growth of freshwater and marine algae (USEPA, 1978; Wong et al., 1984).

Soil treatment with 1,2,4,5-tetrachlorobenzene decreased germination percentages and seedling vigor of barley, oats, and wheat planted in several types of soil (Ameen et al., 1960); 224-4,483 kg 1,2,4,5-tetrachlorobenzene/ha in soil caused almost 100% lethality of cotton seedlings (Adams and Rodriguez-Kabana, 1976).

Human Exposure

In addition to the potential sources of human exposure described above, 1,2,4,5-tetrachlorobenzene has been found in human breast milk at low concentrations (mean, 200 µg/kg) (Jan, 1983). Human adipose tissue samples obtained during autopsies contained low concentrations (mean, 0.02-200 µg/kg) of 1,2,4,5-tetrachlorobenzene (Morita et al., 1975; Williams et al., 1984).

Pharmacokinetics and Metabolism

Studies in several species indicate that 1,2,4,5-tetrachlorobenzene is easily absorbed and slowly metabolized compared with the other two tetrachlorobenzene isomers (1,2,3,4- and 1,2,3,5-) and that it readily accumulates and persists in adipose tissue and in organs with a high fat content.

These differences can be attributed to the molecular configuration of the 1,2,4,5-isomer. Formation of arene oxide intermediates is regarded as the initial step in metabolism of the lower chlorinated benzenes (Kohli et al., 1976). Arene oxides form most easily between adjacent unsubstituted carbon atoms on the benzene ring. Because of steric and electronic hindrance by the bulky electronegative chlorine atoms, arene oxides form with difficulty between chlorinated and unchlorinated carbon atoms or between two chlorinated carbons (Matthews, 1986). Therefore, the molecular configuration of the 1,2,4,5-isomer is less conducive to metabolic transformation than that of the other two isomers.

Jondorf et al. (1958) examined the metabolism of the three isomers of tetrachlorobenzene in female Chinchilla rabbits that had been given a single 0.5 mg/kg oral dose of one of the isomers. During the 6-day observation period, 1,2,4,5-tetrachlorobenzene recovered as the parent compound was distributed as follows: 48% in tissues (25% in fat), 16% in feces, 6.2% in gut contents, and 2% in expired air. 2,3,5,6-Tetrachlorophenol was the predominant metabolite identified in the urine but accounted for only 2% of the administered dose. About 10% and 23% of the initial doses of the 1,2,3,4- and 1,2,3,5-tetrachlorobenzene isomers, respectively, were detected in the tissues. The authors concluded that 1,2,4,5-tetrachlorobenzene was the least metabolized of the three isomers. Similar results were reported by Kohli et al. (1976) after 300 mg/kg doses of individual tetrachlorobenzene isomers were administered to male rabbits by intraperitoneal injection.

When Chu et al. (1984a) gave single oral doses of 10 mg/kg of ^{14}C-labeled tetrachlorobenzene isomers to male Sprague Dawley rats, only 8% of the administered 1,2,4,5-isomer was excreted in the urine and feces by 48 hours, compared with 46% and 51% of the 1,2,3,5- and 1,2,3,4-isomers, respectively. In later studies in male Sprague Dawley rats, with the same dosage regimens, Chu et al. (1987) found the highest compound concentrations in fat, liver, skin, and adrenal gland, with 1,2,4,5-tetrachlorobenzene present at higher concentrations and having greater persistence than the other two isomers. Accumulation of 1,2,4,5-tetrachlorobenzene in the adipose tissue after continuous dietary administration to rats was also demonstrated by Jacobs et al. (1977).

Although residues were also detected in the brain, liver, kidney, heart, and spleen, the highest concentrations of 1,2,4,5-tetrachlorobenzene were present in the perirenal fat of pregnant Sprague Dawley rats given the compound at doses of 50, 100, or 200 mg/kg per day on days 6-15 of gestation (Kacew et al., 1984). Tissue concentrations of the 1,2,4,5-isomer were about 100 times greater than those of the other two isomers, which were tested according to the same protocol.

Fecal and urinary excretion of ^{14}C-labeled 1,2,4,5-tetrachlorobenzene was increased in male Sprague Dawley rats pretreated with phenobarbital or polychlorinated biphenyls (Chu et al., 1986).

Braun et al. (1978) administered 1,2,4,5-tetrachlorobenzene at a dose of 5 mg/kg per day in the diet to beagle dogs for 2 years and found that the compound had a high affinity for fat. At the end of 2 years, 1,2,4,5-tetrachlorobenzene reached 98% and 97% of the calculated steady-state concentrations in fat and plasma, respectively. Marked temporal alterations in the fat to plasma ratio were attributed to differences in the rate of compound elimination from the two compartments.

Schwartz et al. (1987) investigated the metabolism of the three tetrachlorobenzene isomers in male squirrel monkeys given a single oral dose of 50-100 mg/kg of one of the isomers (labeled with carbon-14) twice per week for 3 weeks. The predominant route of elimination was via the feces; urinary excretion was negligible for all three isomers. Only about half as much of the 1,2,4,5-isomer (18% of the administered dose) as of the 1,2,3,4-isomer (38%) and the 1,2,3,5-isomer (36%) was excreted in feces 48 hours after dosing. Several metabolites of the other two isomers were detected in the fecal extracts, but the 1,2,4,5-isomer was excreted exclusively as the unchanged compound. The authors concluded that in the squirrel monkey, tetrachlorobenzene isomers "are not as extensively metabolized as they are in other mammalian species" and that there is no metabolism of the 1,2,4,5-isomer.

1,2,4,5-Tetrachlorobenzene has been reported as a metabolite of other organochlorine compounds, such as hexachlorobenzene or lindane in rats, pheasants, or houseflies (Reed and Forgash, 1970; Saha and Burrage, 1976; Strik, 1986; Artigas et al., 1988).

Short-Term Toxicity

Fomenko (1965) reported that oral single-dose LD_{50} values for 1,2,4,5-tetrachlorobenzene were 1,500 mg/kg in albino rats and rabbits and 1,035-2,650 mg/kg in mice. Other studies yielded oral LD_{50} values of 1,500 and 1,035 mg/kg for unspecified strains of rats and mice, respectively (NIOSH, 1980).

Administration to male rats of 850 or 905 mg/kg 1,2,4,5-tetrachlorobenzene in the diet for 5 days resulted only in mild "degeneration of individual liver cells," whereas administration of 660 mg/kg of the 1,2,3,4-isomer for 10 days induced increased porphyrin and hemoglobin metabolism as well as hepatocellular degeneration (Rimington and Ziegler, 1963).

The short-term toxicity of the three tetrachlorobenzene isomers was investigated in Sprague Dawley rats (Chu et al., 1983, 1984b). The single-dose oral LD_{50} for 1,2,4,5-tetrachlorobenzene was 3,105 mg/kg in male Sprague Dawley rats; in females, the LD_{50} was greater than 2,700 mg/kg. Clinical signs included prostration, loose stools, hypothermia, and coma; deaths occurred 48-72 hours after dosing. The authors suggested that, based on relative LD_{50} values, the isomers could be ranked from most to least toxic as follows: 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene. The same workers also conducted 28-day toxicity studies in which Sprague Dawley rats of each sex were given 0, 0.5, 5, 50, or 500 ppm of one of each of the tetrachlorobenzene isomers in feed. However, in contrast to results from the single-administration studies, 1,2,4,5-tetrachlorobenzene was the most toxic isomer. This was attributed to the greater "tissue accumulation" of the 1,2,4,5-isomer (Chu et al., 1984b).

Chemical-related toxic effects included increases in liver weights, hepatic microsomal enzymes, and serum cholesterol. Histologically, rats had hepatocellular cytoplasmic vacuolation, thyroid follicular epithelial hypertrophy and decreased intrafollicular colloid density, and "eosinophilic inclusions in the proximal convoluted tubule[s] of the renal cortex" (Chu et al., 1983). Dose-dependent accumulation in the fat and liver was greater for 1,2,4,5-tetrachlorobenzene than for the other isomers.

When 75 mg/kg 1,2,4,5-tetrachlorobenzene was administered by gavage to rats for 2 months, changes in liver function and hematologic values were noted, but no compound-related histologic lesions were observed (Fomenko, 1965).

In 13-week feed studies comparing the three tetrachlorobenzene isomers, male and female Sprague Dawley rats were administered one of the three isomers at doses of 0, 0.5, 5, 50, or 500 ppm (Chu et al., 1984b,c). Significant increases

in liver and kidney weights, serum cholesterol, and hepatic microsomal enzyme activities and decreases in hemoglobin and hematocrit values were observed only in rats of each sex receiving the two highest concentrations of the 1,2,4,5-isomer. Histologic lesions similar to those seen in previous 28-day studies (Chu et al., 1983) were present in the kidney, liver, and thyroid gland of rats fed each of three isomers but were most severe in rats given the 1,2,4,5-isomer. As in the 28-day studies, histologic lesions were more severe in male rats (Chu et al., 1984b). The 13-week exposure also resulted in a dose-dependent accumulation of 1,2,4,5-tetrachlorobenzene but not of the other two isomers in the fat and liver.

Long-Term Toxicity and Carcinogenicity

Little information concerning the long-term toxicity or carcinogenicity of 1,2,4,5-tetrachlorobenzene is available. Braun et al. (1978) found a slight increase in serum alkaline phosphatase activity and the total bilirubin concentration in beagle dogs given 1,2,4,5-tetrachlorobenzene in feed for 2 years. In this study, the half-life of 1,2,4,5-tetrachlorobenzene in fat was 111 days, supporting previous reports of the compound's affinity for and persistence in adipose tissue (Jondorf et al., 1958; Chu et al., 1987).

Herren-Freund and Pereira (1986) administered various halogenated benzenes by intraperitoneal injection to Sprague Dawley rats of each sex 1 and 5 weeks after dosing the rats with diethylnitrosamine. 1,2,4,5-Tetrachlorobenzene, given at a dose of 0.25 mmol/kg, was the only halogenated benzene besides hexachlorobenzene that enhanced production of γ-glutamyltranspeptidase (GGT)-positive foci in the liver of rats. This effect was noted only in male rats.

Mechanistic and Interaction Studies

1,2,4,5-Tetrachlorobenzene administration was reported to induce production of hepatic microsomal enzymes in rats, such as cytochrome P450, cytochrome c reductase, δ-aminolevulinic acid synthetase, aminopyrine demethylase, aniline dehydrogenase, ethoxyresorufin deethylase, and aldrin epoxidase (Ariyoshi et al., 1975a,b; Chu et al., 1983, 1984b; Denomme et al., 1983; Kitchin and Ebron, 1983a,b; Kacew et al., 1984; Ikegami et al., 1987). Based on the observed pattern of hepatic microsomal enzyme induction, Chu et al. (1984c) stated that 1,2,4,5-tetrachlorobenzene is a P450-type enzyme inducer, thus resembling phenobarbital more than 3-methylcholanthrene in its activity. The 1,2,4,5-isomer is more potent as a hepatic enzyme inducer than are the other two isomers (Chu et al., 1984b).

In vitro glucuronidation of *p*-nitrophenol and phenolphthalein did not increase in samples of maternal liver from pregnant rats given 30, 100, 300, or 1,000 mg/kg 1,2,4,5-tetrachlorobenzene orally on gestation days 9-13 (Kitchin and Ebron, 1983a).

Oral administration of 250 mg/kg 1,2,4,5-tetrachlorobenzene once per day for 3 days to female Wistar rats resulted in increased liver microsomal protein and phospholipid concentrations, but liver glycogen and triglyceride were not significantly increased (Ariyoshi et al., 1975a,b).

Increased serum cholesterol and/or triglyceride concentrations were noted after administration of 1,2,4,5-tetrachlorobenzene to rats (Chu et al., 1983; Kacew et al., 1984; Ikegami et al., 1987).

Rimington and Ziegler (1963) noted that liver porphyrin values were not increased and urinary porphyrin values were only slightly increased after administration of 1,2,4,5-tetrachlorobenzene to male rats, but they found increased hepatic, fecal, and urinary porphyrins in rats given 1,2,3,4-tetrachlorobenzene. In contrast, Chu et al. (1984b) found no increased liver porphyrin concentrations in male or female Sprague Dawley rats fed diets containing 0.5-500 ppm of one of each of the three tetrachlorobenzene isomers.

Genetic Toxicology

1,2,4,5-Tetrachlorobenzene was tested in Salmonella strains TA98, TA100, TA1535, and TA1537 at concentrations ranging from 0.3 to 1,333 µg/plate (Haworth et al., 1983). The results were negative in either the presence or absence of S9 metabolic activation.

Paradi and Lovenyak (1981) reported that 1,2,4,5-tetrachlorobenzene did not induce an

increased frequency of sex-linked recessive lethal mutations in *Drosophila melanogaster* after exposure of the larvae by feeding at a concentration (not reported) below the LC_{50}. In vitro cytogenetic tests with Chinese hamster ovary cells demonstrated no induction of sister chromatid exchanges (SCEs) or chromosomal aberrations by 1,2,4,5-tetrachlorobenzene with or without S9 (Loveday et al., 1990).

Kiraly et al. (1979) investigated peripheral lymphocytes for chromosomal abnormalities in blood collected from Hungarian workers involved in the production of 1,2,4,5-tetrachlorobenzene. The researchers concluded that 1,2,4,5-tetrachlorobenzene was mutagenic (i.e., clastogenic) to occupationally exposed humans. However, marked methodologic deficiencies render this conclusion questionable.

SCE rates in herring gull embryos were studied in eggs collected from five colonies in the Great Lakes basin and from one relatively pollution-free colony on the Atlantic coast (Ellenton and McPherson, 1983). Relationships were not found between any of the contaminant (including the three tetrachlorobenzenes) levels and the SCE frequencies. There was also no relationship between the contaminant levels in the egg homogenates and mutagenic responses in several in vitro mutation assay systems (i.e., Salmonella assay, Chinese hamster ovary assay, and SCE assay) (Ellenton et al., 1983).

Reproductive Toxicity

The reproductive and teratologic effects of 1,2,4,5-tetrachlorobenzene and the other two tetrachlorobenzene isomers have been studied in rats. The 1,2,4,5-isomer crosses the placental barrier and accumulates in fetal tissues to a greater extent than do the other two isomers (Kacew et al., 1984).

In one study, each of the three isomers was given by gavage to pregnant Sprague Dawley rats in single doses of 50, 100, or 200 mg/kg per day during gestation days 6-15 (Kacew et al., 1984). The dams were killed and the fetuses removed by cesarean section on gestation day 21. The 1,2,4,5-isomer was considered the most toxic because it caused maternal deaths in the 200 mg/kg group. Also, the numbers of live fetuses were decreased in the group receiving 50 mg/kg of the 1,2,4,5-isomer but were decreased only in the groups receiving 200 mg/kg of the other isomers. No compound-related anomalies or histopathologic lesions were seen in fetuses exposed to any of the three tetrachlorobenzene isomers.

The 1,2,4,5-isomer was given orally as a single dose of 0, 30, 100, 300, or 1,000 mg/kg per day to pregnant Sprague Dawley rats on gestation days 9-13, and the dams were killed on day 14 (Kitchin and Ebron, 1983a). No compound-related effects on resorptions, deaths, crown-rump lengths, head lengths, somite numbers, or yolk sac diameters of embryos were noted. In contrast, administration of the 1,2,3,4-isomer according to a similar protocol resulted in growth retardation of embryos in the group given 300 mg/kg per day (because of extensive maternal deaths, embryo characteristics were not evaluated in the group given 1,000 mg/kg per day) (Kitchin and Ebron, 1983b).

Study Rationale

1,2,4,5-Tetrachlorobenzene was nominated for toxicologic evaluation to the National Toxicology Program by the National Cancer Institute; a high priority was based on the high potential for human exposure because of environmental contamination from chemical dump site leaching and pesticide residues and because of its bioaccumulation potential in human tissues and in aquatic organisms used for human food. Another cause for concern is the compound's structural relationship to other chlorinated benzenes that are known carcinogens. No studies on the long-term toxicity or carcinogenicity of 1,2,4,5-tetrachlorobenzene are available. Available data on the short-term toxicity of 1,2,4,5-tetrachlorobenzene in rodents have been derived almost exclusively from studies in Sprague Dawley rats; little or no information is available for mice or F344/N rats. The oral feed route of exposure was chosen because it best resembled the theorized major route of human exposure through contaminated food and/or water. Concurrent studies were performed on pentachlorobenzene (NTP, 1990a).

II. MATERIALS AND METHODS

Procurement and Characterization of 1,2,4,5-Tetrachlorobenzene

1,2,4,5-Tetrachlorobenzene (purity grade, 98%) was obtained in one lot from Aldrich Chemical Company (Milwaukee, WI). The study material was identified as 1,2,4,5-tetrachlorobenzene by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy.

The purity was determined to be greater than 99% by elemental analysis, Karl Fischer water analysis, thin-layer chromatography on silica gel plates, and gas chromatography. Analysis of the study material by full mass scan gas chromatography and mass spectrometry detected no benzene or hexachlorobenzene at a detection level of 500 ppm. In addition, analysis by high resolution gas chromatography/high resolution mass spectrometry with selected ion monitoring indicated that no chlorinated dibenzodioxins or dibenzofurans were detected in the study material (detection limits ranged from 200 to 3,000 ppb for the individual dibenzodioxins and dibenzofurans). The stability of the study material during the toxicology studies was monitored by gas chromatography. No deterioration of the 1,2,4,5-tetrachlorobenzene was noted over the course of the studies.

Preparation and Characterization of Formulated Diets

Formulated diets were prepared by mixing the appropriate amounts of 1,2,4,5-tetrachlorobenzene (w/w) with corn oil until dissolved (final corn oil concentration in feed was 1% for all groups), adding feed to form a premix, and then mixing the premix with feed in a twin-shell blender. The homogeneity and stability of 1,2,4,5-tetrachlorobenzene in feed (0.3 mg/g) were determined by gas chromatography. The chemical in feed was found to be uniformly distributed and stable for at least 3 weeks in the dark at 5° C; the chemical was stable for 1 day when stored under simulated animal room conditions (open to air and light in a rodent cage); a 3% decrease in concentration was observed after 4 days; an 8% decrease was observed after 7 days. During the studies, bulk formulated diets were stored for no longer than 3 weeks at 2°-4° C. Cage feeders were changed twice per week.

Periodic analysis of formulated diets of 1,2,4,5-tetrachlorobenzene was conducted at the study and analytical chemistry laboratories. The 1,2,4,5-tetrachlorobenzene content of the administered diets was determined by gas chromatography after extraction with isooctane. Seventeen formulated diet mixtures were analyzed during the 13-week studies; all samples were within specifications (±10% of the target concentration) (Table 1). The results of the analyses ranged from 91% to 105% of the target concentrations. Two referee analyses confirmed the results obtained by the study laboratory.

Fourteen-Day Study Design

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY) and were held for 11 (rats) or 12 (mice) days before the studies began. The rats were 6-7 weeks old when placed on study, and the mice were 6 weeks old. Groups of five rats and mice of each sex received diets containing 0, 30, 100, 300, 1,000, or 3,000 ppm 1,2,4,5-tetrachlorobenzene and 1% corn oil for 14 days. Further details are presented in Table 2.

TABLE 1. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

Target Concentration (ppm)	Determined Concentration (a) (ppm)
30	29.2 ± 1.8
100	96.3 ± 3.8
300	287 ± 8.8
1,000	953 ± 60
2,000	1,968 ± 108

(a) Mean ± standard deviation for three determinations; for each determination, all samples analyzed in duplicate.

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

Fourteen-Day Studies	Thirteen-Week Studies
Strain and Species F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Study Laboratory Microbiological Associates, Inc.	Microbiological Associates, Inc.
Size of Study Groups 5 males and 5 females of each species; rats housed 5/cage and mice individually housed	Rats--20 males and 20 females, housed 5/cage; mice--10 males and 10 females, individually housed
Doses 0, 30, 100, 300, 1,000, or 3,000 ppm 1,2,4,5-tetrachlorobenzene and 1% corn oil in feed	0, 30, 100, 300, 1,000, or 2,000 ppm 1,2,4,5-tetrachlorobenzene and 1% corn oil in feed
Method of Animal Distribution Assigned to groups such that within groups all weights were approximately equal	Same as 14-d studies
Diet NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum (5%-6% fat content)	Same as 14-d studies
Animal Room Environment Temp--68°-72° F; hum--34%-69%; fluorescent light 12 h/d; at least 12 room air changes/h	Temp--70°-74° F; hum--34%-70%; fluorescent light 12 h/d; at least 10 room air changes/h
Time Held Before Study Rats--11 d; mice--12 d	Same as 14-d studies
Age When Placed on Study Rats--6-7 wk; mice--6 wk	6 wk
Duration of Dosing 14 consecutive d	13 wk
Type and Frequency of Observation Observed 2 × d; weighed initially and 1 × wk thereafter; feed consumption measured 1 × d	Same as 14-d studies
Necropsy, Histologic Examinations, and Supplemental Studies Necropsy performed on all animals; the following tissues were examined for all controls and animals receiving 1,000 or 3,000 ppm: adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal passage and turbinates, pancreas, parathyroid glands, pituitary gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Liver examined for male rats at 300 ppm; organs weighed at necropsy	Necropsy performed on all rats not used for the clinical studies and on all mice; tissues examined for the control and high dose groups were the same as for the 14-d studies except the clitoral or preputial gland was also examined and the spinal cord and sciatic nerve were examined only if neurologic signs were present. Kidneys of all male rats and female rats receiving 300 ppm or more, thyroid glands of all rats, and livers of rats and mice receiving 300 ppm or more were examined microscopically. Serum chemistry analyses performed on 10 rats from each group on d 3, 17, and 45; serum chemical and hematologic analysis performed on these animals at the end of the studies; thyroid function tests, urinalysis, and sperm morphologic or vaginal cytologic examinations performed at the end of the studies on animals not used for hematologic studies; organs weighed at necropsy

Thirteen-Week Study Design

Groups of 20 rats of each sex and 10 mice of each sex were fed diets containing 0, 30, 100, 300, 1,000, or 2,000 ppm 1,2,4,5-tetrachlorobenzene and 1% corn oil for 13 weeks. Ten rats of each sex at each dietary concentration were designated for serum chemistry, hematologic, and thyroid function analysis and urinalysis evaluations. The remaining 10 animals of each sex for each dietary concentration were reserved for toxicologic evaluations, including histopathology, organ weight determinations, and sperm morphology or vaginal cytology studies.

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Taconic Farms. Breeding stock for the foundation colonies of rats and mice at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the study laboratory at 4 weeks of age. The rats were quarantined at the study laboratory for 11 days and mice for 12 days. All animals were placed on study at 6 weeks of age.

All animals were observed two times per day. Body weights were recorded once per week. Blood samples were drawn from the orbital sinus of groups of 10 male and 10 female rats on days 3 or 4, 17 or 18, and 45 or 46 for serum chemistry analyses, including sorbitol dehydrogenase, alanine aminotransferase, creatinine, creatine phosphokinase, γ-glutamyl transferase, and albumin (Baker Centrifichem 400). Serum triiodothronine, free thyroxin, total thyroxin, and thyrotropin concentrations were determined on days 17 or 18, 45 or 46, and 88 or 89 by radioimmunoassay. Sixteen-hour urine samples were collected for urinalysis on days 15 or 16, 43 or 44, and 86 or 87. Appearance, 16-hour volume, specific gravity, glucose and protein concentrations, alkaline phosphatase and aspartate aminotransferase activity (Baker Centrifichem 400), and porphyrin levels (day 86 or 87 only) were determined (Hill et al., 1982); sediment from centrifuged samples was examined microscopically. Blood was drawn from the orbital sinus of the previously bled rats and all surviving mice on day 88 or 89 for serum chemistry determinations and hematologic analysis. Hematologic analyses were performed by manual counts and with a Baker 700 Veterinary Hematology Analyzer and included erythrocyte, leukocyte, reticulocyte, platelet, and differential counts, hemoglobin concentration, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and blood morphology.

Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all rats not bled sequentially for the hematologic studies and on all mice. Evaluation of sperm morphology was performed at necropsy on male rats and male mice that received 0, 30, 300, or 2,000 ppm 1,2,4,5-tetrachlorobenzene (Morrissey et al., 1988). Females given 0, 30, 300, or 2,000 ppm had vaginal smears prepared during the 7 days before necropsy.

For the 12 days prior to terminal kill, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto frosted slides, stained with Toluidine Blue O, and coverslipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial sheets were used to identify the stages of the estrual cycle.

Sperm morphology and motility were determined for males. The right epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis and was weighed. Test yolk buffer (rats, 80 µl) or tyrodes buffer (mice, 80 µl) was applied to two prewarmed slides, and a small cut was made in the distal cauda epididymis. The sperm that effluxed from the epididymis were dispersed throughout the solution, coverslipped, and counted immediately on a warm microscope stage. In fields of 30 sperm or less, the number of moving and nonmoving sperm were counted in 5 fields on each slide.

After sperm sampling for motility estimation, the cauda was placed in phosphate-buffered saline (PBS), gently chopped with a razor blade, and allowed to sit for 15 minutes. The remaining

clumps of tissue were removed, and the solution was mixed gently and heat-fixed at 65° C. Sperm density was then determined using a hemocytometer.

The right testis was frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing dimethyl sulfoxide. Homogenization-resistent spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis and per gram of testis.

Organs and tissues were examined for gross lesions. The liver, right kidney, right testis, brain, heart, thymus, lungs, and seminal vesicles were weighed. Tissues were preserved in 10% neutral buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Tissues and groups examined are listed in Table 2.

Upon completion of the histologic evaluation by the laboratory pathologist, 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 pathology laboratory where quality assessment was performed, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman et al. (1985).

Statistical Analysis

Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends for organ weight, hematologic, serum chemical, and male reproductive system data. If this analysis indicated a significant trend, the nonparametric multiple comparison procedures of Shirley (1977) was used to assess the significance of pairwise comparisons between dosed and control groups. Otherwise, Dunn's test (Dunn, 1964) was used for pairwise comparisons.

Quality Assurance

The studies of 1,2,4,5-tetrachlorobenzene were performed in compliance with Good Laboratory Practice regulations (21 CFR 58). The Quality Assurance Unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the conduct of the studies. The operations of the Quality Assurance Unit were monitored by the NTP, including a site visit during the period of study performance.

III. RESULTS

STUDIES IN RATS

Fourteen-Day Studies

All rats lived to the end of the studies (Table 3). The final mean body weights of rats that received 3,000 ppm were 18% lower than that of controls for males and 15% lower for females. Feed consumption by animals that received 3,000 ppm was about 20% lower than that by controls. Compound-related clinical signs included tremors, lethargy, thin appearance, rough hair coats, ataxia, and chromodacryorrhea in males and females that received 3,000 ppm and rapid breathing in all females that received 3,000 ppm. Absolute and relative liver and kidney weights for males and absolute and relative liver weights for females were increased for animals that received 300 ppm or more. Liver congestion was observed in males that received 1,000 ppm and in males and females that received 3,000 ppm.

TABLE 3. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Dose (e)
MALE							
0	5/5	103 ± 3	178 ± 10	+75 ± 7		15	
30	5/5	100 ± 3	176 ± 5	+76 ± 2	99	14	3.0
100	5/5	105 ± 5	180 ± 9	+76 ± 4	101	15	10.5
300	5/5	107 ± 4	187 ± 6	+80 ± 2	105	15	30.6
1,000	5/5	100 ± 2	174 ± 3	+75 ± 2	98	15	109
3,000	5/5	105 ± 4	146 ± 4	+41 ± 3	82	12	287
FEMALE							
0	5/5	89 ± 3	131 ± 2	+42 ± 1		11	
30	5/5	86 ± 2	119 ± 3	+33 ± 2	91	11	3.2
100	5/5	94 ± 2	135 ± 1	+41 ± 2	103	12	10.5
300	5/5	90 ± 3	133 ± 4	+44 ± 2	102	11	29.6
1,000	5/5	89 ± 2	127 ± 2	+38 ± 2	97	11	102
3,000	5/5	88 ± 5	111 ± 4	+23 ± 1	85	9	271

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day, averaged over the 2-week period; not corrected for scatter.
(e) Estimated milligrams per kilogram per day, based on mean of initial and final body weights

Renal sections from control and exposed male rats were stained with hematoxylin and eosin and Mallory azan stains (Barlow, 1984) and were examined histologically. Large irregular-shaped eosinophilic crystalline structures (abnormal hyaline droplets) were present in the cortical tubular epithelial cytoplasm of exposed animals.

Thirteen-Week Studies

All animals lived to the end of the studies (Table 4). Mean body weights of rats that received 1,000 or 2,000 ppm were lower than those of controls throughout most of the studies (Figure 1). Final mean body weights of rats fed diets containing 1,000 or 2,000 ppm were 10% or 21% lower than that of controls for males and 8% or 16% lower for females. Feed consumption by dosed groups was similar to that by controls. Compound-related clinical signs included hypoactivity and lethargy. Absolute kidney weights were increased at concentrations as low as 300 ppm for males and females, and the relative kidney weights were increased at concentrations as low as 100 ppm for males and 300 ppm for females (Table 5). Absolute liver weights were increased at concentrations as low as 300 ppm for males and 100 ppm for females, and the relative liver weights were increased at concentrations as low as 300 ppm for males and 30 ppm for females.

Hematocrit values, hemoglobin concentration, and erythrocyte count were significantly lower than those of controls for males that received 1,000 or 2,000 ppm (Table A2). The mean cell volume was significantly lower than that of controls for females that received 1,000 or 2,000 ppm. The platelet count was significantly increased for males that received 1,000 or 2,000 ppm. The serum albumin concentration was significantly increased for males exposed at 100 ppm or more and for females exposed at 2,000 ppm (Table A3).

Free thyroxin and total thyroxin concentrations were significantly decreased in males that received dietary concentrations as low as 300 ppm 1,2,4,5-tetrachlorobenzene and in females that received dietary concentrations as low as 30 ppm

TABLE 4. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Dose (e)
MALE							
0	10/10	116 ± 5	334 ± 10	+218 ± 7		16	
30	10/10	122 ± 5	331 ± 4	+210 ± 7	99	16	2.1
100	10/10	117 ± 5	336 ± 4	+219 ± 4	101	16	7.1
300	10/10	119 ± 4	316 ± 6	+197 ± 7	95	16	22.1
1,000	10/10	121 ± 4	299 ± 6	+178 ± 3	90	15	71.4
2,000	10/10	119 ± 4	265 ± 4	+146 ± 3	79	15	156
FEMALE							
0	10/10	101 ± 3	200 ± 3	+100 ± 4		11	
30	10/10	99 ± 3	193 ± 4	+93 ± 2	97	10	2.1
100	10/10	100 ± 3	203 ± 3	+103 ± 2	102	11	7.3
300	10/10	98 ± 3	197 ± 3	+99 ± 2	99	11	22.4
1,000	10/10	95 ± 3	183 ± 3	+88 ± 3	92	11	79.1
2,000	10/10	97 ± 3	168 ± 3	+71 ± 2	84	10	151

(a) Number surviving/number initially in group for animals not bled sequentially during the studies
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day, averaged over the13-week period; not corrected for scatter.
(e) Estimated milligrams per kilogram per day, based on mean of initial and final body weights

TABLE 5. LIVER AND KIDNEY WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Organ	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Body weight (grams)	347 ± 8.3	340 ± 4.2	*296 ± 16.2	333 ± 5.8	**299 ± 6.5	**283 ± 4.9
Right kidney						
Absolute	1,312 ± 39	1,268 ± 18	1,263 ± 27	**1,608 ± 39	**1,970 ± 68	**1,849 ± 74
Relative	3.8 ± 0.07	3.7 ± 0.04	**4.3 ± 0.15	**4.8 ± 0.07	**6.6 ± 0.15	**6.5 ± 0.16
Liver						
Absolute	12,660 ± 380	12,670 ± 180	10,590 ± 1,060	*14,270 ± 370	**17,230 ± 530	**19,170 ± 520
Relative	36.4 ± 0.52	37.2 ± 0.27	35.0 ± 1.75	**42.9 ± 0.68	**57.6 ± 1.20	**67.6 ± 1.17
FEMALE						
Body weight (grams)	201 ± 2.9	191 ± 4.4	207 ± 2.9	199 ± 3.3	*188 ± 3.0	**173 ± 2.7
Right kidney						
Absolute	776 ± 18	734 ± 20	821 ± 16	*844 ± 15	*838 ± 24	*871 ± 25
Relative	3.9 ± 0.07	3.8 ± 0.06	4.0 ± 0.08	**4.2 ± 0.06	**4.5 ± 0.08	**5.0 ± 0.12
Liver						
Absolute	6,445 ± 137	6,610 ± 239	**7,304 ± 151	**7,515 ± 164	**9,512 ± 215	**11,908 ± 312
Relative	32.1 ± 0.43	*34.5 ± 0.84	**35.3 ± 0.63	**37.7 ± 0.50	**50.6 ± 0.83	**68.9 ± 1.55

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*$P<0.05$
**$P<0.01$

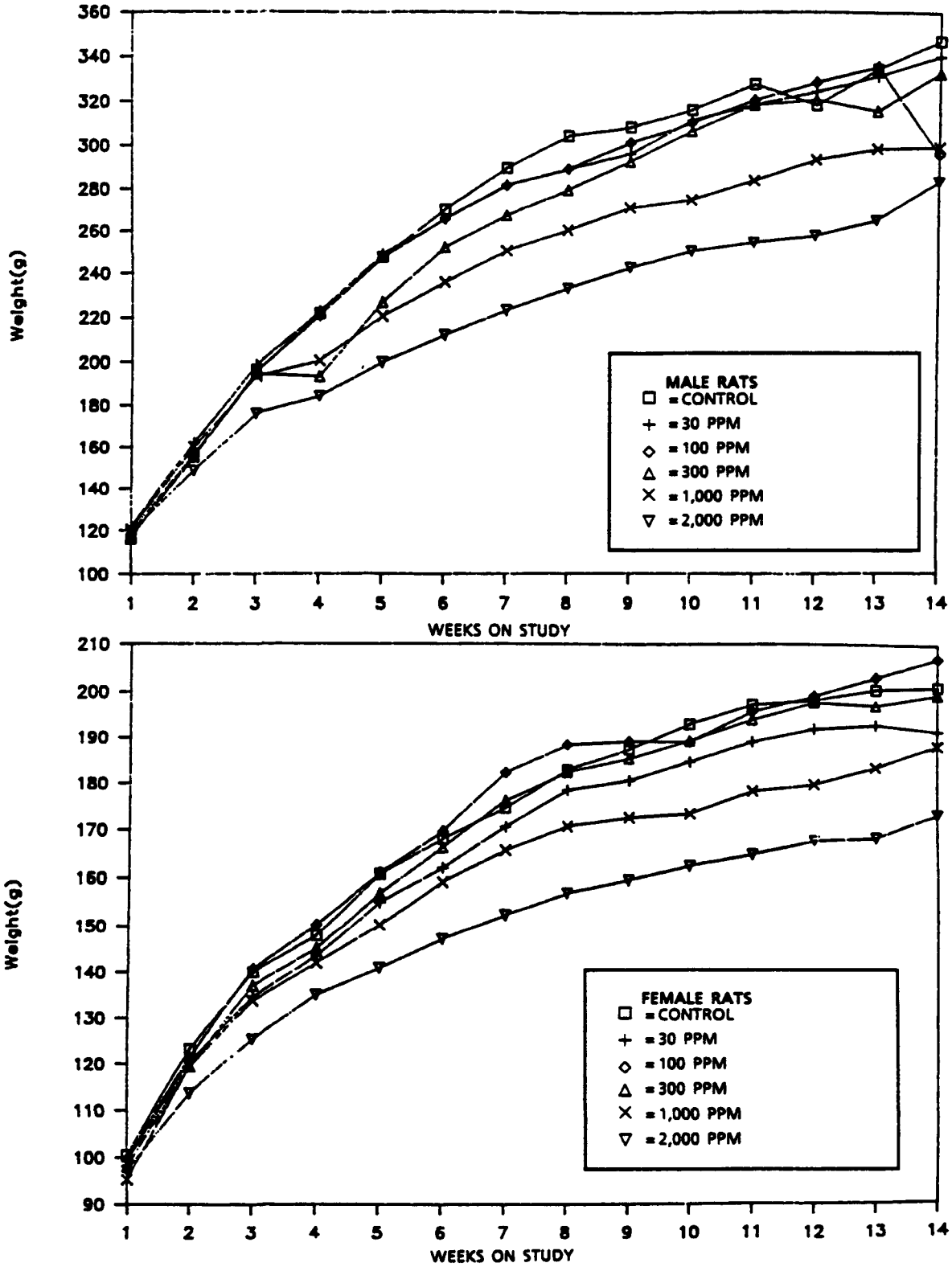


FIGURE 1. GROWTH CURVES FOR RATS ADMINISTERED 1,2,4,5-TETRACHLOROBENZENE IN FEED FOR THIRTEEN WEEKS

(Table 6). Triiodothyronine concentrations were not affected. Thyrotropin concentrations were not adequately measured.

Urinary porphyrin values at the end of the studies were significantly increased for males that received 1,000 or 2,000 ppm and for females that received 2,000 ppm; the increases were 2.5-fold to fourfold. Fluorescence characteristic of porphyrins was not detected when the livers of high dose and control animals was examined under long-wave ultraviolet light.

Urinary glucose concentration for males and females that received 300 ppm or more and urine specific gravity for females that received 1,000 ppm or more were significantly increased (Table A3). Urinary alkaline phosphatase activity was significantly increased for females that received 1,000 or 2,000 ppm and was not clearly dose related; there was no increase for males. Urinary aspartate aminotransferase activity was significantly increased for males at all doses (threefold to elevenfold increases) but not for females. Urinary protein concentration was significantly increased for males and females that received 1,000 and 2,000 ppm.

Right whole and cauda epididymal weights were significantly decreased for rats that received 300 or 2,000 ppm (males that received 1,000 ppm were not examined) (Table A4). Sperm motility was significantly decreased for rats that received 300 or 2,000 ppm (males that received 1,000 ppm were not examined). The length of the estrous cycle was unaffected by compound administration.

Compound-related lesions occurred in the kidney of rats of each sex but were more prominent in males (Table 7). Cortical renal tubular cytoplasmic alteration (abnormal hyaline droplet accumulation) occurred in male rats in the 100- to 2,000-ppm groups and was characterized histologically as intracytoplasmic aggregates of large, eosinophilic, angular inclusions which were increased in number and size compared with those

TABLE 6. SERUM CONCENTRATIONS OF THYROID HORMONES IN RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Analysis/ Day of Study	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Triiodothyronine (ng/dl)						
17/18	96 4 ± 4 04	91 2 ± 6 05	95 9 ± 4 60	84 9 ± 7 81	86 5 ± 5 77	81 5 ± 5 88
45/46	95 6 ± 6 28	81 0 ± 4 62	88 0 ± 4 74	82 7 ± 2 44	99 3 ± 8 52	73 4 ± 5 56
88/89	67 4 ± 5 09	70 0 ± 8 62	67 8 ± 10 61	73 1 ± 8 02	85 0 ± 7 03	80 3 ± 5 78
Free thyroxin (ng/dl)						
3/4	2 1 ± 0 11	2 0 ± 0 16	1 9 ± 0 09	**(b) 1 3 ± 0 15	**0 4 ± 0 04	**0 3 ± 0 02
45/46	2 0 ± 0 19	1 8 ± 0 15	2 2 ± 0 20	**1 3 ± 0 13	**0 6 ± 0 07	**0 2 ± 0 02
88/89	1 7 ± 0 13	1 5 ± 0 11	1 5 ± 0 14	**1 0 ± 0 12	**0 5 ± 0 04	**0 2 ± 0 03
Total thyroxin (micrograms/dl)						
17/18	5 1 ± 0 42	5 1 ± 0 49	4 9 ± 0 38	**3 0 ± 0 16	**1 6 ± 0 13	**1 2 ± 0 11
45/46	5 1 ± 0 29	4 6 ± 0 33	5 1 ± 0 25	**3 4 ± 0 19	**2 0 ± 0 15	**1 3 ± 0 08
88/89	4 2 ± 0 16	4 1 ± 0 39	4 1 ± 0 37	3 3 ± 0 32	**1 9 ± 0 14	**1 3 ± 0 05
FEMALE						
Triiodothyronine (ng/dl)						
17/18	95 2 ± 4 06	104 5 ± 6 93	93 6 ± 5 74	91 5 ± 6 08	82 7 ± 7 35	82 1 ± 7 00
45/46	(b) 98 7 ± 4 87	*75 8 ± 3 51	87 5 ± 4 54	92 9 ± 5 61	93 3 ± 7 72	91 5 ± 7 26
88/89	76 1 ± 6 56	68 7 ± 4 39	68 7 ± 5 39	66 7 ± 6 29	78 4 ± 6 79	82 7 ± 9 20
Free thyroxin (ng/dl)						
3/4	2 0 ± 0 10	*1 7 ± 0 09	*1 6 ± 0 09	**1 1 ± 0 08	**0 4 ± 0 03	**0 2 ± 0 02
45/46	1 6 ± 0 14	**0.9 ± 0 10	*1 3 ± 0 18	**1 0 ± 0 07	**0 4 ± 0 04	**(b) 0 2 ± 0 03
88/89	1 0 ± 0 08	1 0 ± 0 11	0 8 ± 0 09	**0 6 ± 0 10	**0 3 ± 0 03	**0 2 ± 0 03
Total thyroxin (micrograms/dl)						
17/18	4 7 ± 0 32	3 8 ± 0 25	3 8 ± 0 31	**2 5 ± 0 22	**1 4 ± 0 08	**1 2 ± 0 08
45/46	4 6 ± 0 24	**2 8 ± 0 21	**3 8 ± 0 32	**3 2 ± 0 17	**1 6 ± 0 06	**1 2 ± 0 06
88/89	2 8 ± 0 21	2 6 ± 0 24	2 6 ± 0 11	**1 8 ± 0 19	**1 4 ± 0 06	**1 2 ± 0 08

(a) Data for animals bled sequentially on d 3 or 4, 17 or 18, 45 or 46, and 88 or 89, mean ± standard error for groups of 10 animals unless otherwise specified, P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977) IU = international units
(b) Nine animals were examined.
*P<0 05
**P<0 01

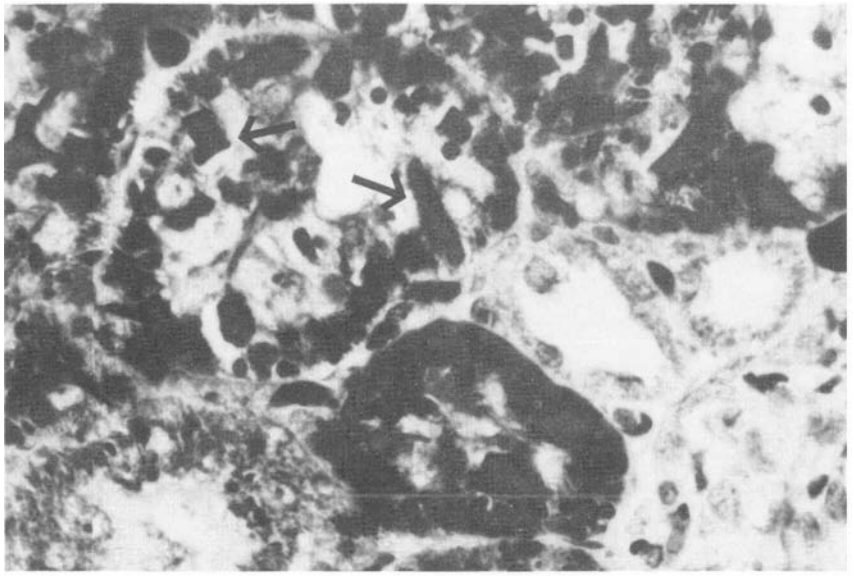


Figure 2. Kidney from a male rat exposed to 2,000 ppm tetrachlorobenzene for 14 days. Note abnormally large, angular hyaline droplets (arrows) in renal cortical epithelial cells. Compare with Figure 3 at same magnification. Maliory-azan stain.

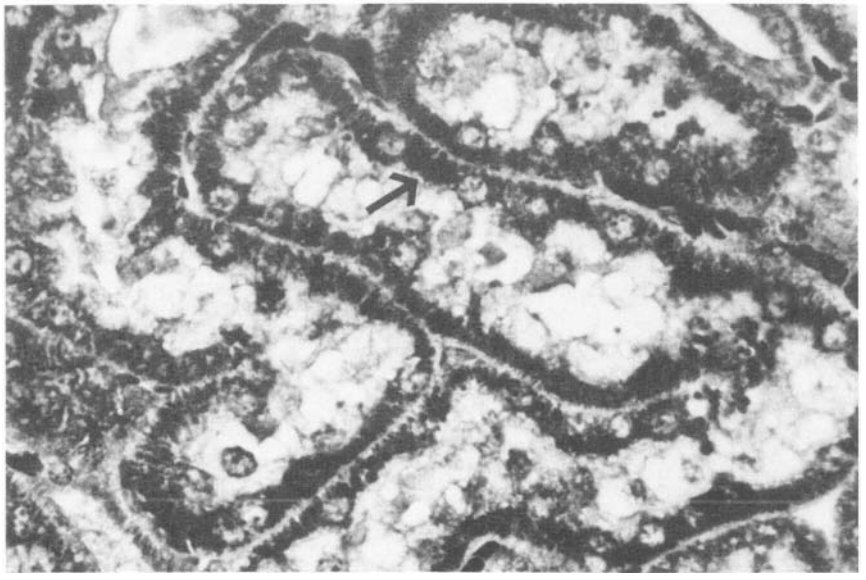


Figure 3. Kidney from a control male rat showing round, uniform hyaline droplets in renal cortical epithelial cells. Compare with Figure 2 at same magnification. Droplets are single or are clustered in apical portion of the cell (arrow). Mallory-azan stain.

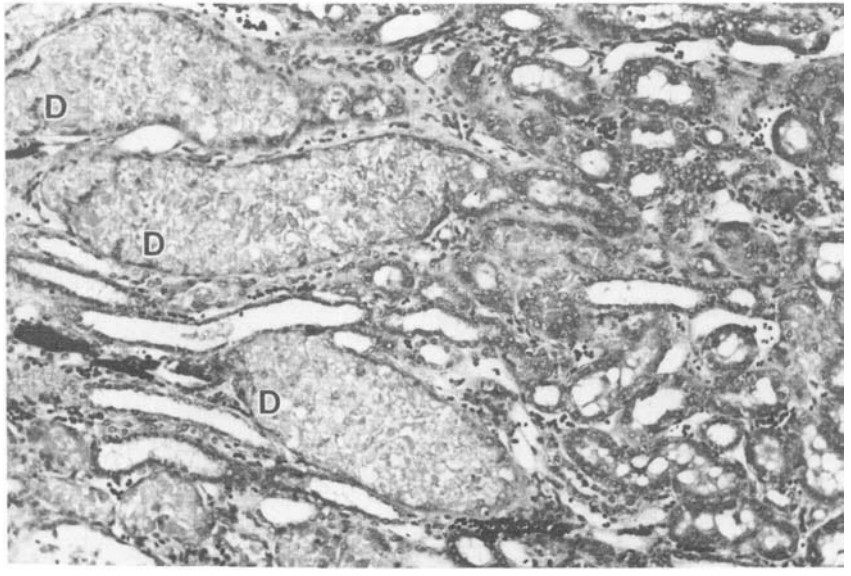


Figure 4. Kidney from a male rat exposed to 2,000 ppm tetrachlorobenzene for 13 weeks. Dilated tubules in the outBr stripe of the outer medulla contain granular cellular debris (D).

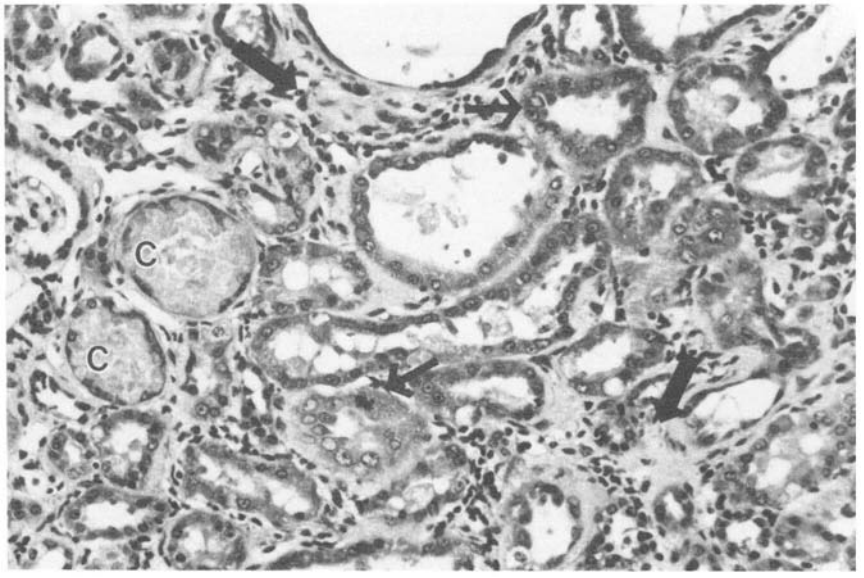


Figure 5. Kidney from a male rat exposed to 2,000 ppm tetrachlorobenzene for 13 weeks. Note granular casts (C), regenerative tubular epithelium (small arrow), and interstitial fibrosis with inflammatory cells (large arrow).

TABLE 7. NUMBERS OF RATS WITH SELECTED LESIONS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Site/Lesion	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Kidney						
Hyaline droplets (abnormal)	0	0	10 (1.0)	10 (2.1)	10 (2.1)	10 (3.2)
Cortical tubular regeneration	10 (1.0)	9 (1.0)	10 (1.7)	10 (2.4)	10 (2.4)	8 (2.1)
Medullary tubular dilatation	0	0	10 (1.0)	10 (2.0)	10 (2.8)	10 (2.5)
Medullary tubular mineralization	0	0	2 (1.0)	10 (1.1)	5 (1.4)	10 (1.0)
Tubular protein casts	3 (1.0)	1 (1.0)	3 (1.0)	0	8 (1.0)	10 (1.4)
Liver						
Centrilobular hypertrophy	0	0	--	0	8 (1.0)	10 (1.1)
Thyroid gland						
Follicular cell hypertrophy	0	0	0	4 (1.0)	10 (1.0)	10 (1.0)
FEMALE						
Kidney						
Cortical tubular regeneration	1 (1.0)	0	2 (1.0)	2 (1.0)	4 (1.0)	6 (1.2)
Tubular protein casts	0	0	0	0	1 (1.0)	7 (1.1)
Cortical tubular pigmentation	0	0	0	0	0	9 (1.5)
Liver						
Centrilobular hypertrophy	0	(b) 0	--	0	5 (1.0)	10 (1.6)
Thyroid gland						
Follicular cell hypertrophy	0	0	2 (1.0)	6 (1.0)	9 (1.0)	10 (1.0)

(a) 10 animals were examined unless otherwise specified. Number in parentheses denotes mean grade of severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
(b) One animal was examined.

in controls (Figure 2). They were morphologically similar to those seen in the kidney of exposed male rats in the 14-day study. Control and 30-ppm males had less abundant small, round hyaline droplets in renal cortical epithelial cells that were morphologically distinct from those in dosed rats (Figure 3).

Dilatation of tubules of the outer stripe of the outer medulla (medullary tubular dilatation) occurred at compound-related increased incidences in male rats given 100-2,000 ppm 1,2,4,5-tetrachlorobenzene. Histologically, the dilated tubules were lined by low cuboidal or flattened epithelium and were often filled by casts composed of eosinophilic, coarsely granular material (Figure 4).

Renal cortical tubular regeneration occurred with increased severity in exposed male rat groups and in female rats administered 100 ppm or more 1,2,4,5-tetrachlorobenzene (Table 7). Histologically, the lesion consisted of multiple foci of cortical tubules lined by cuboidal basophilic epithelial cells supported by basement membranes that were frequently thickened and hyalinized (Figure 5). Regeneration was much more prominent in males than in females.

Mineralization of medullary collecting tubules was seen in male rats in the 100- to 2,000-ppm groups. Microscopically, this lesion consisted of plugs or linear accumulation of basophilic mineralized material in the lumina of collecting tubules of the medulla and renal papilla. Considered collectively, the above lesions in male rats are typical of the pathologic entity known as hyaline droplet or light hydrocarbon nephropathy (Thomas et al., 1985).

Renal tubule protein casts were a distinct lesion observed in all control and most exposed male rat groups and in 1,000- and 2,000-ppm females (see Table 7). These were characterized microscopically as smooth, homogeneous, eosinophilic intraluminal masses. The degree of severity was

minimal to mild in affected animals. Protein casts were considered to be typical of those seen in the spontaneous nephropathy of many strains of laboratory rats (Peter et al., 1986).

Minimal-to-mild pigmentation of renal cortical tubular epithelium was present in female rats in the 2,000-ppm group. Histologically, pigmentation consisted of small, yellow-brown intracytoplasmic granules. The nature of the pigment was not determined.

Centrilobular hepatocellular hypertrophy, characterized by enlarged hepatocytes with increased cytoplasmic eosinophilia, occurred at increased incidences in male and female rats in the 1,000- and 2,000-ppm groups. In all groups, the lesion was of minimal severity.

When examined under long-wave ultraviolet light at the terminal kill, the livers of male or female control or high dose rats did not exhibit fluorescence characteristic of porphyrins.

Thyroid follicular cell hypertrophy was present in male rats in the 300- to 2,000-ppm groups and in female rats in the 100- to 2,000 ppm groups (see Table 7). This lesion was characterized histologically by slight enlargement and increased height of thyroid follicular cells compared with those in controls, often accompanied by cytoplasmic vacuolation and decreased eosinophilia of intraluminal colloid (Figures 6 and 7). In some follicles, small papillary projections composed of follicular epithelium extended into the lumen. In general, these lesions were of minimal severity. Based on histologic findings, the no-effect level (NOEL) for males and females was 30 ppm (see Table 7).

STUDIES IN MICE

Fourteen-Day Studies

All mice that received 3,000 ppm died before the end of the studies (Table 8). The final mean body weights of dosed and control mice were similar.

TABLE 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Dose (e)
MALE							
0	5/5	22.0 ± 0.8	24.0 ± 0.8	+2.0 ± 0.2		4.6	
30	5/5	20.3 ± 0.8	24.0 ± 0.7	+3.7 ± 0.2	100.0	4.6	6.2
100	5/5	22.1 ± 0.6	24.3 ± 0.5	+2.2 ± 0.2	101.3	4.8	20.7
300	5/5	21.3 ± 0.3	23.6 ± 0.3	+2.3 ± 0.4	98.3	4.2	56.1
1,000	5/5	21.3 ± 0.5	23.8 ± 0.5	+2.5 ± 0.1	99.2	4.8	213
3,000	(f) 0/5	21.8 ± 1.0	(g)	(g)	(g)	2.4	(g)
FEMALE							
0	5/5	17.6 ± 0.3	20.3 ± 0.6	+2.7 ± 0.3		4.2	
30	5/5	18.4 ± 0.5	20.6 ± 0.5	+2.2 ± 0.5	101.5	5.8	8.9
100	5/5	17.7 ± 0.5	20.1 ± 0.5	+2.4 ± 0.5	99.0	4.8	25.4
300	5/5	16.9 ± 0.4	20.4 ± 0.3	+3.4 ± 0.3	100.5	4.4	70.8
1,000	5/5	17.6 ± 0.2	20.5 ± 0.4	+2.8 ± 0.3	101.0	5.2	273
3,000	(h) 0/5	17.2 ± 0.5	(g)	(g)	(g)	2.0	(g)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day averaged over the 2-week period; not corrected for scatter
(e) Estimated milligrams per kilogram per day, based on mean of initial and final body weights
(f) All deaths occurred by day 9.
(g) No data are reported due to 100% mortality in this group.
(h) All deaths occurred by day 6.

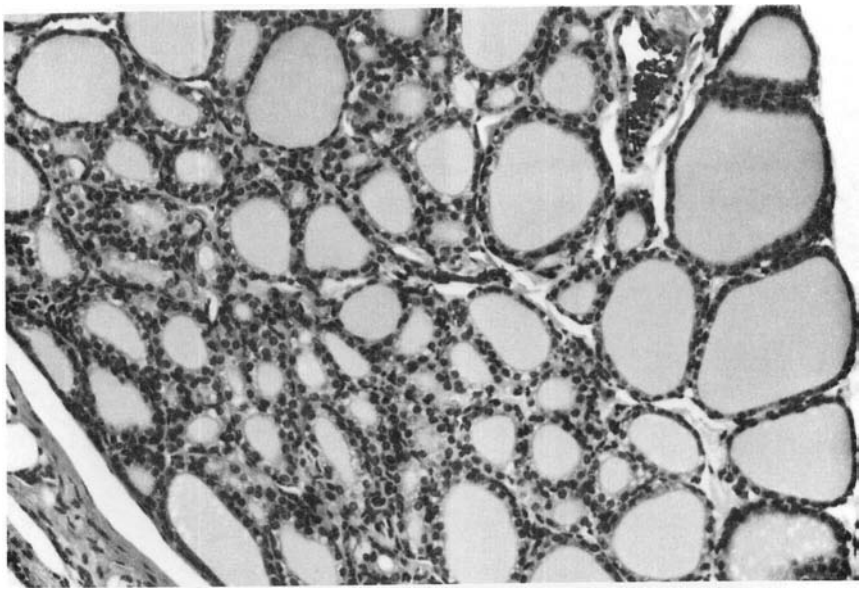


Figure 6. Thyroid gland from a control female rat in the 13-week studies. Follicles are lined by low cuboidal epithelial cells.

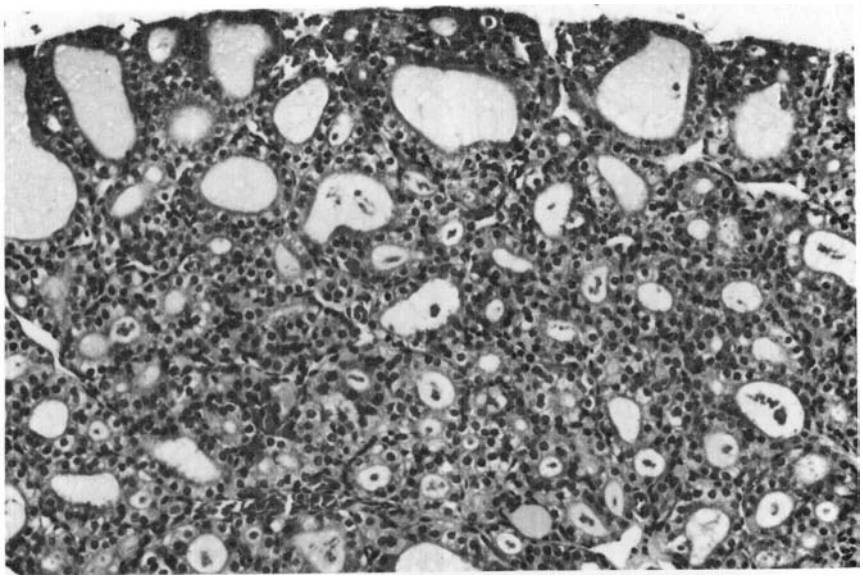


Figure 7. Thyroid gland from a male rat exposed to 2,000 ppm tetrachlorobenzene for 13 weeks. Follicular epithelial cells are increased in height compared with controls photographed at same magnification (see Figure 6).

Compound-related clinical signs included tremors, rapid breathing, lethargy, hunched posture, rough hair coats, dyspnea, and prostration in males and females that received 3,000 ppm and a thin appearance in females that received 3,000 ppm. Absolute and relative liver weights were significantly increased for males that received 1,000 ppm and for females that received 300 or 1,000 ppm. Depletion and necrosis of lymphoid tissue of the spleen, thymus, and lymph nodes were observed in males and females that received 3,000 ppm. These changes are frequently seen in moribund or early-death animals.

Thirteen-Week Studies

Two of 10 female mice that received 2,000 ppm were killed in a moribund condition before the end of the studies (Table 9). Mice that received 2,000 ppm lost weight during week 1. Mean body weights of male mice that received 100 ppm or more and of females that received 2,000 ppm were notably lower than those of controls throughout most of the studies (Figure 8). The final mean body weights of exposed mice were significantly different from those of controls. Feed consumption by males that received 2,000 ppm and females that received 1,000 or 2,000 ppm was lower than that by controls. The absolute liver weights of mice that received 2,000 ppm were about three times those of controls (Table 10). The absolute liver weight was significantly increased at 1,2,4,5-tetrachlorobenzene dietary concentrations as low as 100 ppm for males and 30 ppm for females. The liver weight to body weight ratios were significantly increased at dietary concentrations as low as 100 ppm for males and 1,000 ppm for females. Compound-related clinical signs included tremors in females and prostration, lethargy, hunched posture, and rough hair coats in males and females receiving 2,000 ppm.

TABLE 9. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Dose (e)
MALE							
0	10/10	21.4 ± 0.5	32.1 ± 1.0	+10.7 ± 0.6		3.9	
30	10/10	20.8 ± 0.7	29.7 ± 0.7	+8.8 ± 0.2	92.5	3.8	4.5
100	10/10	21.5 ± 0.4	31.9 ± 0.8	+10.3 ± 0.6	99.4	3.9	14.6
300	10/10	20.7 ± 0.3	29.7 ± 0.7	+9.0 ± 0.4	92.5	3.8	45.2
1,000	10/10	20.5 ± 0.4	30.0 ± 0.7	+9.5 ± 0.4	93.5	3.8	150
2,000	10/10	20.8 ± 0.6	29.6 ± 0.8	+8.8 ± 0.6	92.2	3.5	278
FEMALE							
0	10/10	16.8 ± 0.3	25.4 ± 0.5	+8.6 ± 0.3		4.1	
30	10/10	16.7 ± 0.4	25.1 ± 0.5	+8.4 ± 0.3	98.8	4.2	6.0
100	10/10	17.2 ± 0.4	25.5 ± 0.7	+8.3 ± 0.4	100.4	4.2	19.7
300	10/10	16.9 ± 0.5	25.5 ± 0.7	+8.6 ± 0.5	100.4	4.0	56.6
1,000	10/10	16.8 ± 0.3	25.3 ± 0.6	+8.5 ± 0.4	99.6	3.0	143
2,000	(f) 9/10	16.5 ± 0.3	24.6 ± 0.5	+8.3 ± 0.5	96.9	3.1	302

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams per animal per day averaged over the 13-week period; not corrected for scatter.
(e) Estimated milligrams per kilogram per day, based on mean of initial and final body weights
(f) Week of death: 2; an additional animal died during week 13, after the end of dosing.

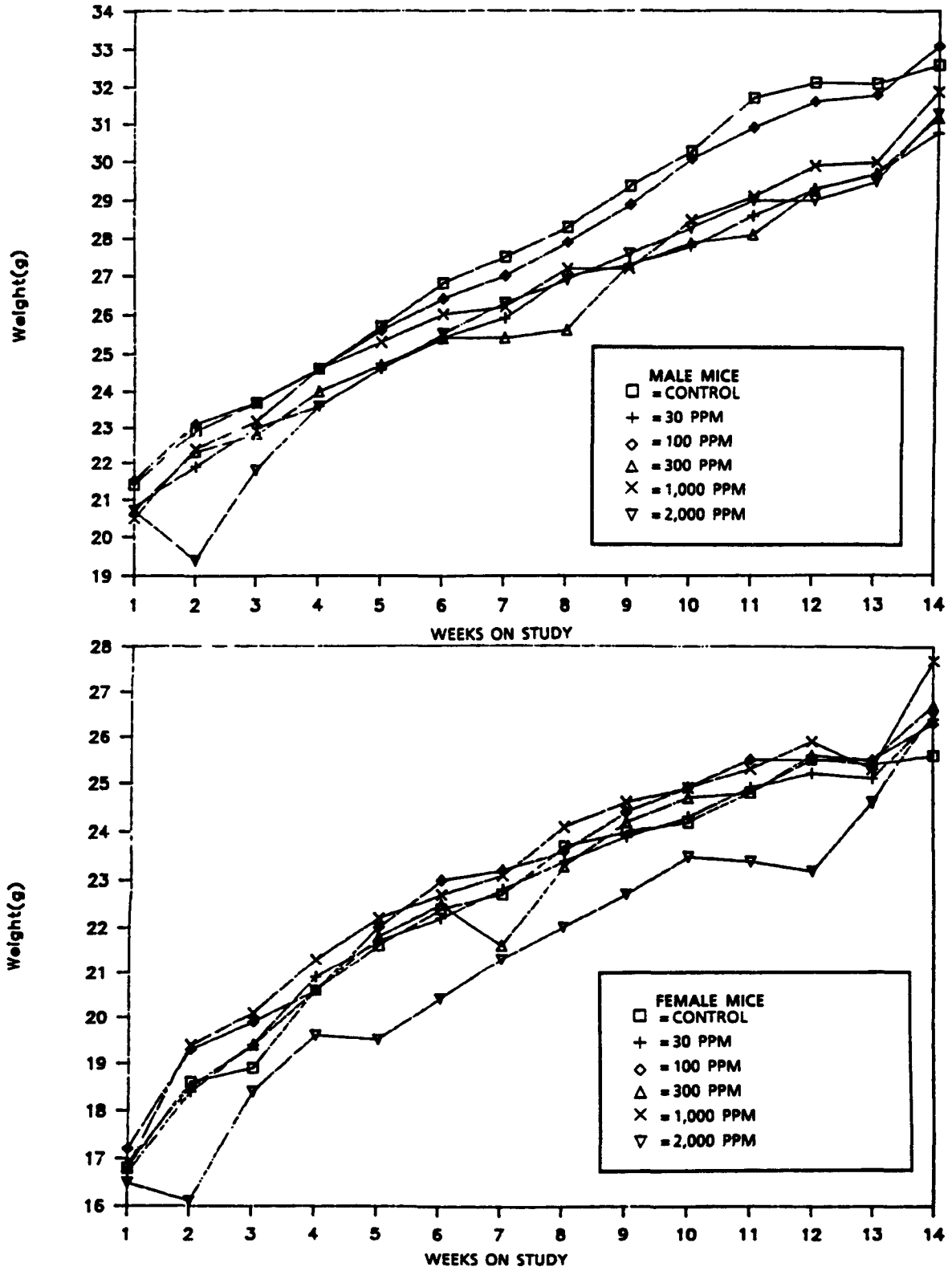


FIGURE 8. GROWTH CURVES FOR MICE ADMINISTERED 1,2,4,5-TETRACHLOROBENZENE IN FEED FOR THIRTEEN WEEKS

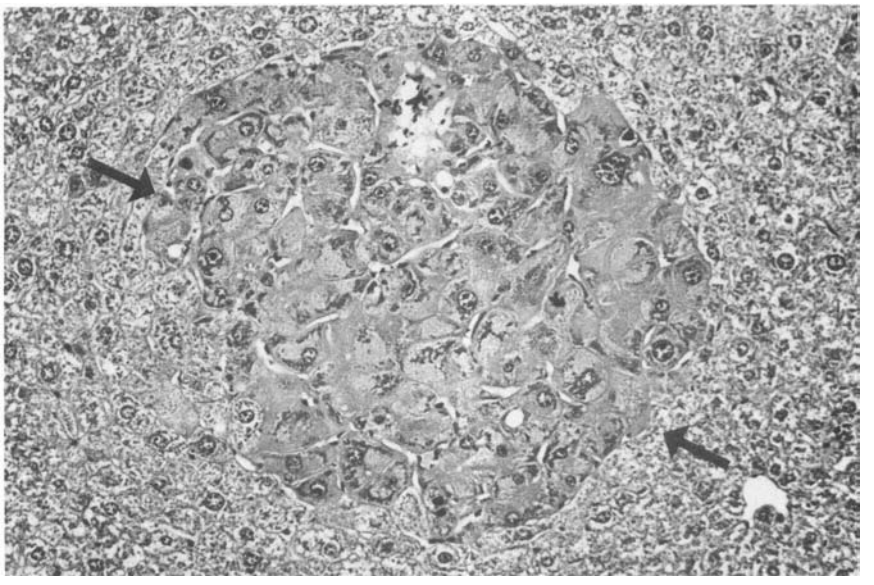


Figure 9. Liver from a male mouse exposed to 2,000 ppm tetrachlorobenzene for 13 weeks. Note focal area of hepatocyte hypertrophy (arrows).

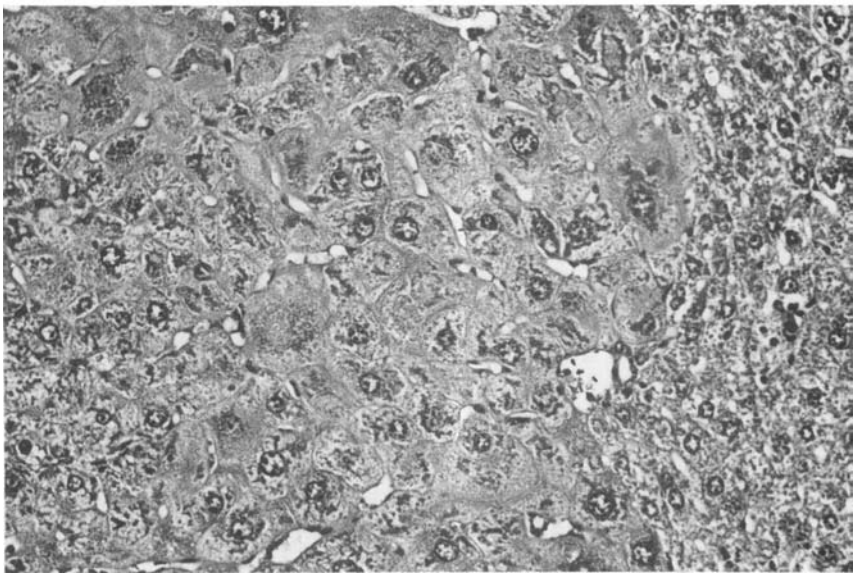


Figure 10. Liver from a male mouse exposed to 2,000 ppm tetrachlorobenzene for 13 weeks. Higher magnification of an area similar to that in Figure 9 shows hepatocellular hypertrophy characterized by enlarged cells with granular or smudgy cytoplasm and large nuclei.

TABLE 10. LIVER AND KIDNEY WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Organ	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Number weighed	10	10	10	10	10	10
Body weight (grams)	32.6 ± 0.96	30.8 ± 0.59	33.1 ± 0.71	31.2 ± 0.66	31.9 ± 0.76	31.3 ± 0.74
Liver						
Absolute	1,373 ± 58	1,367 ± 44	*1,546 ± 42	1,458 ± 60	**2,022 ± 67	**3,700 ± 80
Relative	42.1 ± 1.31	44.4 ± 1.03	*46.8 ± 1.19	*46.6 ± 1.04	**63.3 ± 1.35	**118.4 ± 2.86
FEMALE						
Number weighed	10	10	10	10	10	8
Body weight (grams)	25.6 ± 0.53	26.4 ± 0.55	26.3 ± 0.78	26.7 ± 0.67	*27.7 ± 0.69	26.5 ± 0.29
Right kidney						
Absolute	190 ± 4	199 ± 3	*206 ± 7	196 ± 7	*208 ± 5	*214 ± 9
Relative	7.4 ± 0.16	7.5 ± 0.12	7.8 ± 0.17	7.4 ± 0.21	7.5 ± 0.13	8.1 ± 0.31
Liver						
Absolute	1,183 ± 38	*1,306 ± 36	1,273 ± 52	1,312 ± 63	**2,086 ± 73	**4,171 ± 234
Relative	46.3 ± 1.41	49.4 ± 0.81	48.4 ± 1.43	49.1 ± 1.58	**75.4 ± 1.64	**156.9 ± 7.56

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified); P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

The platelet count was significantly increased for males and females receiving 1,000 ppm or 2,000 ppm (Table A6). Significantly lower values were seen for the hemoglobin concentration and mean corpuscular hemoglobin (2,000-ppm males and females), hematocrit (2,000-ppm females), and mean cell volume (2,000-ppm males and 1,000- and 2,000-ppm females). Significantly higher values were seen for serum sorbitol dehydrogenase activity for males and females receiving 1,000 ppm or 2,000 ppm (up to a fourfold or fivefold increase), serum alanine aminotransferase activity for males and females receiving 2,000 ppm (a twofold or threefold increase), and serum albumin concentration for males and females receiving 2,000 ppm.

The length of the estrous cycle was significantly increased in females receiving 2,000 ppm; females receiving 1,000 ppm were not examined (Table A6). No effects were seen on male reproductive organ weights or sperm evaluations (motility, morphology, or epididymal sperm density).

Compound-related lesions were present in the liver of exposed animals of each sex (Table 11).

Centrilobular hepatocellular hypertrophy was present in male and female mice in the 1,000- and 2,000-ppm groups. Histologically, affected hepatocytes were larger and had moderately increased cytoplasmic eosinophilia and granularity compared with normal hepatocytes (Figures 9 and 10). In general, hypertrophy was of minimal-to-mild severity, with male mice in the 1,000-ppm group exhibiting very subtle histologic changes.

Individual hepatocyte necrosis or degeneration occurred in the liver of 1,000- and 2,000-ppm male mice (Table 11). These lesions occurred in one female mouse in the 30-ppm groups and in several female mice in the 1,000- and 2,000-ppm groups (Table 11).

Histologically, necrotic hepatocytes were rounded and separated from adjacent hepatocytes. Hepatocytes undergoing degeneration were very

TABLE 11. NUMBERS OF MICE WITH SELECTED LESIONS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Site/Lesion	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Liver						
Necrosis	0	0	0	0	0	4 (1.7)
Centrilobular hypertrophy	0	0	0	0	7 (1.0)	10 (1.4)
Degeneration	0	0	0	0	0	9 (1.4)
Heart						
Mineralization	0	0	0	2 (1.5)	0	3 (2.3)
FEMALE						
Liver						
Necrosis	0	1 (1.0)	0	0	1 (1.0)	1 (1.0)
Centrilobular hypertrophy	0	0	0	0	7 (1.0)	9 (1.8)
Degeneration	0	0	0	0	0	5 (1.2)

(a) Ten animals were examined in each group. Number in parentheses denotes mean grade of severity: 1 = minimal; 2 = mild; 3 = moderate.

large with dense homogeneous deeply eosinophilic cytoplasm. Both lesions were of minimal-to-mild severity.

Mineralization of the heart was seen in a few male mice receiving 300 or 2,000 ppm 1,2,4,5-tetrachlorobenzene (see Table 11). Microscopically, there were a few randomly scattered, 200-2,500 μm foci of granular basophilic material within fragmented myocardial fibers; occasional foci had small amounts of osteoid-like eosinophilic material and cells resembling immature osteoblasts. The pathogenesis and relation of this change to compound administration are unclear.

Based on liver lesions, the NOEL is 300 ppm for mice of each sex. (Unlike in mice at the higher doses, the liver necrosis in one female 30-ppm mouse was a focal lesion and was not considered to be exposure related.)

III. DISCUSSION AND CONCLUSIONS

In these studies, the major target organs of toxicity after exposure to 1,2,4,5-tetrachlorobenzene in feed were the kidney and liver in rats and the liver in mice. Minimal thyroid lesions also occurred in rats. Generally, compound-related lesions were more extensive in rats than in mice.

In the 14-day studies, all rats survived to the end, but all mice that received 3,000 ppm 1,2,4,5-tetrachlorobenzene died before the end of the studies. Histologically, lymphoid depletion and necrosis were seen in the spleen, thymus, and lymph nodes of male and female mice in the 3,000-ppm groups. Similar lesions were observed frequently in moribund and early-death mice in other studies.

Compound-related histologic kidney lesions were present in rats of each sex but were most prominent in males. Exposed male rats in the 14-day and 13-week studies exhibited renal cortical tubular cytoplasmic alteration (abnormal hyaline droplet accumulation). Exposed male rats in the 13-week studies also had cortical tubular epithelial regeneration and outer medullary tubule dilatation, granular casts, and mineralization. This spectrum of lesions is compatible with that described for "hydrocarbon or hyaline droplet nephropathy" (Busey and Cockrell, 1984; Thomas et al., 1985; Trump et al., 1985; Short et al., 1986).

Hyaline droplet nephropathy is associated with increased renal cortical tubule resorption of $\alpha_{2\mu}$-globulin, a low molecular weight protein normally produced in the rat liver and excreted by the kidney (Sarkar et al., 1986; Short et al., 1987; Murty et al., 1988). $\alpha_{2\mu}$-Globulin levels

are regulated by androgen hormones and decline in older rats (Roy, 1977), so typical lesions described for hyaline droplet nephropathy occur only in intact young adult male rats (Alden, 1986) or in female rats pretreated with testosterone (Roy, 1977). When administered to rats, compounds such as light hydrocarbons (Trump et al., 1985), unleaded gasoline components (Olson et al., 1987; Short et al., 1987), jet fuel (Bruner, 1984), *d*-limonene (Kanerva et al., 1987; NTP, 1990b), and other chemicals (Dodd et al., 1987; Read et al., 1988) have caused increased incidences of hyaline droplet accumulation and, in some cases, renal epithelial neoplasms (Kitchen, 1984; Phillips and Cockrell, 1984; Alden et al., 1984; Busey and Cockrell, 1984; Halder et al., 1984; Stonard et al., 1986; Dodd et al., 1987).

Chlorinated benzenes have also been implicated in renal toxicity for male rats. Long-term administration of 1,4-dichlorobenzene, but not of 1,2-dichlorobenzene, resulted in abnormal hyaline droplet accumulation and epithelial neoplasms in the kidney of male F344 rats (Charbonneau et al., 1989; NTP, 1985, 1987; Bomhard et al., 1988). Renal epithelial neoplasms also occurred in rats fed hexachlorobenzene (Lambrecht et al., 1983).

"Eosinophilic inclusions" (apparently hyaline droplets) were noted in the cytoplasm of renal cortical epithelial cells in male rats fed 1,2,4,5-tetrachlorobenzene in the diet for 28 days or 13 weeks (Chu et al., 1983, 1984c).

Although the exact pathogenesis of hyaline droplet nephropathy is unknown, binding of chemicals to $\alpha_{2\mu}$-globulin is postulated to lead to formation of complexes that are resorbed but not readily degraded by renal cortical tubules (Trump et al., 1985). With light microscopy, the resulting phagolysosomes are detected as abnormal hyaline droplets in cortical tubule epithelial cells (Short et al., 1987). Cortical epithelial cell necrosis and subsequent tubular regeneration result from hyaline droplet accumulation (Swenberg et al., 1989).

In addition, homogeneous eosinophilic protein casts were observed in renal tubules in all groups of exposed male rats and in females in the two highest dose groups. This lesion was not considered to be part of the hyaline droplet nephropathy complex but rather to be a manifestation of the spontaneous nephropathy commonly seen in laboratory rats (Peter et al., 1986), which was exacerbated by 1,2,4,5-tetrachlorobenzene exposure.

Female rats in the 2,000-ppm group had minimal-to-mild aggregates of intracytoplasmic yellow-brown pigment in the renal cortical epithelial cells. This pigment was not positively identified but could have been composed of porphyrin compounds. Although other chlorinated benzenes such as hexachlorobenzene cause marked porphyria in several species (Elder, 1978), 1,2,4,5-tetrachlorobenzene appears to be at best weakly porphyrinogenic in the rat (Billi et al., 1986).

Urinary glucose concentrations were increased in male and female rats in the higher dose groups and may have been related to renal tubular epithelial damage. Increases in urinary glucose have been reported previously in male F344 rats with hyaline droplet nephropathy (Phillips and Cockrell, 1984).

In the 13-week studies, mild-to-minimal hepatocellular centrilobular hypertrophy and individual hepatocyte degeneration and necrosis were noted in the livers of exposed male and female mice in the two highest dose groups. Similar histologic lesions, as well as hepatocellular degeneration and necrosis, were noted in male and female $B6C3F_1$ mice given 1,4-dichlorobenzene by gavage for 13 weeks or 2 years (NTP, 1987). Significant increases in serum sorbitol dehydrogenase activity in males and females receiving 1,000 or 2,000 ppm 1,2,4,5-tetrachlorobenzene and of alanine aminotransferase activity in males and females receiving 2,000 ppm are indicative of mild hepatocellular damage (Duncan and Prasse, 1977).

Compound-related increased incidences of hepatocellular hypertrophy were observed in the liver of male and female rats in the 1,000- and 2,000-ppm groups. Hepatocellular hypertrophy was observed previously in male and female rats exposed to 1,2,4,5-tetrachlorobenzene in feed for 28 days or 13 weeks (Chu et al., 1983, 1984c). Hepatocellular hypertrophy in rats also occurred after administration of compounds such as chlorinated benzenes and halogenated biphenyls

which induce hepatic microsomal enzymes; ultrastructurally, the light microscopic changes correlate with increased amounts of smooth endoplasmic reticulum (Kuiper-Goodman and Grant, 1986; Strik et al., 1986). Chlorinated benzenes such as 1,4-dichlorobenzene are also known to cause hepatocellular degeneration and necrosis in male and female F344/N rats (NTP, 1987). In the current studies, hepatocellular changes may have been morphologic manifestations of metabolic enzyme induction.

Exposure-related histologic changes were present in the thyroid gland of male and female rats in the 13-week studies. Minimal follicular cell hypertrophy and decreased intraluminal colloid density occurred in rats given 1,2,4,5-tetrachlorobenzene. Similar lesions were noted previously in rats administered 1,2,4,5-tetrachlorobenzene orally for 23 days (Chu et al., 1983, 1984b). Free thyroxin and total thyroxin concentrations were significantly decreased in females in all exposed groups and in males in the 300- to 2,000-ppm groups (Table A3). These data are strongly suggestive of a moderate primary hypothyroxemia.

Induction of thyroid proliferative lesions (hyperplasia, hypertrophy, or adenoma) in animals and humans is a well-documented effect of many polyhalogenated aromatic hydrocarbons such as hexachlorobenzene (Cabral et al., 1977; Peters et al., 1982), pentachlorobenzene (NTP, 1990a), 1,4-dichlorobenzene (NTP, 1987), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (NTP, 1982), and polychlorinated biphenyls (Capen and Martin, 1989).

Decreased serum thyroxin levels were reported in rats exposed to polychlorinated biphenyls (Bastomsky et al., 1976; Collins and Capen, 1980). Conjugation to glucuronic acid and biliary excretion of thyroxin was also accelerated, presumably due to compound-related induction of hepatic thyroxine-UDP-glucuronyltransferase (Bastomsky and Murthy, 1976; McClain, 1989). Collins et al. (1977) proposed that lower thyroxin levels after polychlorinated biphenyl administration are due to the combined effects of direct toxicity on thyroid follicular cells and potentiation of peripheral metabolism. Whether similar factors played a causative role in the thyroid hormone abnormalities noted in these studies was not determined.

Hematocrit values, hemoglobin concentration, erythrocyte count, and/or mean cell volume were significantly lower in male and/or female rats in the two highest dose groups.

Significantly decreased values for hemoglobin concentration, mean corpuscular hemoglobin, hematocrit, and mean cell volume were observed in male and/or female mice in the 1,000- and 2,000-ppm groups (Table A6). These changes indicate a moderate, poorly regenerative, microcytic anemia that is clearly present in rats of each sex and in female mice and is marginal in male mice. The increased serum albumin concentration observed in several exposure groups of rats and mice indicated possible dehydration, so the anemia may have been even more severe than indicated by the absolute hematologic values. Similar changes in hematologic values occurred in male F344/N rats given 1,4-dichlorobenzene by gavage for 13 weeks (NTP, 1987).

The exact cause of the anemia was not determined. However, a mild compound-related disturbance of porphyrin metabolism may have been involved in the pathogenesis of the anemia, since porphyrins are precursors in heme biosynthesis (Bonkowsky, 1982). Interference in heme biosynthesis has been implicated as an important pathogenic mechanism in hexachlorobenzene-induced porphyria in humans (De Matteis, 1986). Decreased erythropoietin production by the damaged kidneys, considered to be an important pathogenic factor in the anemia of renal failure (Anagnostou et al., 1981), also could have contributed to the hematologic derangements observed in these studies.

The pathogenesis and relationship to exposure of the myocardial mineralization observed in a few male mice that received 300 or 2,000 ppm 1,2,4,5-tetrachlorobenzene are not clear. Multifocal mineralization of myocardium and skeletal muscle occurred in B6C3F_1 mice of each sex given 1,2-dichlorobenzene (NTP, 1985).

The NOEL for histologic lesions in the 13-week studies was 30 ppm for male and female rats. The NOEL for histologic lesions in male and female mice was 300 ppm.

V. REFERENCES

1. Adams, J.R.; Rodriguez-Kabana, R. (1976) The effects of 1,2,4,5-tetrachlorobenzene on plant parasitic and free-living nematodes. J. Alabama Acad. Sci. 47:134 (Abstr.).

2. Alden, C.L. (1986) A review of unique male rat hydrocarbon nephropathy. Toxicol. Pathol. 14:109-111.

3. Alden, C.L.; Kanerva, R.L.; Ridder, G.; Stone, L.C. (1984) The pathogenesis of the nephrotoxicity of volatile hydrocarbons in the male rat. Mehlman, M.A.; Hemstreet, G.P., III; Thorpe, J.J.; Weaver, N.K., Eds.: Advances in Modern Environmental Toxicology, Vol. VII. Renal Effects of Petroleum Hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., pp. 107-120.

4. Ameen, O.A.; Day, A.D.; Hamilton, K.C. (1960) Effect of 1,2,4,5-tetrachlorobenzene on the germination and seedling vigor of barley, oats, and wheat. Agron. J. 52:87-89.

5. Anagnostou, A.; Fried, W.; Kurtzman, N.A. (1981) Hematological consequences of renal failure. Brenner, B.M.; Rector, F.C., Eds.: The Kidney, 2nd ed. Philadelphia: W.B. Saunders Co., pp. 2184-2212.

6. Ariyoshi, T.; Idiquchi, K.; Ishizuka, Y.; Iwasaki, K.; Arakaki, M. (1975a) Relationship between chemical structure and activity. I. Effects of the number of chlorine atoms in chlorinated benzenes on the components of drug-metabolizing systems and the hepatic constituents. Chem. Pharm. Bull. 28:817-823.

7. Ariyoshi, T.; Idiquchi, K.; Iwasaki, K.; Arakaki, M. (1975b) Relation between chemical structure and activity. II. Influences of isomers in dichlorobenzene, trichlorobenzene, and tetrachlorobenzene on the activities of drug-metabolizing enzymes. Chem. Pharm. Bull. 23:824-830.

8. Artigas, F.; Martinez, E.; Gelpi, E. (1988) Organochlorine pesticides by negative ion chemical ionization. Brain metabolites of lindane. Biomed. Environ. Mass Spectrom. 16:279-284.

9. Barlow, W. (1984) An improved Heidenhain's azan technique using "SUSA" fixation. Histo-Logic 14:223-224.

10. Bastomsky, C.H.; Murthy, P.V.N. (1976) Enhanced *in vitro* hepatic glucuronidation of thyroxine in rats following cutaneous application or ingestion of polychlorinated biphenyls. Can. J. Physiol. Pharmacol. 54:23-26.

11. Bastomsky, C.H.; Murthy, P.V.N.; Banovac, K. (1976) Alterations in thyroxine metabolism produced by cutaneous application of microscope immersion oil: Effects due to polychlorinated biphenyls. Endocrinology 98:1309-1314.

12. Billi, S.C.; Koss, G.; San Martin de Viale, L.C. (1986) Ability of several hexachlorobenzene metabolites to decrease rat-liver porphyrinogen carboxy-lyase and to produce porphyrin accumulation in chick-embryo liver. Morris, C.R.; Cabral, J.R.P., Eds.: Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications No. 77. Lyon, France: International Agency for Research on Cancer, pp. 471-476.

13. Bomhard, E.; Luckhaus, G.; Voigt, W.-H.; Loeser, E. (1988) Induction of light hydrocarbon nephropathy by *p*-dichlorobenzene. Arch. Toxicol. 61:433-439.

14. Bonkowsky, H.L. (1982) Porphyrin and heme metabolism and the porphyrias. Zakim, D.; Boyer, T.D., Eds.: Hepatology: A Textbook of Liver Diseases. Philadelphia: W.B. Saunders Co., pp. 351-393.

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

16. Braun, W.H.; Sung, L.Y.; Keyes, D.G.; Kociba, R.J. (1978) Pharmacokinetic and toxicological evaluation of dogs fed 1,2,4,5-tetrachlorobenzene in the diet for two years. J. Environ. Pathol. Toxicol. 2:225-233.

17. Bruckmann, P.; Kersten, W.; Funcke, W.; Balfanz, E.; Konig, J.; Theisen, J.; Ball, M.; Papke, O. (1988) The occurrence of chlorinated and other organic trace compounds in urban air. Chemosphere 17:2363-2380.

18. Bruner, R.H. (1984) Pathologic findings in laboratory animals exposed to hydrocarbon fuels of military interest. Mehlman, M.A.; Hemstreet, G.P., III; Thorpe, J.J.; Weaver, N.K., Eds.: Advances in Modern Environmental Toxicology, Vol. VII. Renal Effects of Petroleum Hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., pp. 133-140.

19. Busey, W.M.; Cockrell, B.Y. (1984) Non-neoplastic exposure-related renal lesions in rats following inhalation of unleaded gasoline vapors. Mehlman, M.A.; Hemstreet, G.P., III; Thorpe, J.J.; Weaver, N.K., Eds.: Advances in Modern Environmental Toxicology, Vol. VII. Renal Effects of Petroleum Hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., pp. 57-64.

20. Cabral, J.R.P.; Shubik, P.; Mollner, T.; Raitano, F. (1977) Carcinogenic activity of hexachlorobenzene in hamsters. Nature 269:510-511.

21. Capen, C.C.; Martin, S.L. (1989) The effects of xenobiotics on the structure and function of thyroid follicular and C-cells. Toxicol. Pathol. 17:266-293.

22. Charbonneau, M.; Strasser, J.; Lock, E.A.; Turner, M.J.; Swenberg, J.A. (1989) 1,4-Dichlorobenzene-induced nephrotoxicity: Similarity with unleaded gasoline (UG)-induced renal effects. Bach, P.; Lock, E.A., Eds.: Nephrotoxicity: Extrapolation from In Vitro to In Vivo and from Animals to Man. New York: Plenum Press, pp. 557-562.

23. Chu, I.; Villeneuve, D.; Secours, V.; Valli, V.E. (1983) Comparative toxicity of 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene in the rat: Results of acute and subacute studies. J. Toxicol. Environ. Health 11:663-677.

24. Chu, I.; Villeneuve, D.; Viau, A.; Barnes, C.R.; Benoit, F.M.; Qin, Y.H. (1984a) Metabolism of 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene in the rat. J. Toxicol. Environ. Health 13:777-786.

25. Chu, I.; Villeneuve, D.C.; Valli, V.E. (1984b) Comparative toxicity and metabolism of tetrachlorobenzene isomers. Kaiser, K.L.E., Ed.: QSAR in Environmental Toxicology. Dordrecht, The Netherlands: D. Reidel Publishing Company, pp. 17-37.

26. Chu, I.; Villeneuve, D.; Valli, V.E.; Secours, V.E. (1984c) Toxicity of 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene in the rat: Results of a 90-day feeding study. Drug Chem. Toxicol. 7: 113-127.

27. Chu, I.; Villeneuve, D.C.; Yagminas, A.; Valli, V.E. (1986) Effect of phenobarbital and polychlorinated biphenyls on the toxicity and disposition of 1,2,4,5-tetrachlorobenzene in the rat. J. Environ. Sci. Health B21:229-242.

28. Chu, I.; Villeneuve, D.C.; Murdoch, D.J.; Viau, A. (1987) Tissue distribution and elimination of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene in the rat. Kaiser, K.L.E., Ed.: QSAR in Environmental Toxicology, Proceedings of the 2nd International Workshop. Dordrecht, The Netherlands: D. Reidel Publishing Company, pp. 55-60.

29. Collins, W.T.; Capen, C.C. (1980) Ultrastructural and functional alterations of the rat thyroid gland produced by polychlorinated biphenyls compared with iodide excess and deficiency, and thyrotropin and thyroxine administration. Virchows Arch. [B] 33:213-231.

30. Collins, W.T., Jr.; Capen, C.C.; Kasza, L.; Carter, C.; Dailey, R.E. (1977) Effect of polychlorinated biphenyl (PCB) on the thyroid gland of rats. Am. J. Pathol. 89:119-136.

31. The Condensed Chemical Dictionary (1981) 10th ed. Hawley, G.G., Ed. New York: Van Nostrand Reinhold Company, p. 1003.

32. De Matteis, F. (1986) Experimental hepatic porphyria caused by hexachlorobenzene: Mechanism of the metabolic block. Morris, C.R.; Cabral, J.R.P., Eds.: Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications No. 77. Lyon, France: International Agency for Research on Cancer, pp. 427-431.

33. Denomme, M.A.; Leece, B.; Gyorkos, J.; Homonko, K.; Safe, S. (1983) Polychlorinated benzene and phenol congeners as inducers of rat hepatic drug-metabolizing enzymes in immature male Wistar rats. Can. J. Physiol. Pharmacol. 61:1063-1070.

34. Dodd, D.E.; Losco, P.E.; Troup, C.M.; Pritts, I.M.; Tyler, T.R. (1987) Hyalin droplet nephrosis in male Fischer-344 rats following inhalation of diisobutyl ketone. Toxicol. Ind. Health 3:443-457.

35. Duncan, J.R.; Prasse, R.W. (1977) Veterinary Laboratory Medicine: Clinical Pathology. Ames: Iowa State University Press, pp. 81-83.

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

37. Dunnett, C.W. (1980) Pairwise multiple comparisons in the unequal variance case. J. Am. Stat. Assoc. 75:796-800.

38. Elder, G.H. (1978) Porphyria caused by hexachlorobenzene and other polyhalogenated aromatic hydrocarbons. De Matteis, F.; Aldridge, W.N., Eds.: Handbook of Experimental Pharmacology, Vol. 44. Heme and Hemoproteins. New York: Springer-Verlag, pp. 157-200.

39. Ellenton, J.A.; McPherson, M.F. (1983) Mutagenicity studies on herring gulls from different locations on the Great Lakes. I. Sister chromatid exchange rates in herring-gull embryos. J. Toxicol. Environ. Health 12:317-324.

40. Ellenton, J.A.; McPherson, M.F.; Maus, K.L. (1983) Mutagenicity studies on herring gulls from different locations on the Great Lakes: II. Mutagenic evaluation of extracts of herring gull eggs in a battery of in vitro mammalian and microbial tests. J. Toxicol. Environ. Health 12: 325-336.

41. Ellenton, J.A.; Brownlee, L.J.; Hollebone, B.R. (1985) Aryl hydrocarbon hydroxylase levels in herring gull embryos from different locations on the Great Lakes. Environ. Toxicol. Chem. 4:615-622.

42. Fomenko, V.N. (1965) Determination of the maximum permissible concentration of tetrachlorobenzene in water basins. Hyg. Sanit. 30:8-15.

43. Halder, C.A.; Warne, T.M.; Hatoum, N.S. (1984) Renal toxicity of gasoline and related petroleum naphthas in male rats. Mehlman, M.A.; Hemstreet, G.P., III; Thorpe, J.J.; Weaver, N.K., Eds.: Advances in Modern Environmental Toxicology, Vol. VII. Renal Effects of Petroleum Hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., pp. 73-87.

44. Hallett, D.J.; Norstrom, R.J.; Onuska, F.I.; Comba, M.E. (1982) Incidence of chlorinated benzenes and chlorinated ethylenes in Lake Ontario Herring Gulls. Chemosphere 11:277-285.

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

46. Herren-Freund, S.L.; Pereira, M.A. (1986) Carcinogenicity of by-products of disinfection in mouse and rat liver. Environ. Health Perspect. 69:59-65.

47. Hill, R., Jr.; Bailey, S.; Needham, L. (1982) Development and utilization of a procedure for measuring urinary porphyrins by HPLC. J. Chromatogr. 232:251-60.

48. Ikegami, S.; Tsuchihashi, F.; Ohno, M.; Nishide, E. (1987) Relationship between chemical structure of chlorinated benzenes and their effect on hepatic and serum lipid components in rats. Shokuhin Eiseigaku Zasshi 28:436-444.

49. Jacobs, A.; Hellmund, E.; Eberle, S.H. (1977) Speicherung von organischen Spurenverunreinigungen des Wassers im Korper: Zeit- und Dosisabhangigkeit der Akkumulation von Hexachlorbenzol und Tetrachlorbenzolderivaten im Rattenversuch. Vom Wasser 48:255-272.

50. Jaffe, R.; Hites, R.A. (1986) Anthropogenic, polyhalogenated, organic compounds in nonmigratory fish from the Niagara River area and tributaries to Lake Ontario. J. Great Lakes Res. 12:63-71.

51. Jan, J. (1983) Chlorobenzene residues in human fat and milk. Bull. Environ. Contam. Toxicol. 30:595-599.

52. Jan, J.; Malnersic, S. (1980) Chlorinated benzene residues in fish in Slovenia (Yugoslavia). Bull. Environ. Contam. Toxicol. 24:824-827.

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

54. Jondorf, W.R.; Parke, D.V.; Williams, R.T. (1958) Studies in detoxication. 76. The metabolism of halogenobenzenes. 1:2:3:4-, 1:2:3:5-, and 1:2:4:5-tetrachlorobenzenes. Biochem. J. 69:181-189.

55. Kacew, S.; Ruddick, J.A.; Parulekar, M.; Valli, V.E.; Chu, I.; Villeneuve, D.C. (1984) A teratological evaluation and analysis of fetal tissue levels following administration of tetrachlorobenzene isomers to the rat. Teratology 29:21-27.

56. Kanerva, R.L.; Ridder, G.M.; Lefever, F.R.; Alden, C.L. (1987) Comparison of short-term renal effects due to oral administration of decalin or *d*-limonene in young adult male Fischer-344 rats. Food Chem. Toxicol. 25:345-353.

57. Kiraly, J.; Szentesi, I.; Ruzicska, M.; Czeize, A. (1979) Chromosome studies in workers producing organophosphorus insecticides. Arch. Environ. Contam. Toxicol. 8:309-319.

58. Kitchen, D.N. (1984) Neoplastic renal effects of unleaded gasoline in Fischer 344 rats. Mehlman, M.A.; Hemstreet, G.P., III; Thorpe, J.J.; Weaver, N.K., Eds.: Advances in Modern Environmental Toxicology, Vol. VII. Renal Effects of Petroleum Hydrocarbons. Princeton, NJ: Princeton, Scientific Publishers, Inc., pp. 65-72.

59. Kitchin, K.T.; Ebron, M.T. (1983a) Maternal hepatic effects of 1,2,4,5-tetrachlorobenzene in the rat. Environ. Res. 32:134-144.

60. Kitchin, K.T.; Ebron, M.T. (1983b) Maternal hepatic and embryonic effects of 1,2,3,4-tetrachlorobenzene in the rat. Toxicology 26:243-256.

61. Kohli, J.; Jones, D.; Safe, S. (1976) The metabolism of higher chlorinated benzene isomers. Can. J. Biochem. 54:203-208.

62. Kuiper-Goodman, T.; Grant, D.L. (1986) Subchronic toxicity of hexachlorobenzene in the rat: Clinical, biochemical, morphological and morphometric findings. Morris, C.R.; Cabral, J.R.P., Eds.: Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications No. 77. Lyon, France: International Agency for Research on Cancer, pp. 343-348.

63. Lambrecht, R.W.; Erturk, E.; Grunden, E.E.; Peters, H.A.; Morris, C.R.; Bryan, G.T. (1983) Renal tumors in rats (R) chronically exposed to hexachlorobenzene (HCB). Carcinogenesis 24:59 (Abstr.).

64. Loveday, K.S.; Anderson, B.E.; Resnick, M.A.; Zeiger, E. (1990) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals. Environ. Molec. Mutagen. (in press).

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

66. Matthews, H.B. (1986) Factors determining hexachlorobenzene distribution and persistence in higher animals. Morris, C.R.; Cabral, J.R.P., Eds.: Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications No. 77. Lyon, France: International Agency for Research on Cancer, pp. 253-260.

67. McClain, R.M. (1989) The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: Implications for thyroid gland neoplasia. Toxicol. Pathol. 17: 294-306.

68. Melancon, M.J.; Lech, J.J. (1985) The uptake, distribution and elimination of di-, tri-, tetra-, and pentachlorobenzene in rainbow trout. Fed. Proc. 44:516 (Abstr.).

69. Morita, M.; Mimura, S.; Ohi, G.; Yagyu, H. (1975) A systematic determination of chlorinated benzenes in human adipose tissue. Environ. Pollut. 9:175-179.

70. Morrissey, R.E.; Schwetz, B.A.; Lamb, J.C., IV; Ross, M.D.; Teague, J.L.; Morris, R.W. (1988) Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. Fundam. Appl. Toxicol. 11:343-358.

71. Murty, C.V.R.; Olson, M.J.; Garg, B.D.; Roy, A.K. (1988) Hydrocarbon-induced hyaline droplet nephropathy in male rats during senescence. Toxicol. Appl. Pharmacol. 96:380-392.

72. National Institute for Occupational Safety and Health (NIOSH) (1980) Registry of Toxic Effects of Chemical Substances, February, 1982.

73. National Toxicology Program (NTP) (1982) Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Osborne-Mendel Rats and B6C3F$_1$ Mice. NTP Technical Report No. 209. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD. 195 p.

74. National Toxicology Program (NTP) (1985) Toxicology and Carcinogenesis Studies of 1,2-Dichlorobenzene (o-Dichlorobenzene) in F344/N Rats and B6C3F$_1$ Mice. NTP Technical Report No. 255. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD. 195 p.

75. National Toxicology Program (NTP) (1987) Toxicology and Carcinogenesis Studies of 1,4-Dichlorobenzene in F344/N Rats and B6C3F$_1$ Mice. NTP Technical Report No. 319. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. 198 p.

76. National Toxicology Program (NTP) (1990a) Toxicity Studies of Pentachlorobenzene in F344/N Rats and B6C3F$_1$ Mice. NTP Toxicity Report No. 6. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. 48 p.

77. National Toxicology Program (NTP) (1990b) Toxicology and Carcinogenesis Studies of *d*-Limonene in F344/N Rats and B6C3F$_1$ Mice. NTP Technical Report No. 347. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. 165 p.

78. Olie, K.; Lustenhouwer, J.W.A.; Hutzinger, O. (1980) Polychlorinated dibenzo-*p*-dioxins and related compounds in incinerator effluents. Hutzinger, O.; Frei, R.W.; Merian, E.; Pocchiari, F., Eds.: Chlorinated Dioxins and Related Compounds: Impact on the Environment. Oxford: Pergamon Press, pp. 227-244.

79. Oliver, B.G. (1987) Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ. Sci. Technol. 21:785-790.

80. Oliver, B.G.; Nicol, K.D. (1982) Chlorobenzenes in sediments, water, and selected fish from Lakes Superior, Huron, Erie, and Ontario. Environ. Sci. Technol. 16:532-536.

81. Oliver, B.G.; Niimi, A.J. (1983) Bioconcentration of chlorobenzenes from water by rainbow trout: Correlations with partition coefficients and environmental residues. Environ. Sci. Technol. 17:287-291.

82. Oliver, B.G.; Charlton, M.N. (1984) Chlorinated organic contaminants on settling particulates in the Niagara River vicinity of Lake Ontario. Environ. Sci. Technol. 18:903-908.

83. Olson, M.J.; Garg, B.D.; Murty, C.V.R.; Roy, A.K. (1987) Accumulation of α_{2u}-globulin in the renal proximal tubules of male rats exposed to unleaded gasoline. Toxicol. Appl. Pharmacol. 90:43-51.

84. Onuska, F.I.; Terry, K.A. (1985) Determination of chlorinated benzenes in bottom sediment samples by WCOT column gas chromatography. Anal. Chem. 57:801-805.

85. Paradi, E.; Lovenyak, M. (1981) Genetic effects of pesticides in *Drosophila melanogaster*. Acta Biol. Acad. Sci. Hung. 32:119-122.

86. Pereira, W.E.; Rostad, C.E.; Chiou, C.T.; Brinton, T.I.; Barber, L.B., II; Demcheck, D.K.; Demas, C.R. (1988) Contamination of estuarine water, biota, and sediment by halogenated organic compounds: A field study. Environ. Sci. Technol. 22:772-778.

87. Peter, C.P.; Burek, J.D.; van Zwieten, M.J. (1986) Spontaneous nephropathies in rats. Toxicol. Pathol. 14:91-99.

88. Peters, H.A.; Gocman, A.; Cripps, D.J.; Bryan, G.T.; Dogramzci, I. (1982) Epidemiology of hexachlorobenzene-induced porphyria in Turkey. Clinical and laboratory follow-up after 25 years. Arch. Neurol. 39:744-749.

89. Phillips, R.D.; Cockrell, B.Y. (1984) Effect of certain light hydrocarbons on kidney function and structure in male rats. Mehlman, M.A.; Hemstreet, G.P., III; Thorpe, J.J.; Weaver, N.K., Eds.: Advances in Modern Environmental Toxicology, Vol. VII. Renal Effects of Petroleum Hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., pp. 89-105.

90. Read, N.G.; Astbury, P.J.; Morgan, R.J.I.; Parsons, D.N.; Port, C.J. (1988) Induction and exacerbation of hyaline droplet formation in the proximal tubular cells of the kidneys from male rats receiving a variety of pharmacological agents. Toxicology 52:81-101.

91. Reed, W.T.; Forgash, A.J. (1970) Metabolism of lindane to organic-soluble products by houseflies. J. Agric. Food Chem. 18:475-481.

92. Rimington, C.; Ziegler, G. (1963) Experimental porphyria in rats induced by chlorinated benzenes. Biochem. Pharmacol. 12:1387-1397.

93. Roy, A.K. (1977) Early events in the steroidal regulation of α_{2u} globulin in rat liver: Evidence for both androgenic and estrogenic induction. Eur. J. Biochem. 73:537-543.

94. Saha, J.G.; Burrage, R.H. (1976) Residues of lindane and its metabolites in eggs, chicks and body tissues of hen pheasants after ingestion of lindane carbon-14 via treated wheat seed or gelatin capsules. J. Environ. Sci. Health [B] Bull. 1:67-93.

95. Sarkar, F.H.; Mancini, M.A.; Nag, A.C.; Roy, A.K. (1986) Cellular interactions in the hormonal induction of α_{2u}-globulin in rat liver. J. Endocrinol. 111:205-208.

96. Schwartz, H.; Chu, I.; Villeneuve, D.C.; Benoit, F.M. (1987) Metabolism of 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-tetrachlorobenzene in the squirrel monkey. J. Toxicol. Environ. Health 22:341-350.

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

98. Short, B.G.; Burnett, V.L.; Swenberg, J.A. (1986) Histopathology and cell proliferation induced by 2,2,4-trimethylpentane in the male rat kidney. Toxicol. Pathol. 14:194-203.

99. Short, B.G.; Burnett, V.L.; Cox, M.G.; Bus, J.S.; Swenberg, J.A. (1987) Site-specific renal cytotoxicity and cell proliferation in male rats exposed to petroleum hydrocarbons. Lab. Invest. 57:564-577.

100. Stonard, M.D.; Phillips, P.G.N.; Foster, J.R.; Simpson, M.G.; Lock, E.A. (1986) α_{2U}-Globulin: Measurement in rat kidney following administration of 2,2,4-trimethylpentane. Toxicology 41:161-168.

101. Strik, J.J.T.W.A. (1986) Subacute toxicity of hexachlorobenzene. Morris, C.R.; Cabral, J.R.P., Eds.: Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications No. 77. Lyon, France: International Agency for Research on Cancer, pp. 335-342.

102. Swenberg, J.A.; Short, B.; Borghoff, S.; Strasser, J.; Charbonneau, M. (1989) The comparative pathobiology of α_{2u}-globulin nephropathy. Toxicol. Appl. Pharmacol. 97:35-46.

103. Thomas, F.B.; Halder, C.A.; Holdsworth, C.E.; Cockrell, B.Y. (1985) Hydrocarbon nephropathy in male rats. Temporal and morphologic characterization of the renal lesions. Bach, P.H.; Lock, E.A., Eds.: Renal Heterogeneity and Target Cell Toxicity. New York: John Wiley & Sons, pp. 477-480.

104. Toxicological Information Response Center/ Oak Ridge National Laboratory (TIRC/ORNL) (1979) Chemicals Identified in Dumps: List compiled by Toxicological Information Response Center, Information Center Complex, Oak Ridge National Laboratory.

105. Trump, B.F.; Jones, T.W.; Lipsky, M.M. (1985) Light hydrocarbon nephropathy. Bach, P.H.; Lock, E.A., Eds.: Renal Heterogeneity and Target Cell Toxicity. New York: John Wiley & Sons, pp. 493-504.

106. U.S. Environmental Protection Agency (USEPA) (1976) Preliminary Scoring of Selected Organic Air Pollutants. EPA-450/3-77-008a.

107. U.S. Environmental Protection Agency (USEPA) (1978) In-Depth Studies on Health and Environmental Impacts of Selected Water Pollutants. EPA-68-01-4646. Washington, DC.

108. U.S. Environmental Protection Agency (USEPA) (1979) List of Chemicals Found in the Love Canal: Compiled by Hazardous Waste Enforcement Task Force, Office of Enforcement, USEPA, Washington, DC.

109. U.S. Environmental Protection Agency (USEPA) (1980) TSCA Chemical Assessment Series, Assessment of Testing Needs: Chlorinated benzenes. EPA-560/11-80-014. Office of Pesticides and Toxic Substances, USEPA, Washington, DC.

110. Van Leeuwen, C.J.; Griffioen, P.S.; Vergouw, W.H.A.; Maas-Diepeveen, J.L. (1985) Differences in susceptibility of early life stages of rainbow trout (*Salmo gairdneri*) to environmental pollutants. Aquat. Toxicol. 7:59-78.

111. Viau, A.C.; Studak, S.M.; Karasek, F.W. (1984) Comparative analysis of hazardous compounds on fly-ash from municipal waste incineration by gas chromatography/mass spectrometry. Can. J. Chem. 62:2140-2145.

112. Williams, D.T.; LeBel, G.L.; Junkins, E. (1984) A comparison of organochlorine residues in human adipose tissue autopsy samples from two Ontario municipalities. J. Toxicol. Environ. Health 13:19-29.

113. Wong, P.T.S.; Chau, Y.K.; Rhamey, J.S.; Docker, M. (1984) Relationship between water solubility of chlorobenzenes and their effects on a freshwater green alga. Chemosphere 13:991-996.

APPENDIX

ORGAN WEIGHT, HEMATOLOGIC, SERUM CHEMISTRY, URINALYSIS, AND REPRODUCTIVE SYSTEM DATA FOR RATS AND MICE IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE

TABLE A1. ORGAN WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Organ	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Body weight (grams)	347 ± 8.3	340 ± 4.2	*296 ± 16.2	333 ± 5.8	**299 ± 6.5	**283 ± 4.9
Brain						
Absolute	1,995 ± 26	1,983 ± 19	1,978 ± 15	*1,913 ± 28	**1,871 ± 31	**1,883 ± 27
Relative	5.8 ± 0.13	5.8 ± 0.07	6.9 ± 0.41	5.8 ± 0.12	*6.3 ± 0.12	**6.7 ± 0.13
Heart						
Absolute	1,168 ± 24	*1,084 ± 25	**1,001 ± 39	**1,072 ± 30	*1,060 ± 35	**1,043 ± 20
Relative	3.4 ± 0.05	3.2 ± 0.06	3.4 ± 0.08	3.2 ± 0.07	3.5 ± 0.06	**3.7 ± 0.04
Right kidney						
Absolute	1,312 ± 39	1,268 ± 18	1,263 ± 27	**1,608 ± 39	**1,970 ± 68	**1,849 ± 74
Relative	3.8 ± 0.07	3.7 ± 0.04	**4.3 ± 0.15	**4.8 ± 0.07	**6.6 ± 0.15	**6.5 ± 0.16
Liver						
Absolute	12,660 ± 380	12,670 ± 180	10,590 ± 1,060	*14,270 ± 370	**17,230 ± 530	**19,170 ± 520
Relative	36.4 ± 0.52	37.2 ± 0.27	35.0 ± 1.75	**42.9 ± 0.68	**57.6 ± 1.20	**67.6 ± 1.17
Lung						
Absolute	1,858 ± 101	1,798 ± 58	1,699 ± 65	1,886 ± 111	1,613 ± 72	*1,547 ± 70
Relative	5.3 ± 0.22	5.3 ± 0.18	5.8 ± 0.25	5.7 ± 0.36	5.4 ± 0.32	5.5 ± 0.26
Right testis						
Absolute	1,535 ± 32	1,480 ± 36	1,493 ± 21	1,524 ± 24	1,543 ± 23	1,472 ± 15
Relative	4.4 ± 0.10	4.4 ± 0.09	*5.1 ± 0.22	4.6 ± 0.09	**5.2 ± 0.11	**5.2 ± 0.10
Seminal vesicles						
Absolute	583 ± 72	612 ± 61	408 ± 33	564 ± 57	563 ± 60	507 ± 42
Relative	1.7 ± 0.21	1.8 ± 0.17	1.4 ± 0.08	1.7 ± 0.17	1.9 ± 0.18	1.8 ± 0.14
Thymus						
Absolute	353 ± 22	341 ± 16	*248 ± 30	**261 ± 8	288 ± 23	324 ± 15
Relative	1.01 ± 0.047	1.00 ± 0.036	0.82 ± 0.063	*0.79 ± 0.028	0.96 ± 0.068	1.15 ± 0.058
FEMALE						
Body weight (grams)	201 ± 2.9	191 ± 4.4	207 ± 2.9	199 ± 3.3	*188 ± 3.0	**173 ± 2.7
Brain						
Absolute	1,835 ± 20	1,798 ± 23	1,819 ± 34	1,815 ± 28	1,806 ± 24	**1,735 ± 26
Relative	9.2 ± 0.16	9.4 ± 0.17	8.8 ± 0.21	9.1 ± 0.13	9.6 ± 0.18	**10.0 ± 0.12
Heart						
Absolute	739 ± 18	696 ± 16	736 ± 18	742 ± 30	717 ± 12	714 ± 20
Relative	3.7 ± 0.08	3.6 ± 0.08	3.6 ± 0.13	3.7 ± 0.13	3.8 ± 0.07	**4.1 ± 0.08
Right kidney						
Absolute	776 ± 18	734 ± 20	821 ± 16	*844 ± 15	*838 ± 24	*871 ± 25
Relative	3.9 ± 0.07	3.8 ± 0.06	4.0 ± 0.08	**4.2 ± 0.06	**4.5 ± 0.08	**5.0 ± 0.12
Liver						
Absolute	6,445 ± 137	6,610 ± 239	**7,304 ± 151	**7,515 ± 164	**9,512 ± 215	**11,908 ± 312
Relative	32.1 ± 0.43	*34.5 ± 0.84	**35.3 ± 0.63	**37.7 ± 0.50	**50.6 ± 0.83	**68.9 ± 1.55
Lung						
Absolute	1,279 ± 54	1,467 ± 86	1,287 ± 46	1,421 ± 77	1,329 ± 61	**1,011 ± 36
Relative	6.4 ± 0.29	7.7 ± 0.38	6.2 ± 0.18	7.1 ± 0.34	7.0 ± 0.25	5.9 ± 0.22
Thymus						
Absolute	294 ± 16	276 ± 12	301 ± 15	309 ± 14	291 ± 14	251 ± 17
Relative	1.5 ± 0.08	1.4 ± 0.06	1.5 ± 0.08	1.6 ± 0.06	1.6 ± 0.08	1.5 ± 0.09

(a) Data for animals not bled sequentially during the studies; mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*$P<0.05$
**$P<0.01$

TABLE A2. HEMATOLOGIC DATA FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Analysis	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Number examined (b)	7	8	5	6	8	8
Leukocytes (10^3/µl)	6.8 ± 0.25	7.8 ± 0.33	7.0 ± 0.20	6.1 ± 0.24	7.0 ± 0.20	7.3 ± 0.30
Lymphocytes (10^3/µl)	5.4 ± 0.26	6.2 ± 0.30	5.7 ± 0.13	4.8 ± 0.27	5.5 ± 0.12	6.1 ± 0.30
Segmented neutrophils (10^3/µl)	1.4 ± 0.10	1.5 ± 0.28	1.3 ± 0.10	1.2 ± 0.16	1.4 ± 0.10	1.1 ± 0.11
Monocytes (10^3/µl)	0.07 ± 0.042	0.08 ± 0.049	0.04 ± 0.024	0.05 ± 0.022	0.05 ± 0.038	0.06 ± 0.026
Eosinophils (10^3/µl)	0.07 ± 0.029	0.14 ± 0.026	0.06 ± 0.024	0.08 ± 0.017	0.08 ± 0.025	0.08 ± 0.025
Hematocrit (percent)	45.2 ± 0.62	45.7 ± 0.45	43.8 ± 0.26	44.1 ± 0.79	**40.6 ± 0.47	**39.4 ± 0.58
Hemoglobin (g/dl)	16.8 ± 0.18	17.2 ± 0.18	16.5 ± 0.06	16.6 ± 0.24	**15.1 ± 0.22	**14.8 ± 0.22
Mean corpuscular hemoglobin (pg)	35.8 ± 0.43	36.8 ± 0.42	37.1 ± 0.27	36.3 ± 0.47	35.8 ± 0.24	35.3 ± 0.30
Mean corpuscular hemoglobin concentration (g/dl)	37.2 ± 0.32	37.8 ± 0.45	37.5 ± 0.14	37.6 ± 0.53	37.2 ± 0.28	37.6 ± 0.30
Mean cell volume (µ^3)	48.3 ± 0.42	48.9 ± 0.35	49.2 ± 0.37	48.2 ± 0.48	48.1 ± 0.44	47.0 ± 0.38
Platelets (10^3/µl)	(c) 623 ± 9.6	(c) 648 ± 16.9	(c) 655 ± 12.5	(c) 634 ± 13.3	**(c) 739 ± 14.5	**(c) 774 ± 6.6
Erythrocytes (10^6/µl)	9.4 ± 0.19	9.4 ± 0.08	*8.9 ± 0.07	9.2 ± 0.17	**8.5 ± 0.14	**8.4 ± 0.10
Reticulocytes (10^6/µl)	0.19 ± 0.024	0.18 ± 0.011	0.20 ± 0.014	0.18 ± 0.011	(d) 0.15 ± 0.006	0.15 ± 0.005
FEMALE						
Number examined (b)	5	9	6	8	7	7
Leukocytes (10^3/µl)	6.2 ± 0.51	5.7 ± 0.24	6.2 ± 0.44	5.1 ± 0.31	5.7 ± 0.53	5.8 ± 0.46
Lymphocytes (10^3/µl)	5.0 ± 0.37	4.5 ± 0.19	4.9 ± 0.19	4.3 ± 0.23	4.7 ± 0.40	5.0 ± 0.49
Segmented neutrophils (10^3/µl)	1.1 ± 0.19	1.1 ± 0.10	(e) 0.8 ± 0.13	0.8 ± 0.09	0.9 ± 0.22	0.8 ± 0.11
Monocytes (10^3/µl)	0.06 ± 0.024	0.08 ± 0.036	0.08 ± 0.048	0.05 ± 0.027	0.04 ± 0.020	0.00 ± 0.000
Eosinophils (10^3/µl)	0.04 ± 0.024	0.04 ± 0.024	0.05 ± 0.022	0.05 ± 0.019	0.04 ± 0.030	0.06 ± 0.043
Hematocrit (percent)	43.8 ± 1.50	45.0 ± 0.40	44.4 ± 0.33	45.4 ± 0.76	46.3 ± 0.95	43.7 ± 0.43
Hemoglobin (g/dl)	17.3 ± 0.26	16.8 ± 0.17	16.9 ± 0.11	17.1 ± 0.14	17.3 ± 0.44	16.4 ± 0.17
Mean corpuscular hemoglobin (pg)	41.5 ± 1.19	39.3 ± 0.30	40.0 ± 0.21	39.4 ± 0.46	**37.8 ± 0.23	**36.7 ± 0.36
Mean corpuscular hemoglobin concentration (g/dl)	39.6 ± 1.06	*37.4 ± 0.19	38.2 ± 0.20	37.7 ± 0.39	*37.3 ± 0.33	*37.6 ± 0.20
Mean cell volume (µ^3)	52.4 ± 0.24	52.4 ± 0.29	52.2 ± 0.31	52.1 ± 0.23	**50.6 ± 0.30	**49.0 ± 0.44
Platelets (10^3/µl)	(c) 656 ± 16.1	(c) 654 ± 12.7	(c) 646 ± 7.9	(c) 617 ± 10.5	(c) 688 ± 54.6	(c) 656 ± 12.8
Erythrocytes (10^6/µl)	8.4 ± 0.29	8.7 ± 0.13	8.5 ± 0.05	8.7 ± 0.15	9.1 ± 0.20	9.0 ± 0.14
Reticulocytes (10^6/µl)	0.17 ± 0.008	0.16 ± 0.007	(e) 0.15 ± 0.005	0.17 ± 0.010	0.15 ± 0.006	*0.14 ± 0.006

(a) Mean ± standard error; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977); results for animals bled on d 88 or 89. These animals had also been bled sequentially during the studies.
(b) Unless otherwise specified
(c) Ten animals were examined.
(d) Seven animals were examined.
(e) Five animals were examined.
*P<0.05
**P<0.01

TABLE A3. SERUM CHEMISTRY AND URINALYSIS DATA FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Analysis/ Day of Study	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Albumin (g/dl)						
3/4	4 4 ± 0 05	4 5 ± 0 07	4 5 ± 0 06	4 5 ± 0 06	4 5 ± 0 12	4 5 ± 0 07
17/18	4 4 ± 0 05	4 4 ± 0 06	4 4 ± 0 03	(b) 4 4 ± 0 03	**4 7 ± 0 07	**5 0 ± 0 07
45/46	4 3 ± 0 05	4 4 ± 0 09	**4 6 ± 0 05	**4 7 ± 0 07	**4 9 ± 0 07	**5 0 ± 0 17
88/89	4 3 ± 0 04	4 4 ± 0 03	*4 5 ± 0 04	**4 5 ± 0 07	**4 9 ± 0 05	**5 0 ± 0 09
Alanine aminotransferase (IU/liter)						
3/4	32 6 ± 0 72	*37 8 ± 1 67	35 0 ± 1 84	(c) 35 1 ± 1 50	36 5 ± 1 69	35 4 ± 1 67
17/18	31 9 ± 0 75	29 6 ± 1 65	28 7 ± 0 63	(b) 29 6 ± 1 78	29 7 ± 1 97	38 6 ± 2 08
45/46	51 0 ± 2 61	49 7 ± 2 45	**42 4 ± 1 23	**37 5 ± 1 26	**33 4 ± 1 45	**47 8 ± 2 19
88/89	49 5 ± 4 62	46.8 ± 2 53	43.8 ± 2 46	38 6 ± 1 63	41 8 ± 2.14	48.2 ± 1 01
Creatine phosphokinase (IU/liter)						
3/4	329 ± 58 5	272 ± 20 3	258 ± 30.2	295 ± 36 3	(c) 288 ± 28 8	264 ± 14 4
17/18	(c) 177 ± 14 7	196 ± 23 0	178 ± 29 9	(b) 151 ± 21 3	188 ± 19 8	226 ± 23 0
45/46	153 ± 20 7	125 ± 18 4	111 ± 7 3	120 ± 28 3	129 ± 23 5	137 ± 12 6
88/89	175 ± 25 6	293 ± 42 7	199 ± 27 4	278 ± 71 4	226 ± 33 9	*276 ± 12 2
Creatinine (mg/dl)						
3/4	0 59 ± 0 013	0 58 ± 0 019	0 61 ± 0 019	0 59 ± 0 016	0 62 ± 0 020	0 63 ± 0 013
17/18	0 63 ± 0 032	0 60 ± 0 055	0 69 ± 0 039	(b) 0 66 ± 0 021	*0 72 ± 0 019	0 71 ± 0 021
45/46	0 63 ± 0 061	0 64 ± 0 019	0 67 ± 0 017	0 62 ± 0 047	0 60 ± 0 038	0 64 ± 0 018
88/89	0 63 ± 0 007	0 66 ± 0 011	**0 69 ± 0 010	**0 71 ± 0 016	**0 80 ± 0 010	**0 77 ± 0 015
gamma-Glutamyl transferase (IU/liter)						
3/4	3 8 ± 0 87	4 8 ± 0 57	2 8 ± 0 88	4 9 ± 0 23	2 3 ± 0 78	3 4 ± 0 78
45/46	0 20 ± 0 200	0 20 ± 0 200	0 80 ± 0 327	0 10 ± 0 100	0 00 ± 0 000	0 70 ± 0 260
Sorbitol dehydrogenase (IU/liter)						
3/4	4 3 ± 0 75	5 3 ± 1 01	3 8 ± 1 00	3 9 ± 0 92	4 9 ± 1 16	4 2 ± 0 68
17/18	7 8 ± 0 68	8 5 ± 1 13	6 1 ± 0 35	(b) 9 9 ± 0 73	*11 0 ± 0 97	**13 8 ± 0 70
45/46	9 7 ± 1 36	7 9 ± 1 21	10 3 ± 1.65	7 1 ± 0 62	10 1 ± 1 10	12 1 ± 1 66
88/89	10 0 ± 1 28	8 5 ± 0 50	8 5 ± 0 48	8.3 ± 0 58	12 4 ± 1 52	**13 0 ± 0 45
Triiodothyronine (ng/dl)						
17/18	96 4 ± 4 04	91 2 ± 6 05	95 9 ± 4 60	84 9 ± 7 81	86 5 ± 5 77	81 5 ± 5 88
45/46	95 6 ± 6 28	81 0 ± 4 62	88 0 ± 4 74	82 7 ± 2 44	99 3 ± 8 52	73 4 ± 5 56
88/89	67 4 ± 5 09	70 0 ± 8 62	67 8 ± 10 61	73 1 ± 8 02	85 0 ± 7 03	80 3 ± 5 78
Free thyroxin (ng/dl)						
3/4	2 1 ± 0 11	2 0 ± 0 16	1 9 ± 0 09	**(c) 1 3 ± 0 15	**0 4 ± 0 04	**0 3 ± 0 02
45/46	2 0 ± 0 19	1 8 ± 0 15	2 2 ± 0 20	**1 3 ± 0 13	**0 6 ± 0 07	**0 2 ± 0 02
88/89	1 7 ± 0 13	1 5 ± 0 11	1 5 ± 0 14	**1 0 ± 0 12	**0 5 ± 0 04	**0 2 ± 0 03
Total thyroxin (micrograms/dl)						
17/18	5 1 ± 0 42	5 1 ± 0 49	4 9 ± 0 38	**3 0 ± 0 16	**1 6 ± 0 13	**1 2 ± 0 11
45/46	5 1 ± 0 29	4 6 ± 0 33	5 1 ± 0 25	**3 4 ± 0 19	**2 0 ± 0 15	**1 3 ± 0 08
88/89	4 2 ± 0 16	4 1 ± 0 39	4 1 ± 0 37	3 3 ± 0 32	**1 9 ± 0 14	**1 3 ± 0 05
Urinary porphyrin (nmol/16 h)						
86/87	(d) 1 44 ± 0 153	(c) 1 66 ± 0 136	(c) 1 36 ± 0 061	1 70 ± 0 078	*4 54 ± 0 347	*5 20 ± 0 478
Urine specific gravity						
15/16	(e) 1 025 ± 0 004	(f) 1 025 ± 0 005	(d) 1 015 ± 0 001	(g) 1 022 ± 0 001	(e) 1 031 ± 0 002	(e) 1 036 ± 0 002
43/44	1 029 ± 0 003	1 024 ± 0 002	1 022 ± 0 001	1 025 ± 0 002	1 030 ± 0 002	*1 036 ± 0 001
86/87	(c) 1 037 ± 0 004	(c) 1 030 ± 0 003	1 028 ± 0 006	1 029 ± 0 001	*1 023 ± 0 002	(d) 1 030 ± 0 002
Urinary alkaline phosphatase (IU/liter)						
15/16	(e) 176 ± 34 8	(f) 244 ± 54 9	(d) 352 ± 175 6	(g) 203 ± 1 0	(e) 216 ± 31 7	(e) 195 ± 17 4
43/44	177 ± 13 1	160 ± 13 0	156 ± 12 8	156 ± 15 2	181 ± 16 9	192 ± 24 5
86/87	(c) 169 ± 12 2	(c) 168 ± 16 2	194 ± 33 9	159 ± 9 4	*114 ± 9 5	(d) 179 ± 25 9
Urinary aspartate aminotransferase (IU/liter)						
15/16	(e) 6 9 ± 1 57	*(f) 14 4 ± 2 58	**(d) 37 9 ± 5 68	**(g) 66 0 ± 1 00	**(e) 103 9 ± 7 17	**(e) 82 3 ± 13 87
43/44	14 6 ± 1 71	*21 6 ± 1 73	**35 6 ± 3 11	**72 4 ± 7 61	**85 9 ± 8 05	**82 4 ± 10 91
86/87	(c) 17 0 ± 1 45	*(c) 24 0 ± 2 78	**35 5 ± 7 14	**50 9 ± 3 81	**41 9 ± 3 27	**(d) 59 8 ± 6 14
Urinary glucose (mg/dl)						
15/16	(e) 13 6 ± 3 02	(f) 14.3 ± 3 91	(d) 5 3 ± 1 37	(g) 8.7 ± 7 30	(e) 25 0 ± 4 68	*(e) 25 8 ± 3 37
43/44	14 0 ± 2 10	10 5 ± 2 16	17 6 ± 1 27	**28 6 ± 2 46	**24 5 ± 3 87	**28 4 ± 2 01
86/87	(c) 18 7 ± 2 54	(c) 15 8 ± 2 87	24 3 ± 5 10	**41 5 ± 3 44	**30 5 ± 2 99	**(d) 38 3 ± 4 46
Urinary protein (mg/dl)						
15/16	(e) 137 ± 26 3	(f) 131 ± 33 7	(d) 100 ± 15 9	(g) 118 ± 22 0	*(h) 253 ± 26 4	*(e) 274 ± 48 6
43/44	160 ± 31 7	151 ± 34 3	121 ± 22 6	197 ± 26 7	*294 ± 38 4	*314 ± 43 1
86/87	(c) 107 ± 6 3	(c) 128 ± 26 7	139 ± 32 4	118 ± 3 7	177 ± 28 1	*(d) 217 ± 29 6
Urine volume (ml/16 h)						
15/16	(h) 5 9 ± 0 91	(f) 7 6 ± 1 36	(d) 10 5 ± 1 35	(g) 6 4 ± 1 70	(e) 5 8 ± 0 64	(e) 5 4 ± 0 56
43/44	7 7 ± 1 07	10 4 ± 1 50	10 6 ± 0 63	8 0 ± 0 44	(c) 6 2 ± 0 44	*4 9 ± 0 41
86/87	(c) 6 2 ± 1 30	(c) 6 9 ± 1.22	9 0 ± 1 61	6 9 ± 0 40	**10 3 ± 0 90	(d) 7 4 ± 1 15

TABLE A3. SERUM CHEMISTRY AND URINALYSIS DATA FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (Continued)

Analysis/ Day of Study	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
FEMALE						
Albumin (g/dl)						
3/4	4.5 ± 0.08	4.6 ± 0.03	4.6 ± 0.07	4.7 ± 0.06	4.5 ± 0.05	4.5 ± 0.07
17/18	4.5 ± 0.07	4.5 ± 0.08	(c) 4.4 ± 0.07	4.4 ± 0.05	4.6 ± 0.06	**5.1 ± 0.08
45/46	4.5 ± 0.06	4.4 ± 0.05	4.4 ± 0.11	4.5 ± 0.11	4.8 ± 0.09	**5.5 ± 0.10
88/89	4.6 ± 0.07	4.6 ± 0.09	4.6 ± 0.04	4.7 ± 0.05	*4.8 ± 0.17	**5.3 ± 0.06
Alanine aminotransferase (IU/liter)						
3/4	31.8 ± 1.25	32.9 ± 1.52	31.5 ± 0.72	32.2 ± 1.67	30.0 ± 1.64	32.5 ± 1.64
17/18	26.4 ± 0.76	26.7 ± 0.92	(c) 26.8 ± 0.88	25.0 ± 0.58	29.9 ± 2.38	*32.7 ± 2.44
45/46	32.0 ± 1.19	34.6 ± 1.23	33.3 ± 1.03	34.0 ± 0.63	34.5 ± 0.98	34.6 ± 1.84
88/89	36.7 ± 3.23	37.7 ± 2.31	35.6 ± 2.11	35.2 ± 1.97	(c) 29.0 ± 1.20	38.6 ± 1.61
Creatine phosphokinase (IU/liter)						
3/4	301 ± 34.3	354 ± 33.2	373 ± 41.3	274 ± 32.9	281 ± 35.6	360 ± 45.4
17/18	(c) 254 ± 49.5	225 ± 42.6	(c) 246 ± 50.4	189 ± 28.0	172 ± 22.6	196 ± 33.1
45/46	144 ± 17.3	122 ± 9.5	110 ± 13.1	144 ± 16.6	191 ± 52.5	169 ± 29.4
88/89	148 ± 12.9	194 ± 26.1	213 ± 32.9	236 ± 32.8	184 ± 24.7	188 ± 33.7
Creatinine (mg/dl)						
3/4	0.55 ± 0.013	0.60 ± 0.016	0.57 ± 0.012	0.59 ± 0.014	0.60 ± 0.017	0.58 ± 0.017
17/18	0.56 ± 0.017	0.60 ± 0.023	(c) 0.60 ± 0.023	0.57 ± 0.021	0.57 ± 0.014	0.55 ± 0.016
45/46	0.57 ± 0.029	0.56 ± 0.021	0.58 ± 0.018	0.54 ± 0.016	0.54 ± 0.011	*0.50 ± 0.029
88/89	0.62 ± 0.031	0.61 ± 0.006	0.68 ± 0.043	0.61 ± 0.015	0.62 ± 0.026	0.58 ± 0.005
gamma-Glutamyl transferase (IU/liter)						
3/4	4.5 ± 0.75	4.3 ± 0.60	4.0 ± 0.63	3.9 ± 0.86	4.3 ± 0.63	4.3 ± 0.79
45/46	0.60 ± 0.306	0.10 ± 0.100	0.60 ± 0.427	0.40 ± 0.267	0.30 ± 0.300	1.90 ± 0.482
Sorbitol dehydrogenase (IU/liter)						
3/4	5.5 ± 0.70	6.0 ± 0.63	4.1 ± 0.62	5.5 ± 0.65	4.8 ± 1.03	5.2 ± 0.73
17/18	6.9 ± 0.69	7.3 ± 0.47	(c) 7.1 ± 0.39	7.2 ± 0.42	7.9 ± 0.64	7.7 ± 0.58
45/46	7.9 ± 1.22	8.2 ± 0.47	9.3 ± 1.01	8.8 ± 1.07	7.4 ± 0.67	10.6 ± 1.98
88/89	5.9 ± 0.48	**8.6 ± 0.50	**7.9 ± 0.67	**8.1 ± 0.71	*7.1 ± 0.61	**8.2 ± 0.36
Triiodothyronine (ng/dl)						
17/18	95.2 ± 4.06	104.5 ± 6.93	93.6 ± 5.74	91.5 ± 6.08	82.7 ± 7.35	82.1 ± 7.00
45/46	(c) 98.7 ± 4.87	*75.8 ± 3.51	87.5 ± 4.54	92.9 ± 5.61	93.3 ± 7.72	91.5 ± 7.26
88/89	76.1 ± 6.56	68.7 ± 4.39	68.7 ± 5.39	66.7 ± 6.29	78.4 ± 6.79	82.7 ± 9.20
Free thyroxin (ng/dl)						
3/4	2.0 ± 0.10	*1.7 ± 0.09	*1.6 ± 0.09	**1.1 ± 0.08	**0.4 ± 0.03	**0.2 ± 0.02
45/46	1.6 ± 0.14	**0.9 ± 0.10	*1.3 ± 0.18	**1.0 ± 0.07	**0.4 ± 0.04	**(c) 0.2 ± 0.03
88/89	1.0 ± 0.08	1.0 ± 0.11	0.8 ± 0.09	**0.6 ± 0.10	**0.3 ± 0.03	**0.2 ± 0.03
Total thyroxin (micrograms/dl)						
17/18	4.7 ± 0.32	3.8 ± 0.25	3.8 ± 0.31	**2.5 ± 0.22	**1.4 ± 0.08	**1.2 ± 0.08
45/46	4.6 ± 0.24	**2.8 ± 0.21	**3.8 ± 0.32	**3.2 ± 0.17	**1.6 ± 0.06	**1.2 ± 0.06
88/89	2.8 ± 0.21	2.6 ± 0.24	2.6 ± 0.11	**1.8 ± 0.19	**1.4 ± 0.06	**1.2 ± 0.08
Urinary porphyrin (nmol/16 h)						
86/87	(d) 0.90 ± 0.100	1 13 ± 0.149	(d) 1.50 ± 0.183	0.97 ± 0.059	1.69 ± 0.258	*(c) 2.23 ± 0.718
Urine specific gravity						
15/16	(e) 1.014 ± 0.001	(d) 1.017 ± 0.001	(h) 1.013 ± 0.002	(e) 1.019 ± 0.002	**(e) 1.027 ± 0.002	**(e) 1.035 ± 0.004
43/44	1.021 ± 0.002	1.019 ± 0.002	1.015 ± 0.002	(c) 1.020 ± 0.003	1.028 ± 0.002	**1.035 ± 0.003
86/87	1.022 ± 0.002	(c) 1.021 ± 0.002	(c) 1.017 ± 0.002	(c) 1.028 ± 0.003	*(c) 1.031 ± 0.004	**(c) 1.032 ± 0.002
Urinary alkaline phosphatase (IU/liter)						
15/16	(h) 64.5 ± 12.09	(d) 69.1 ± 7.38	(h) 82.5 ± 27.98	(h) 104.2 ± 32.35	(e) 101.1 ± 17.14	*(e) 121.6 ± 11.33
43/44	69.8 ± 9.18	76.1 ± 9.97	43.9 ± 9.09	(c) 66.4 ± 8.83	*110.1 ± 12.64	*104.3 ± 10.28
86/87	72.5 ± 10.22	(c) 71.3 ± 6.66	(c) 112.0 ± 35.86	(c) 115.8 ± 15.49	**(c) 128.3 ± 14.46	*(d) 115.1 ± 10.98
Urinary aspartate aminotransferase (IU/liter)						
15/16	(h) 5.8 ± 2.20	(d) 6.6 ± 1.67	(h) 6.8 ± 1.64	(e) 9.6 ± 3.64	(e) 4.0 ± 1.53	(e) 6.3 ± 2.46
43/44	4.2 ± 1.79	0.9 ± 0.90	0.7 ± 0.70	(c) 1.7 ± 1.67	0.0 ± 0.00	2.4 ± 1.25
86/87	3.5 ± 1.27	(c) 6.0 ± 1.68	(d) 2.8 ± 1.37	(c) 1.3 ± 0.88	(c) 1.1 ± 1.11	(d) 3.0 ± 1.48
Urinary glucose (mg/dl)						
15/16	(e) 2.8 ± 0.65	**(d) 7.9 ± 0.76	(h) 5.1 ± 1.86	**(e) 9.5 ± 1.31	**(e) 10.6 ± 1.31	**(e) 13.1 ± 2.74
43/44	5.2 ± 0.97	6.9 ± 1.19	4.4 ± 0.98	(c) 8.1 ± 1.21	**10.0 ± 1.10	*9 5 ± 1.58
86/87	7.4 ± 1.58	(c) 8.3 ± 1.09	(c) 7.2 ± 1.06	*(c) 11.8 ± 1.40	*(c) 11.1 ± 1.56	*(c) 11.4 ± 1.62
Urinary protein (mg/dl)						
15/16	(e) 17.0 ± 1.38	(d) 16.4 ± 2.03	(h) 15.5 ± 1.96	(e) 21.1 ± 3.26	(e) 23.3 ± 2.81	*(e) 31.3 ± 5.48
43/44	12.1 ± 2.77	13.1 ± 1.12	10.5 ± 1.98	(c) 13.6 ± 3.02	*18.5 ± 1.85	**32.6 ± 3.99
86/87	15.3 ± 2.56	(c) 16.6 ± 3.44	(c) 12.0 ± 1.71	(c) 22.6 ± 2.68	*(c) 26.3 ± 3.82	**(c) 154.8 ± 60.91
Urine volume (ml/16 h)						
15/16	(e) 8.5 ± 0.79	*(d) 6.3 ± 0.61	(f) 8.4 ± 1.78	(e) 6.5 ± 0.90	**(e) 4.3 ± 0 48	**(e) 4.1 ± 0.37
43/44	7.4 ± 1.28	9.0 ± 1.35	10.1 ± 1.61	(c) 7.5 ± 1.30	4.9 ± 0 76	*4.3 ± 0.37
86/87	5.8 ± 0.76	(c) 4.8 ± 0.56	(d) 6.9 ± 0.66	(c) 3.9 ± 0.46	*(c) 3.7 ± 0.47	(c) 4.1 ± 0.27

(a) Data for animals bled sequentially on d 3 or 4, 17 or 18, 45 or 46, and 88 or 89; mean ± standard error for groups of 10 animals unless otherwise specified; P values vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977). IU = international units.
(b) N = 11
(c) Nine animals were examined.
(d) Eight animals were examined.
(e) Seven animals were examined.
(f) Five animals were examined.
(g) Two animals were examined.
(h) Six animals were examined.
*P<0.05
**P<0.01

TABLE A4. REPRODUCTIVE SYSTEM DATA FOR MALE RATS IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

	Control	30 ppm	300 ppm	2,000 ppm
Abnormal sperm (percent)	0.74 ± 0.119	0.83 ± 0.133	0.76 ± 0.078	0.94 ± 0.133
Caudal weight (mg)	201 ± 4	197 ± 4	*185 ± 4	**153 ± 5
Right epididymal weight (mg)	453 ± 5	442 ± 14	**421 ± 5	**380 ± 8
Sperm density (10^6/g cauda)	451 ± 20	455 ± 23	458 ± 19	477 ± 11
Sperm motility (percent) (b)	74.8 ± 0.58	72.9 ± 0.71	*72.2 ± 0.93	**70.1 ± 0.46

(a) Mean ± standard error for groups of 10 animals; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977); these animals were not bled sequentially.
(b) N = 20
*P<0.05
**P<0.01

TABLE A5. ORGAN WEIGHTS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Organ	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Number weighed (b)	10	10	10	10	10	10
Body weight (grams)	32.6 ± 0.96	30.8 ± 0.59	33.1 ± 0.71	31.2 ± 0.66	31.9 ± 0.76	31.3 ± 0.74
Brain						
Absolute	465 ± 5	459 ± 6	456 ± 4	465 ± 7	459 ± 9	455 ± 5
Relative	14.3 ± 0.36	15.0 ± 0.30	13.8 ± 0.27	15.0 ± 0.47	14.5 ± 0.44	14.6 ± 0.28
Heart						
Absolute	159 ± 5	155 ± 4	164 ± 5	152 ± 3	158 ± 5	149 ± 5
Relative	4.9 ± 0.14	5.0 ± 0.12	5.0 ± 0.17	4.9 ± 0.04	5.0 ± 0.15	4.7 ± 0.10
Right kidney						
Absolute	290 ± 12	291 ± 9	293 ± 5	293 ± 7	308 ± 12	300 ± 14
Relative	8.9 ± 0.21	9.4 ± 0.20	8.9 ± 0.17	9.4 ± 0.20	9.7 ± 0.41	9.5 ± 0.27
Liver						
Absolute	1,373 ± 58	1,367 ± 44	*1,546 ± 42	1,458 ± 60	**2,022 ± 67	**3,700 ± 80
Relative	42.1 ± 1.31	44.4 ± 1.03	*46.8 ± 1.19	*46.6 ± 1.04	**63.3 ± 1.35	**118.4 ± 2.86
Lung						
Absolute	181 ± 5	198 ± 8	199 ± 9	(c) 186 ± 6	(c) 206 ± 12	173 ± 5
Relative	5.6 ± 0.22	*6.4 ± 0.23	6.0 ± 0.25	(c) 5.9 ± 0.20	(c) 6.4 ± 0.27	5.5 ± 0.10
Right testis						
Absolute	118 ± 2	118 ± 2	127 ± 2	116 ± 3	119 ± 4	121 ± 2
Relative	3.6 ± 0.10	3.8 ± 0.05	3.8 ± 0.04	3.7 ± 0.07	3.7 ± 0.13	3.9 ± 0.06
Seminal vesicle						
Absolute	161 ± 8	143 ± 9	151 ± 10	153 ± 10	154 ± 6	120 ± 11
Relative	5.0 ± 0.27	4.7 ± 0.33	4.5 ± 0.22	4.9 ± 0.24	4.8 ± 0.18	*3.8 ± 0.37
Thymus						
Absolute	45.3 ± 2.41	39.9 ± 2.21	46.5 ± 2.26	45.1 ± 2.48	44.4 ± 2.48	47.2 ± 3.35
Relative	1.4 ± 0.07	1.3 ± 0.07	1.4 ± 0.07	1.5 ± 0.08	1.4 ± 0.08	1.5 ± 0.12
FEMALE						
Number weighed (b)	10	10	10	10	10	8
Body weight (grams)	25.6 ± 0.53	26.4 ± 0.55	26.3 ± 0.78	26.7 ± 0.67	*27.7 ± 0.69	26.5 ± 0.29
Brain						
Absolute	478 ± 8	482 ± 3	481 ± 4	472 ± 5	470 ± 6	**432 ± 7
Relative	18.7 ± 0.47	18.3 ± 0.32	18.4 ± 0.52	17.8 ± 0.36	**17.1 ± 0.35	**16.3 ± 0.29
Heart						
Absolute	135 ± 5	133 ± 2	144 ± 5	135 ± 5	142 ± 5	126 ± 3
Relative	5.3 ± 0.23	5.0 ± 0.13	5.5 ± 0.14	5.0 ± 0.11	5.1 ± 0.15	*4.7 ± 0.10
Right kidney						
Absolute	190 ± 4	199 ± 3	*206 ± 7	196 ± 7	*208 ± 5	*214 ± 9
Relative	7.4 ± 0.16	7.5 ± 0.12	7.8 ± 0.17	7.4 ± 0.21	7.5 ± 0.13	8.1 ± 0.31
Liver						
Absolute	1,183 ± 38	*1,306 ± 36	1,273 ± 52	1,312 ± 63	**2,086 ± 73	**4,171 ± 234
Relative	46.3 ± 1.41	49.4 ± 0.81	48.4 ± 1.43	49.1 ± 1.58	**75.4 ± 1.64	**156.9 ± 7.56
Lung						
Absolute	(c) 194 ± 11	187 ± 5	193 ± 9	186 ± 5	207 ± 10	(d) 156 ± 10
Relative	(c) 7.6 ± 0.45	7.1 ± 0.14	7.3 ± 0.19	7.0 ± 0.20	7.5 ± 0.29	**(d) 5.9 ± 0.39
Thymus						
Absolute	48 ± 4	(e) 50 ± 3	52 ± 2	54 ± 3	(e) 54 ± 3	43 ± 3
Relative	1.9 ± 0.15	(e) 1.9 ± 0.10	2.0 ± 0.08	2.0 ± 0.14	(e) 2.0 ± 0.06	1.6 ± 0.13

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified); P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Unless otherwise specified
(c) The lungs of nine animals were weighed.
(d) The lungs of seven animals were weighed.
(e) Nine thymuses were weighed.
*$P<0.05$
**$P<0.01$

TABLE A6. HEMATOLOGIC, SERUM CHEMISTRY, AND REPRODUCTIVE SYSTEM DATA FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF 1,2,4,5-TETRACHLOROBENZENE (a)

Analysis	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	2,000 ppm
MALE						
Number weighed or examined (b)	10	10	10	10	10	10
Leukocytes (10^3/microliter)	7.7 ± 0.79	7.1 ± 0.76	7.1 ± 0.66	8.0 ± 0.89	7.8 ± 0.43	8.0 ± 0.58
Lymphocytes (10^3/microliter)	5.8 ± 0.63	5.5 ± 0.62	5.2 ± 0.48	6.2 ± 0.75	5.7 ± 0.37	6.2 ± 0.46
Segmented neutrophils (10^3/microliter)	1.8 ± 0.22	1.5 ± 0.17	1.8 ± 0.19	1.8 ± 0.21	2.1 ± 0.17	1.8 ± 0.17
Monocytes (10^3/microliter)	0.02 ± 0.013	0.04 ± 0.022	0.06 ± 0.029	0.04 ± 0.027	0.07 ± 0.021	0.04 ± 0.022
Hematocrit (percent)	45.0 ± 0.66	45.3 ± 0.82	46.0 ± 0.61	44.8 ± 0.77	45.2 ± 0.42	43.0 ± 0.64
Hemoglobin (g/dl) (c)	16.8 ± 0.17	16.9 ± 0.10	16.9 ± 0.17	16.8 ± 0.13	16.8 ± 0.13	**(d) 15.8 ± 0.24
Mean corpuscular hemoglobin (pg)	35.4 ± 0.41	35.0 ± 0.25	35.0 ± 0.24	35.6 ± 0.26	34.9 ± 0.23	**33.3 ± 0.35
Mean corpuscular hemoglobin concentration (g/dl)	37.8 ± 0.42	37.6 ± 0.55	37.5 ± 0.29	38.0 ± 0.28	37.7 ± 0.26	41.9 ± 3.72
Mean cell volume (microns^3)	46.9 ± 0.10	46.5 ± 0.64	46.5 ± 0.17	46.9 ± 0.18	46.3 ± 0.33	**43.6 ± 0.31
Platelets (10^3/microliter)	(d) 812 ± 23	775 ± 23	811 ± 18	839 ± 17	**960 ± 23	**1,161 ± 40
Erythrocytes (10^6/microliter)	9.6 ± 0.15	9.7 ± 0.12	9.8 ± 0.12	9.6 ± 0.14	9.8 ± 0.12	9.9 ± 0.16
Reticulocytes (10^6/microliter)	(e) 0.15 ± 0.016	(e) 0.17 ± 0.008	(f) 0.16 ± 0.010	(e) 0.16 ± 0.014	(g) 0.16 ± 0.009	(h) 0.15 ± 0.016
Albumin (g/dl)	3.2 ± 0.03	3.2 ± 0.07	3.2 ± 0.05	3.2 ± 0.05	3.2 ± 0.07	**3.6 ± 0.09
Alanine aminotransferase (IU/liter)	(d) 33.0 ± 3.04	(d) 38.8 ± 3.59	54.1 ± 12.59	(d) 27.4 ± 2.04	(d) 46.1 ± 6.80	**(d) 71.7 ± 9.56
Sorbitol dehydrogenase (IU/liter)	19.1 ± 1.51	21.0 ± 1.72	*(d) 25.1 ± 2.34	20.6 ± 1.00	**32.3 ± 3.55	**83.3 ± 11.83
Abnormal sperm (percent)	1.8 ± 0.19	1.7 ± 0.28	--	1.9 ± 0.16	--	1.3 ± 0.14
Sperm density (millions)	967 ± 73	768 ± 60	--	906 ± 37	--	836 ± 73
Sperm motility (percent)	74.6 ± 1.88	68.9 ± 2.85	--	74.0 ± 2.40	--	70.0 ± 2.44
Caudal weight (mg)	20 ± 1	18 ± 1	--	19 ± 1	--	18 ± 1
Right epididymal weight (mg)	46 ± 1	45 ± 1	--	44 ± 1	--	44 ± 2
FEMALE						
Number examined (b)	10	10	10	10	10	8
Leukocytes (10^3/microliter)	5.8 ± 0.53	6.1 ± 0.71	6.4 ± 0.65	7.2 ± 0.72	6.8 ± 0.57	5.9 ± 0.32
Lymphocytes (10^3/microliter)	4.7 ± 0.38	4.7 ± 0.54	5.2 ± 0.54	5.5 ± 0.57	5.4 ± 0.42	4.7 ± 0.29
Segmented neutrophils (10^3/microliter)	1.0 ± 0.18	1.4 ± 0.19	1.2 ± 0.18	1.5 ± 0.22	1.3 ± 0.20	1.1 ± 0.15
Monocytes (10^3/microliter)	0.01 ± 0.010	0.03 ± 0.015	0.02 ± 0.013	**0.09 ± 0.023	*0.05 ± 0.017	0.05 ± 0.027
Hematocrit (percent)	44.0 ± 0.75	44.5 ± 0.39	44.3 ± 0.87	44.4 ± 0.53	42.7 ± 0.51	**38.1 ± 1.11
Hemoglobin (g/dl)	16.6 ± 0.25	16.8 ± 0.13	16.6 ± 0.27	16.6 ± 0.13	16.5 ± 0.17	**15.0 ± 0.37
Mean corpuscular hemoglobin (pg)	35.2 ± 0.25	35.2 ± 0.21	34.8 ± 0.26	35.1 ± 0.35	35.0 ± 0.33	**31.8 ± 0.17
Mean corpuscular hemoglobin concentration (g/dl)	37.8 ± 0.22	37.5 ± 0.20	37.6 ± 0.30	37.5 ± 0.29	*38.7 ± 0.35	**39.5 ± 0.25
Mean cell volume (microns^3)	46.6 ± 0.16	46.9 ± 0.28	46.3 ± 0.42	46.9 ± 0.18	**45.4 ± 0.22	**40.4 ± 0.26
Platelets (10^3/microliter)	779 ± 19	783 ± 19	771 ± 11	781 ± 24	**895 ± 25	**1,282 ± 103
Erythrocytes (10^6/microliter)	9.4 ± 0.18	9.5 ± 0.09	9.6 ± 0.18	9.5 ± 0.12	9.4 ± 0.12	9.5 ± 0.25
Reticulocytes (10^6/microliter)	(f) 0.15 ± 0.006	(i) 0.17 ± 0.021	(h) 0.15 ± 0.011	(e) 0.16 ± 0.012	(g) 0.15 ± 0.005	(h) 0.16 ± 0.019
Albumin (g/dl)	3.1 ± 0.08	3.3 ± 0.04	3.3 ± 0.07	3.1 ± 0.05	3.2 ± 0.05	**4.0 ± 0.10
Alanine aminotransferase (IU/liter)	31.2 ± 5.02	24.2 ± 1.86	(d) 27.0 ± 3.10	33.0 ± 5.39	31.4 ± 3.85	**91.6 ± 19.04
Sorbitol dehydrogenase (IU/liter)	(d) 15.7 ± 0.50	**20.0 ± 1.58	(d) 16.9 ± 0.92	(d) 19.6 ± 2.02	**(d) 30.4 ± 1.12	**88.3 ± 7.86
Estrous cycle length (days) (j)	4.00 ± 0.00	3.89 ± 0.11	--	4.22 ± 0.15	-	**5.00 ± 0.26
Number clearly determined	(k) 9	(k) 9		(k) 9		(l) 6

(a) Mean ± standard error; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977) IU = international units.
(b) Unless otherwise specified
(c) N = 18 or 20
(d) Nine animals were examined.
(e) Five animals were examined.
(f) Six animals were examined.
(g) Seven animals were examined.
(h) Four animals were examined.
(i) Two animals were examined.
(j) Mean ± standard error in days for those animals in which the length of the estrous cycle could be clearly determined; groups of 10 animals were examined unless otherwise specified; P values vs. the controls by Dunnett's test (Dunnett, 1980).
(k) Estrous cycle longer than 7 days or unclear in 1/10 animals
(l) Estrous cycle longer than 7 days or unclear in 3/9 animals; 9 animals were examined.
*P<0.05
**P<0.01