TOXICITY STUDIES OF

PENTACHLOROBENZENE

(CAS NO. 608-93-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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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).

NTP REPORT ON THE

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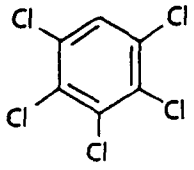

PENTACHLOROBENZENE

CAS No. 608-93-5

C_6HCl_5 Molecular weight 250.3

Synonyms: 1,2,3,4,5-Pentachlorobenzene; quintochlorobenzene

ABSTRACT

Toxicology studies were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice of each sex to pentachlorobenzene (99% pure) in feed for 15 days or 13 weeks.

Exposure concentrations were 0, 100, 330, 1,000, 3,300, or 10,000 ppm pentachlorobenzene in the 15-day studies (five animals of each sex per group per species). All rats that received 10,000 ppm and all mice that received 3,300 or 10,000 ppm died. Of the exposed rats that survived to the end of the studies, males had an accumulation of abnormal hyaline droplets in the renal cortical epithelium and males and females had centrilobular hepatocellular hypertrophy. Chemical-related lesions were not observed in exposed mice.

Exposure concentrations were 0, 33, 100, 330, 1,000, or 2,000 ppm pentachlorobenzene in the 13-week studies (10 animals of each sex per group per species). No compound-related deaths occurred. Body weights of exposed rats but not of mice were lower than those of controls. In male rats, dose-related histologic lesions included renal tubular epithelial hyaline droplet formation 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." Exacerbation of spontaneous nephropathy characterized by renal tubular cell regeneration and homogeneous intratubular protein casts was seen in rats of each sex. Urinary protein concentration was increased in male and female rats in the 1,000- and 2,000-ppm groups; this change was especially prominent in males. Urinary glucose concentration was increased in male rats in the 330- to 2,000-ppm groups and in female rats in the 1,000- and 2,000-ppm groups. Centrilobular hepatocellular hypertrophy was observed in exposed male and female rats. Unidentified yellow-brown pigment granules were present in hepatocytes and renal tubular epithelium in exposed animals of each sex but were more prominent in females. These granules possibly contained porphyrins. The only exposure-related histologic lesion in mice of either sex was centrilobular hepatocellular hypertrophy. Significant, but not dose-related, increases of liver porphyrin concentrations were observed in exposed male rats; female rats in the 2,000-ppm group also had increased liver porphyrin concentrations. Liver porphyrin concentrations were significantly increased in the 1,000- and 2,000-ppm groups of mice of each sex. Increased sorbitol dehydrogenase concentrations in exposed rats and mice of each sex were attributed to mild hepatocyte injury.

Minimal thyroid follicular cell hypertrophy was also present in male and female rats in the 1,000- and 2,000-ppm groups. Free thyroxin and total thyroxin concentrations were significantly decreased in exposed male and female rats; these data indicate moderate hypothyroxinemia in exposed animals.

Hematologic findings in exposed rats included decreased hematocrit, hemoglobin concentration, erythrocyte count (males), mean corpuscular hemoglobin, mean erythrocyte volume, and mean corpuscular hemoglobin concentration; these findings are consistent with a mild-to-moderate anemia that is microcytic (decreased mean cell volume), hypochromic (decreased mean corpuscular hemoglobin concentration, females), and poorly regenerative (slight-to-no change in reticulocyte counts).

The no-observed effect levels (NOELs) for histologic lesions were 33 ppm for male rats and 330 ppm for female rats. The NOEL for histologic lesions in female mice was 100 ppm. An NOEL was not reached for male mice.

CONTRIBUTORS

The NTP Report on the Toxicity Studies of Pentachlorobenzene is based on the 15-day and 13-week studies that began in February 1986 at EG&G Mason Research Institute (Worcester, MA).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of pentachlorobenzene 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.

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

On November 20, 1989, the draft report on the toxicity studies of pentachlorobenzene 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.

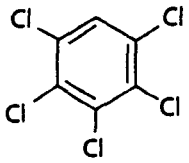
Dr. M.M. McDonald, NIEHS, introduced the short-term toxicity studies by reviewing the rationale, experimental design, and results.

Dr. Klaassen, a principal reviewer, said that this represented a good toxicity study, with the main effects observed in the liver, thyroid gland, and male rat kidney. He said that a table for organ weights and more consistent presentation would be helpful. Dr. Klassen asked for more discussion of chemical effects on the thyroid and whether the effects were direct or indirect. Dr. M. Thompson, NIEHS, said that the data did not support a direct effect.

Dr. Davis, a second principal reviewer, said that an explanation for the ventral body swelling in mice at the highest dose would be of interest. Dr. McDonald said that the increase in liver weight and size would probably account for the swelling. Dr. Davis asked for more precise definitions of the severity code for lesions.

Dr. Silbergeld commented that monitoring of defects on porphyrin metabolism might be a sensitive indicator of toxic response, yet the method used to measure porphyrins was an insensitive one. She noted the observation of severe neurotoxicity in high dose mice, yet no attempt to quantify these observations. Dr. Scala said that since many of these short-term toxicity studies were self-contained studies, it would be important to characterize toxic signs as completely as possible. Dr. R. Yang, NIEHS, noted that the emphasis of the design had been more on range finding for further long-term evaluations.

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



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C_6HCl_5 Molecular weight 250.3

Synonyms: 1,2,3,4,5-Pentachlorobenzene; quintochlorobenzene

I. INTRODUCTION

Chemical and Physical Properties

Pentachlorobenzene can be produced commercially by catalytic chlorination of any tetrachlorobenzene or by heating trichloroethylene to 700° C. It is a white, crystalline, solid material at room temperature, has a pleasant aroma, and like other halogenated benzenes, is lipophilic and hydrophobic. It is insoluble in water and cold alcohol but is soluble in hot alcohol, benzene, ether, carbon tetrachloride, chloroform, and carbon disulfide (USEPA, 1980a). Pentachlorobenzene melts at 86° C and boils at 277° C at atmospheric pressure. Its specific gravity at 16.5° C is 1.8342 (Sittig, 1981; Sax, 1984).

Production and Environmental Contamination

Pentachlorobenzene has been used primarily as a precursor in the synthesis of the fungicide pentachloronitrobenzene and as a flame retardant (USEPA, 1980b). Approximately 1.4×10^6 kg was produced in 1972. Pentachlorobenzene is no longer produced commercially in or imported into the United States (USEPA, 1983).

Contamination of water and aquatic sediment by pentachlorobenzene can occur through industrial discharge, leaching from toxic waste disposal sites, or as a result of degradation of other organochlorine compounds such as lindane and hexachlorobenzene (Villaneuva et al., 1974; USEPA, 1980b; Oliver and Nicol, 1982; Oliver and Charlton, 1984; Silkworth et al., 1984; Onuska and Terry, 1985; Charlton and Oliver, 1986; Takazawa and Strobel, 1986; Vogelgesang, 1986; Pereira et al., 1988). Pentachlorobenzene was detected in fly ash samples from municipal and hazardous waste incinerators (Olie et al., 1980; Elceman et al., 1981; Viau et al., 1984; Schreiner et al., 1986) and in ambient air near industrial complexes and chemical waste disposal sites (Bruckner et al., 1973; Barkley et al., 1980; Pellizzari, 1982; Bruckmann et al., 1988). It is also known to be an impurity of hexachlorobenzene and pentachloronitrobenzene (Kuchar et al., 1969; Dunn et al., 1978; Rozman et al., 1979; Strik, 1986). Pentachlorobenzene is estimated to have a half-life of 194-345 days in experimentally contaminated soil samples; persistence of pentachlorobenzene residues for at least 2-3 years in soil samples from agricultural sites was observed (Beck and Hansen, 1974). Pentachlorobenzene was detected in soil samples, potatoes, and carrots from fields treated with pentachloronitrobenzene.

Ecotoxicology

Pentachlorobenzene residues were found in wild mammals (Brunn et al., 1985; Somers et al., 1987) and in birds and their eggs (Hallett et al., 1982; Ellenton et al., 1985; Fox et al., 1988) from various geographic locations. Residues were also detected in freshwater, estuarine, and salt water organisms used as human food (Ofstad et

al., 1978; Bjerk and Brevik, 1980; Jan and Malnersic, 1980; Oliver and Niimi, 1983; Kuehl et al., 1984; Jaffe and Hites, 1986; Pereira et al., 1988; Swackhamer and Hites, 1988; Niimi and Oliver, 1989). Bioaccumulation of pentachlorobenzene by oligochaete worms was demonstrated under laboratory conditions and in the field (Oliver, 1987). Pentachlorobenzene generally exhibited a higher bioconcentration factor than other chlorinated benzenes, when tested in various experimental systems using guppies (Konemann and van Leeuwen, 1980), rainbow trout (Oliver and Niimi, 1983; Melancon and Lech, 1985), and fathead minnows (Carlson and Kosian, 1987), an effect attributed to its higher degree of chlorine substitution (Oliver and Niimi, 1983; Matthews, 1986).

Human Exposure

In addition to the sources of human exposure described above, low concentrations of pentachlorobenzene were detected in various foodstuffs during market basket surveys (Gartrell et al., 1985a,b), in edible fish and shellfish (Ofstad et al., 1978; Jan, 1983; Swackhamer and Hites, 1988), and in animal and poultry fat and eggs (Greve, 1973). Low levels (mean 22 ng pentachlorobenzene per gram of milkfat) were also found in human breast milk (Mes et al., 1986). Human adipose tissue samples obtained during autopsies contained low (0-70 ng/kg) concentrations of pentachlorobenzene (Mes et al., 1982; Williams et al., 1984).

Pharmacokinetics and Metabolism

Pentachlorobenzene is not readily metabolized in most species, probably because its high degree of chlorine substitution inhibits formation of the arene-oxide intermediates important in the metabolism of the less-chlorinated benzenes (Matthews, 1986). Other mechanisms, such as reductive dehalogenation, may be more significant in the metabolism of the higher chlorinated benzenes, such as pentachlorobenzene (Mehendale et al., 1975; Takazawa and Strobel, 1986).

Parke and Williams (1960) found that, in female Chinchilla rabbits, 60% of the administered dose could be isolated from gut contents and feces or 47% from the subcutis 3-4 days after administration of 0.5 g/kg pentachlorobenzene by gavage or subcutaneous injection, respectively; 10%-20% was found in expired air. Only traces of metabolites (including pentachlorophenol) were found in the urine. The authors state that pentachlorobenzene "is not readily metabolized."

Administration of 60-75 mg/kg pentachlorobenzene to male rabbits by intraperitoneal injection resulted in urinary excretion of about 1% of the administered dose as the metabolites pentachlorophenol and 2,3,4,5-tetrachlorophenol (Kohli et al., 1976).

Rozman et al. (1979) administered a single oral dose of 0.5 mg/kg [^{14}C]pentachlorobenzene to two male and two female rhesus monkeys and determined urinary and fecal excretion for 40 days, after which one male and one female monkey were killed and examined for tissue distribution of pentachlorobenzene and metabolites. About 95% of the administered pentachlorobenzene was absorbed. Metabolites included pentachlorophenol, 1,2,3,4,-tetrachlorobenzene, and several tetrachlorophenols. About twice as much pentachlorobenzene and its metabolites were excreted in feces than in urine. Tissue concentrations were highest in fat and bone marrow, followed by lymph nodes and the thymus, adrenal cortex, and large intestine.

Koss and Koransky (1978) examined urine and feces of female rats after a single intraperitoneal injection of 403 µM/kg [sic] pentachlorobenzene. In both urine and feces, most of the material present was in the form of hydrophilic metabolites, including pentachlorophenol, leading the authors to conclude that pentachlorobenzene "undergoes an almost complete biodegradation." In another study, male Wistar rats were dosed with 8 mg/kg pentachlorobenzene by gavage for 19 days (Engst et al., 1976). Gas chromatographic analysis revealed the presence of pentachlorobenzene and several metabolites, including

pentachlorophenol and various tetrachlorophenols, in pooled weekly samples of urine and feces. Pentachlorobenzene and/or its metabolites were also found in the kidney, liver, and blood.

Villeneuve and Khera (1975) demonstrated placental transfer of pentachlorobenzene by administering 50, 100, or 200 mg/kg by gavage to pregnant Wistar rats on days 6-15 of gestation. On day 22, the dams were killed and the fetuses removed. Gas chromatographic analysis of fetal tissues revealed dose-related pentachlorobenzene accumulation in brain and liver as well as in whole fetuses. Maternal tissues had highest concentrations of pentachlorobenzene in the fat, followed by the liver, brain, heart, kidney, and spleen.

Dose-related accumulation of pentachlorobenzene in adipose tissue of Sherman rats was reported by Linder et al. (1980). Male rats were fed diets containing pentachlorobenzene at concentrations of 125 or 1,000 ppm for 100 days; female rats received 125, 250, 500, or 1,000 ppm for 180 days. Perirenal fat residues of pentachlorobenzene were 1.5-2.2 times the concentrations in feed. Umegaki and Ichikawa (1988) also noted highest concentrations of pentachlorobenzene in the fat of rats given 40-250 mg/kg of the compound (route unspecified) for 5 days, with lower concentrations detected in liver, kidney, and brain. The major metabolite of pentachlorobenzene in the liver was pentachlorophenol.

Single-Dose Toxicity

LD_{50} values for pentachlorobenzene administered by gavage to Sherman rats and Swiss Webster mice were reported by Linder et al. (1980) and are summarized in Table 1. Clinical signs included tremors, decreased activity, and weakness in both species. Some rats also exhibited reddish stains around the mouth, nose, and eyes and had dark yellow urine. Survival times were 2-4 days for mice and 5-12 days for rats. Grossly, the rat liver, kidney, and adrenal gland appeared enlarged. When the gastrointestinal tract in rats was examined under longwave ultraviolet light, slight reddish fluorescence was observed.

Linder et al. (1980) also evaluated the dermal absorption of pentachlorobenzene. No clinical signs of toxicity were observed for 2 weeks after application of a single dose of 2,500 mg/kg pentachlorobenzene dissolved in xylene to the shaved backs of male and female Sherman rats.

Administration of 50-100 mg/kg pentachlorobenzene by gavage to pregnant CD®-1 mice resulted in increased absolute and relative maternal liver weights (Courtney et al., 1977).

Short-Term Toxicity

Linder et al. (1980) also studied the short-term toxicity of pentachlorobenzene in Sherman rats. Female rats were given 125, 250, 500, or 1,000 ppm pentachlorobenzene in feed for 180 days, and male rats were given 125 or 1,000 ppm for 100 days. After 67 days of exposure, female rats were bred to untreated males and underwent pregnancy and lactation during the remainder of the study. No deaths or clinical signs of toxicity were noted throughout the study period. Body weight gains and feed consumption of exposed animals did not vary significantly from those of controls. Absolute and relative liver weights were increased for the high dose group of each

TABLE 1. SELECTED LD_{50} VALUES FOR PENTACHLOROBENZENE (a)

Species	Route of Exposure	Sex	Age	LD_{50} (mg/kg)
Rat	Oral	Male	Adult	1,125
Rat	Oral	Female	Adult	1,080
Rat	Oral	Female	Weanling	940
Mouse	Oral	Male	Adult	1,175
Mouse	Oral	Female	Adult	1,370

(a) Sherman rats and Swiss Webster mice; Linder et al. (1980)

sex, and relative kidney weights were increased for the 1,000-ppm group of each sex. No fluorescence characteristic of porphyrin was observed when viscera of male and female rats were examined under ultraviolet light. Urinary porphyrin excretion was not increased in exposed female rats compared with that in controls. Total liver porphyrin was slightly increased in 1,000-ppm female rats compared with that in controls, but this was "not judged to be a porphyrinogenic response" and was considered "of doubtful significance." (Male rats were not evaluated for urinary porphyrin concentrations.)

Histologically, female rats in the 500- and 1,000-ppm groups and males in the 1,000-ppm group exhibited hypertrophy of the centrilobular hepatocytes (Linder et al., 1980). Male rats exhibited dose-related renal lesions consisting of hyaline droplet accumulation in the cortical tubule epithelium, atrophic cortical tubules, cortical interstitial lymphocytic foci, and cortical tubular dilatation with granular or hyaline casts. Renal cortical tubular eosinophilic casts and hyaline droplet accumulation were reported for one female rat (1,000 ppm).

Chu et al. (1983) administered 5, 50, or 500 ppm pentachlorobenzene in the diet for 28 days to male and female Sprague Dawley rats. Absolute liver weights, serum cholesterol concentration, and sorbitol dehydrogenase activity were increased in high dose male rats, and hepatic microsomal enzyme activity was increased in the liver of high dose animals of each sex. However, liver porphyrin concentrations were not increased in dosed animals. Compound-related histologic lesions included enlargement and cytoplasmic eosinophilia of centrilobular hepatocytes, thyroid follicular cell hypertrophy, and decreased thyroid follicular colloid density in each sex and "eosinophilic inclusions in the proximal convoluted tubules of the renal cortex" in males.

Long-Term Toxicity and Carcinogenicity

Pentachlorobenzene did not promote diethylnitrosamine-initiated γ-glutamyl transferase-positive foci in rat liver (Herren-Freund and Pereira, 1986).

Mechanistic and Interaction Studies

Several studies have demonstrated that pentachlorobenzene induces production of various hepatic enzymes in rats. Short-term effects on hepatic enzymes were investigated by Ariyoshi et al. (1975) by the administration of 250 mg/kg pentachlorobenzene by gavage once per day for 3 days to female Wistar rats. The liver of dosed animals had increased activity of cytochrome P450 microsomal enzymes, δ-aminolevulinic acid synthetase, aniline hydoxylase, and aminopyrine demethylase. Increased hepatic microsomal protein and triglyceride content were also observed, whereas glycogen levels were decreased. Other studies in rats have corroborated these results (Goldstein et al., 1982; Chu et al., 1983; Ikegami et al., 1987).

These findings have led to general agreement that pentachlorobenzene is a "phenobarbital-type" inducer, resembling phenobarbital more than 3-methyl-cholanthrene in the types of hepatic enzymes it induces (Goldstein et al., 1982; Denomme et al., 1983; Li et al., 1986). Pentachlorobenzene was shown to induce preferentially the same subtypes of P450 cytochromes (known as P450b and P450e in rats) as phenobarbital (Goldstein et al., 1986).

Other drug-metabolizing hepatic enzymes that were increased after administration of pentachlorobenzene by intraperitoneal injection in male Wistar rats were 4-dimethylaminoantipyrine and aldrin epoxidase (Denomme et al., 1983).

Other polyhalogenated aromatic hydrocarbons, notably hexachlorobenzene, are well documented to cause porphyrin metabolism derangements in humans and laboratory animals (Peters et al., 1982). Inhibition of uroporphyrinogen decarboxylase, either directly or via a reactive metabolite, is generally accepted as the underlying pathogenic mechanism (Debets et al., 1980; De Matteis, 1986). However, reports concerning the porphyrinogenic potential of pentachlorobenzene are somewhat conflicting.

Levels of δ-aminolevulinic acid synthetase, which catalyzes the rate-limiting step of porphyrin synthesis, were increased in rats

administered pentachlorobenzene (Ariyoshi et al., 1975). Pentachlorobenzene caused increases in porphyrin levels in chick-embryo liver (Billi et al., 1986). Addition of pentachlorobenzene to chick-embryo hepatocyte cultures pretreated with 3-methylcholanthrene resulted in modest increases in uroporphyrins, attributed to inhibition of uroporphyrinogen decarboxylase (Sinclair et al., 1986).

In contrast, increased porphyrin production after pentachlorobenzene administration has not been detected in several other studies. Significant increases in porphyrin levels did not occur in the liver of chick embryos inoculated with pentachlorobenzene in ova (Billi and San Martin de Viale, 1985). Abnormal porphyrin accumulation did not occur when chick embryo hepatocyte cultures pretreated with the microsomal enzyme inducer β-naphthoflavone were inoculated with pentachlorobenzene (Debets et al., 1981).

Goerz et al. (1978) reported that dietary exposure of female rats to 500 ppm pentachlorobenzene for 60 days did not result in an increase in urinary excretion of porphyrins. Linder et al. (1980) concluded that dietary exposure to 1,000 ppm pentachlorobenzene for 180 days did not result in a biologically significant increase in urinary or liver porphyrins in female Sherman rats.

No increase in liver porphyrin was noted when male and female rats were given diets containing 5, 50, or 500 ppm pentachlorobenzene for 28 days (Chu et al., 1983).

Genetic Toxicology

Pentachlorobenzene was not mutagenic in a Salmonella gene mutation assay conducted in four strains with and without S9 (Haworth et al., 1983). Pentachlorobenzene was also negative in Chinese hamster ovary cell assays for induction of sister chromatid exchanges and chromosomal aberrations (NTP unpublished data). Peripheral blood smears from the current 13-week study animals were analyzed for incidence of micronucleated normochromic erythrocytes; no significant increase in micronuclei was observed for any of the exposure groups (NTP unpublished data).

The metabolites of pentachlorobenzene (chlorobenzene, tetrachlorophenols, tetrachlorobenzenes, trichlorophenols, trichlorobenzenes, pentachlorophenol, tetrachlorohydroquinone) were also negative for gene mutation induction in Salmonella (Haworth et al., 1983; Zeiger et al., 1988; NTP unpublished data). Some of the metabolites, however, in contrast to pentachlorobenzene, have shown evidence of clastogenic activity in vitro (Galloway et al., 1987; NTP unpublished data).

Reproductive Toxicology

Pentachlorobenzene has been shown to cross the placental barrier and accumulate in fetal tissues in rats and other species (Villeneuve and Khera, 1975; Aly and Fassbender, 1984).

Teratogenicity of pentachlorobenzene was evaluated by Khera and Villeneuve (1975) in pregnant Wistar rats given single doses of 50, 100, or 200 mg/kg pentachlorobenzene once per day on days 6-15 of gestation. Compared with those in controls, the mean number of live fetuses per litter and mean fetal weight were decreased at 200 mg/kg. At 200 mg/kg, there were also higher incidences of supernumerary ribs and unossified or nonaligned sternebrae.

Linder et al. (1980) studied reproductive toxicity of pentachlorobenzene in Sherman rats. Pentachlorobenzene was given in feed at 125 or 1,000 ppm for 100 days to males and at 125, 250, 500, or 1,000 ppm for 180 days to females. At day 67 of chemical exposure, the animals of each sex were mated with controls. Fertility and fecundity were unaffected. Litters sired by exposed males did not exhibit exposure-related effects. Offspring of dams exposed to pentachlorobenzene at high concentrations were smaller than those of controls and exhibited significant preweaning mortality. Tremors were observed in pups of exposed dams. Weanling rats from high concentration groups had increased relative liver weights and histologically detected hepatocellular enlargement.

Courtney et al. (1977) administered pentachlorobenzene by gavage to pregnant CD®-1 mice at

50, 100, or 200 mg/kg on gestation days 6-15. Decreased fetal viability and teratogenicity were not observed.

Study Rationale

Pentachlorobenzene is a slowly metabolized, biologically persistent compound structurally related to other polychlorinated benzenes, dioxins, and biphenyls well known to be highly toxic and/or carcinogenic in animals and humans. Pentachlorobenzene is no longer produced commercially in the United States, but long-term human exposure may result from its documented persistence in ground water, aquatic sediments, and soil and its bioconcentration in aquatic and terrestrial plants and animals used as human food. The present studies were undertaken following the designation of pentachlorobenzene as a priority chemical for toxicologic testing by the Interagency Agreement (Superfund Project) between the National Toxicology Program (NTP) and the EPA.

Although some pharmacokinetic and toxicity studies of pentachlorobenzene are available for rats, very little toxicity data exist for mice. No long-term toxicity or carcinogenicity studies of pentachlorobenzene are readily available for any species. The current studies supplement available short-term toxicity data for rats.

Since the greatest human exposure to pentachlorobenzene might result from water supplies contaminated by leaching from dump sites or degradation of other persistent organochlorines such as hexachlorobenzene, the preferred route of administration would be through drinking water. However, because of pentachlorobenzene's poor water solubility, formulated diets were selected as the route of administration in these studies. Concurrent studies were conducted on 1,2,4,5-tetrachlorobenzene (NTP, 1990a).

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PENTACHLOROBENZENE

A single-study lot of pentachlorobenzene was prepared by milling material from three suppliers: ICN K&K Labs (Plainview, NY), Lancaster Synthesis (Windham, NH), and Chemical Dynamics Corporation (South Plainfield, NJ). Purity and identity analyses were conducted by Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on the analyses performed in support of the pentachlorobenzene studies are on file at the National Institutes of Environmental Health Sciences.

The composite material was identified as pentachlorobenzene 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, and gas chromatography. Analysis by high resolution gas chromatography/high resolution mass spectrometry/selected ion monitoring indicated that no chlorinated dibenzodioxins or dibenzofurans were detected in the study material (detection limits ranged from 100 to 1,000 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 pentachlorobenzene was noted over the course of the studies.

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diets were prepared by mixing the appropriate amounts of pentachlorobenzene (w/w) with feed in a twin-shell blender. The homogeneity and stability of pentachlorobenzene in feed (0.1 mg/g) were determined

by gas chromatographic analysis performed on isooctane extracts of feed mixtures. The chemical in feed was found to be homogeneously distributed and to be stable for at least 21 days when stored in the dark at room temperature and for at least 7 days when stored open to air and light in a rodent cage. During the studies, formulated diets were stored for no longer than 3 weeks at 4° ± 3° C.

Periodic analysis of formulated diets of pentachlorobenzene by gas chromatography was conducted at the study and analytical chemistry laboratories. Three complete sets of formulated diet mixtures were analyzed by the study laboratory during the 13-week studies; all samples were within specifications (± 10% of the target concentration) (Table 2). The results of the analyses ranged from 91% to 102% of the target concentrations. A single referee analysis conducted by the analytical laboratory confirmed the results obtained by the study laboratory.

FIFTEEN-DAY STUDY DESIGN

Male and female F344/N rats and male and female $B6C3F_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) and were held for 11-14 days before the studies. The rodents were 6 weeks old when placed on study. Groups of five rats and five mice of each sex received diets containing 0, 100, 330, 1,000, 3,300, or 10,000 ppm pentachlorobenzene for 15 days. Further details are presented in Table 3.

THIRTEEN-WEEK STUDY DESIGN

Groups of 20 rats and 20 mice of each sex were fed diets containing 0, 33, 100, 330, 1,000, or 2,000 ppm pentachlorobenzene for 13 weeks. Ten animals in each group were designated for supplemental studies and scheduled for sequential bleeding during the studies.

Source and Specification of Animals

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 (Germantown, NY). 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 4-5 weeks of age. The rats were quarantined at the study laboratory for 13-20 days and mice for 13-21 days. All animals were placed on study at 6-7 weeks of age.

Clinical Examinations and Pathology

All animals were observed twice per day. Body weights were recorded once per week. Blood samples were obtained by puncture of the retro-orbital sinus of CO_2-anesthetized animals from

TABLE 2. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE

Target Concentration (ppm)	Determined Concentration (a) (ppm)
33	31.2 ± 0.9
100	95.6 ± 3.9
330	320 ± 12
1,000	999 ± 28
2,000	1,957 ± 95

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

TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FIFTEEN-DAY AND THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE

Fifteen-Day Studies	Thirteen-Week Studies
Strain and Species	
F344/N rats, $B6C3F_1$ mice	F344/N rats, $B6C3F_1$ mice
Animal Source	
Simonsen Laboratories (Gilroy, CA)	Taconic Farms (Germantown, NY)
Study Laboratory	
EG&G Mason Research Institute	EG&G Mason Research Institute
Size of Study Groups	
5 males and 5 females of each species, rats housed 5 per cage, mice individually caged	20 males and 20 females of each species, rats housed 5 per cage, mice individually caged
Doses	
0, 100, 330, 1,000, 3,300, or 10,000 ppm pentachlorobenzene in feed	0, 33, 100, 330, 1,000, or 2,000 ppm pentachlorobenzene in feed
Method of Animal Distribution	
Assigned to groups such that for a given sex and species all cage weights were approximately equal	Animals distributed to weight classes and then assigned to groups by a table of random numbers
Diet	
NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 15-d studies
Animal Room Environment	
Temp--20°-23° C, hum--36%-49%; fluorescent light 12 h/d; 10 room air changes/h	Temp -19°-23° C; hum--34%-56%, fluorescent light 12 h/d, 10 room air changes/h
Time Held Before Study	
Rats--11-12 d, mice--13-14 d	Rats--13-14 d (male) or 17-20 d (female), mice--13-14 d (male) or 20-21 d (female)
Duration of Dosing	
15 d	13 wk
Age When Killed	
8 wk	Male rats and male mice--19 wk, female rats and female mice--20 wk
Type and Frequency of Observation	
Observed 2 × d; weighed initially, at d 8, and at necropsy	Observed 2 × d, weighed initially and 1 × wk thereafter
Necropsy, Histologic Examinations, and Supplemental Studies	
Necropsy performed on all animals; the following tissues were examined histologically for control and 3,300- and 10,000-ppm rats and 1,000-, 3,300-, and 10,000-ppm mice: adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland (rats), rectum, salivary glands, skin, small and large intestine, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder	Necropsy performed on all animals, 10 males and 10 females from the control and high dose groups of each species were examined histologically; tissues examined were the same as those for the 15-d studies except that bone and bone marrow specimens were always taken from the femur Liver and kidney examined for all male rats, thyroid gland examined for all rats, and liver examined for all mice In addition, organ weights and liver porphyrin concentrations determined and sperm morphologic exams conducted at necropsy; vaginal cytologic exams conducted on females for 7 d before necropsy For animals not examined histologically, organ weights obtained and hematologic exams performed on d 90, and serum thyroid hormone concentrations determined on d 15, 45, and 90, serum chemistry analysis performed on d 3, 15, and 90 for rats and on d 90 for mice, urinalysis performed on d 3, 15, and 90 for rats

male and 10 female rats of the supplemental groups on days 3, 15, and 45 for hematologic and serum chemistry analysis. Serum chemistry analyses were performed on a Gemini clinical chemistry analyzer by standard methodology and included sorbitol dehydrogenase, alanine aminotransferase, creatinine phosphokinase, and γ-glutamyl transferase activities and creatinine and albumin concentrations. Hematologic analyses were performed on a Baker Series 7000 Cell Counter and included erythrocyte and leukocyte counts, hemoglobin concentration, hematocrit, mean cell volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

Leukocyte differentials and platelet counts were performed and blood cell morphology evaluated from Wright-Giemsa stained blood smears. Reticulocyte counts were performed on new methylene blue-stained whole blood smears.

Urine was collected over a 16-hour period for urinalysis on days 3, 15, and 90 for rats. Blood sampling preceded the beginning of urine collection by several hours. Sediment from centrifuged urine samples was evaluated microscopically, and glucose and protein concentrations determinations were performed on a Gemini clinical chemistry analyzer using standard methodology. Thyroid hormone determinations were performed for groups of 10 rats and 10 mice of each sex on days 15, 45, and 90. NML Tri-Tab RIA kits (Nuclear Medical Laboratories, Dallas, TX) were used to quantitate triiodothyronine and thyroxin; Clinical Assays Gammacoat RIA kits (Travenol-Genentech Diagnostics, Cambridge, MA) were used to quantitate free thyroxin, and reagents provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases were used to quantitate thyrotropin. On day 90, the rats and mice were anesthetized with carbon dioxide and blood was drawn from the retroorbital sinus for serum chemical determinations and hematologic analysis.

Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals not bled sequentially. Blood was drawn from mice for evaluation of micronuclei. Sperm morphology, motility, density, and head count were evaluated for male rats and male mice that received 0, 33, 330, or 2,000 ppm pentachlorobenzene; vaginal smears to identify stages of the estrual cycle were prepared during the 7 days before necropsy for females that received 0, 33, 330, or 2,000 ppm. Full experimental procedures are described in Morrissey et al., (1988).

Organs and tissues were examined for gross lesions. 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 3. The liver, right kidney, brain, heart, thymus, lung, and right testis of all rodents were weighed. Liver porphyrin content was determined by a fluorometric procedure (Poh-Fitzpatrick et al., 1974).

Upon completion of the histologic evaluation by the laboratory pathologist, 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 METHODS

Analysis of Continuous Variables: Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends for organ weight, serum chemistry, hematologic, urinalysis, and male reproductive system data. If this analysis indicated a significant trend, the nonparametric multiple comparison procedure 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 pentachlorobenzene were performed in compliance with Good Laboratory Practice regulations (21 CFR 58). The Quality Assurance Unit of EG&G Mason Research Institute 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

Fifteen-Day Studies

All rats that received 10,000 ppm pentachlorobenzene died (males by day 7, females by day 6) (Table 4). The final mean body weight of rats at 3,300 ppm was 23% lower than that of the controls for males and 15% lower for females. Feed consumption by dosed groups was generally lower than that by controls during week 1 and greater during week 2.

The absolute liver weights and the liver weight to body weight ratios were significantly increased in all dosed groups of rats except the 100-ppm females; at 3,300 ppm, the mean absolute liver weights were about twice those of controls. Absolute kidney weights of males were slightly (10%-20%) but significantly increased at 100, 330, and 1,000 ppm, and the kidney weight to body weight ratios were significantly increased for all dosed male groups; the significantly lower absolute kidney weights of males and females that received 3,300 ppm were probably a consequence of the notably lower weight gain. Absolute thymus, heart, and lung weights were significantly decreased for males and females receiving 3,300 ppm, but not at lower doses, and the decreases may have been a consequence of the notably lower weight gain; the relative organ weights were only marginally affected at any dose.

In male rats, renal lesions consisted of excessive accumulation of abnormal hyaline droplets in the cytoplasm of the cortical tubular epithelium. Results of the review of original sections and additional hematoxylin and eosin and Mallory-azan-stained (Barlow, 1984) kidney sections are shown in Table 5. The abnormal hyaline droplets were abundant, large, eosinophilic, angular, crystalline structures present in the cytoplasm of cortical epithelial cells of exposed male rats. Control and 100-ppm animals had less abundant, small, uniformly round cytoplasmic hyaline droplets like those commonly observed in untreated young male rats and morphologically different from those in males in the higher dosed groups. The reason for the absence of abnormal hyaline droplets in male rats in the 10,000-ppm group and in three of five rats in the 3,300-ppm group may have been related to the early deaths and decreased synthesis of $\alpha_{2\mu}$-globulin related to greater toxicity at these highest doses.

Male rats in the 330- and 1,000-ppm groups and female rats in the 1,000-ppm group had minimal centrilobular hepatocellular hypertrophy, which consisted of enlarged hepatocytes with abundant homogeneous eosinophilic cytoplasm (Table 5). Male and female rats given 10,000 ppm pentachlorobenzene in feed died during the studies and may not have lived long enough to develop these hepatic lesions.

Marked depletion of thymic lymphocytes and mild-to-moderate hyperkeratosis of the forestomach occurred in males and females receiving 10,000 ppm; forestomach acanthosis was present in one female receiving 10,000 ppm.

TABLE 4. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FIFTEEN-DAY FEED STUDIES OF PENTACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Compound Consumption (e)
MALE							
0	5/5	114	188	+74		89	--
100	5/5	116	197	+81	104.8	96	10
330	5/5	117	188	+71	100.0	92	30
1,000	5/5	116	183	+67	97.3	95	95
3,300	5/5	116	145	+29	77.1	92	304
10,000	(f) 0/5	115	(g)	(g)	(g)	(g)	(g)
FEMALE							
0	5/5	108	139	+31		88	--
100	5/5	109	140	+31	100.7	85	9
330	5/5	107	142	+35	102.2	93	31
1,000	5/5	105	142	+37	102.2	91	91
3,300	5/5	106	118	+12	84.9	88	290
10,000	(h) 0/5	106	(g)	(g)	(g)	(g)	(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 of feed per kilogram body weight per day averaged over the 2-wk period; not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights; not corrected for scatter.
(f) Day of death: 6,6,6,6,7
(g) No data are reported due to 100% mortality in this group.
(h) Day of death: 5,5,6,6,6

TABLE 5. NUMBERS OF RATS WITH SELECTED LESIONS IN THE FIFTEEN-DAY FEED STUDIES OF PENTACHLOROBENZENE (a)

Site/Lesion	Control	100 ppm	330 ppm	1,000 ppm	3,300 ppm	10,000 ppm
MALE						
Kidney						
Hyaline droplets (b)	0	1	5	5	2	0
Liver						
Centrilobular hypertrophy	0	0	0	5	5	0
FEMALE						
Liver						
Centrilobular hypertrophy	0	0	0	0	5	0

(a) Five animals were examined in each group of each sex.
(b) Diagnosed as cytoplasmic alteration by the study pathologist in the 13-week studies.

Thirteen-Week Studies

No compound-related deaths occurred in the studies (Table 6). Mean body weights of male rats that received 1,000 and 2,000 ppm and all dosed groups of female rats were lower than those of controls throughout the studies (Figure 1). The initial mean body weight of female rats that received 330 ppm was 7% lower than that of controls, and body weights of this group remained lower than all other groups throughout the studies. The final mean body weight of males that received 2,000 ppm was 13% lower than that of the controls; for females that received 330, 1,000, or 2,000 ppm, final mean body weights were 6%-9% lower.

Compound-related increases in the absolute organ weights and organ weight to body weight ratios were seen for the kidney and liver of both males and females (Table 7). Absolute and relative weights of other organs are given in Table A1.

Absolute kidney weights were increased in rats given feed containing dietary concentrations as low as 330 ppm for males and 1,000 ppm for females, and relative kidney weights in males and females were increased at concentrations as low as 100 ppm. Absolute liver weights were increased at concentrations as low as 100 ppm for males and females, and relative liver weights were increased at concentrations as low as 33 ppm (a no-observed-effect concentration was not reached) for males and 100 ppm for females.

Hematocrit values, hemoglobin concentration, mean corpuscular hemoglobin, and mean cell volume were all significantly decreased for males and females that received 1,000 or 2,000 ppm (Table A2). The serum albumin concentration was significantly increased for males that received 1,000 or 2,000 ppm and for females that received 330 ppm or more. The reticulocyte (2,000-ppm group only) and platelet counts and creatinine concentration were significantly increased for males that received 1,000 or 2,000

TABLE 6. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS EXAMINED HISTOLOGICALLY IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Compound Consumption (e)
MALE							
0	10/10	134 ± 5	344 ± 7	+209 ± 5		17	--
33	10/10	131 ± 3	341 ± 8	+210 ± 7	99	17	2 4
100	10/10	132 ± 4	344 ± 9	+212 ± 7	100	16	6 7
330	10/10	131 ± 4	341 ± 5	+211 ± 5	99	16	22
1,000	10/10	132 ± 4	331 ± 8	+199 ± 6	96	17	73
2,000	10/10	130 ± 3	299 ± 6	+170 ± 3	87	17	159
FEMALE							
0	10/10	125 ± 3	207 ± 15	+81 ± 5		11	--
33	10/10	125 ± 3	199 ± 3	+73 ± 3	96	11	2.2
100	10/10	124 ± 2	196 ± 3	+73 ± 3	95	11	6.9
330	10/10	116 ± 3	188 ± 5	+72 ± 5	91	11	24
1,000	10/10	125 ± 3	194 ± 2	+69 ± 2	94	11	69
2,000	10/10	125 ± 2	192 ± 2	+67 ± 2	93	13	164

(a) Number surviving/number initially in group for animals not bled sequentially
(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 of feed per animal per day averaged over the 13-wk period for both bled and nonbled groups (combined); not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights; not corrected for scatter.

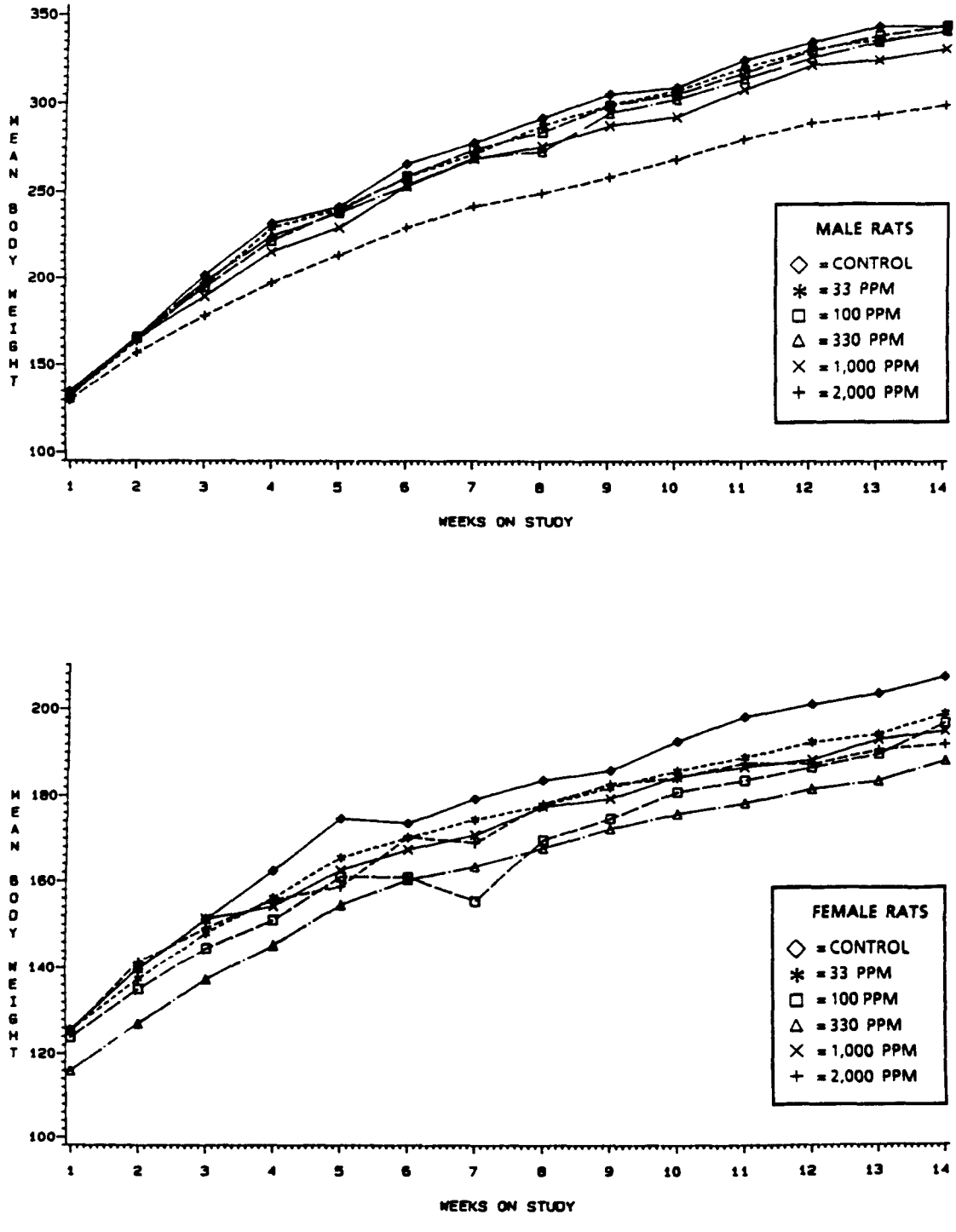


FIGURE 1. GROWTH CURVES FOR RATS FED DIETS CONTAINING PENTACHLOROBENZENE FOR THIRTEEN WEEKS AND EXAMINED HISTOLOGICALLY (NOT BLED)

TABLE 7. ORGAN WEIGHTS FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Organ	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE (b)						
Body weight (grams)	354 ± 7.1	339 ± 7.9	344 ± 8.5	345 ± 5.6	335 ± 8.3	**299 ± 6 8
Right kidney						
Absolute	1,307 ± 39	1,314 ± 22	1,362 ± 40	**1,607 ± 43	**1,889 ± 53	**1,427 ± 36
Relative	3.7 ± 0.09	3.9 ± 0.05	*4.0 ± 0.07	**4.7 ± 0.12	**5.6 ± 0.09	**4.8 ± 0.13
Liver						
Absolute	12,740 ± 370	12,990 ± 340	*14,620 ± 630	**14,330 ± 230	**19,750 ± 1,000	**21,870 ± 750
Relative	36.0 ± 0.76	*38.4 ± 0.76	**42.4 ± 1.05	**41.5 ± 0.53	**58.7 ± 1.99	**73.1 ± 1 40
FEMALE (b)						
Body weight (grams)	209 ± 5.3	200 ± 2.9	197 ± 3.1	**189 ± 4.5	*195 ± 2.2	**193 ± 2 2
Right kidney						
Absolute	745 ± 28	712 ± 15	791 ± 15	676 ± 22	**886 ± 18	**911 ± 12
Relative	3.6 ± 0.11	3.6 ± 0.06	**4.0 ± 0 09	3.6 ± 0.04	**4.5 ± 0.06	**4.7 ± 0 07
Liver						
Absolute	7,010 ± 167	6,676 ± 171	*7,544 ± 106	6,706 ± 241	**10,787 ± 296	**13,783 ± 325
Relative	33.6 ± 0.39	33.3 ± 0.57	**38.4 ± 0.83	**35.4 ± 0.68	**55.3 ± 1.32	**71.5 ± 1.73

(a) Mean ± standard error in milligrams (absolute) or milligrams per gram (relative) 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).
(b) Data for animals examined histologically but not bled sequentially during the study
*P<0 05
**P<0.01

ppm; for females, no effect was seen on the reticulocyte count and creatinine concentration, and significantly lower values were obtained for the platelet count in all dosed groups. These values are considered to be within normal variation. Serum sorbitol dehydrogenase activity was significantly increased for males at 1,000 and 2,000 ppm and for females at dietary concentrations as low as 100 ppm, and γ-glutamyl transferase activity was significantly increased at 2,000 ppm at some time points. The increased serum sorbitol dehydrogenase activity was considered to be compound related, but the γ-glutamyl transferase activity could not definitely be related to compound administration because of the large variation in individual values.

The urinary glucose concentration was significantly increased for male rats in the 330- to 2,000-ppm groups and for female rats in the 1,000- to 2,000-ppm groups. Urinary protein concentration was increased in male and female rats in the 1,000- and 2,000-ppm groups; this finding was especially pronounced in males (Table A2). Urine volume was significantly increased for males receiving 1,000 or 2,000 ppm and for females receiving 2,000 ppm on day 90. These urinary effects were clearly compound related.

Free thyroxin and total thyroxin concentrations in serum were significantly decreased for males at all pentachlorobenzene concentrations (down to 33 ppm) and for females at concentrations down to 100 ppm. The thyrotropin concentration was significantly increased for males and females that received 1,000 or 2,000 ppm. Significant changes in concentrations of triiodothyronine were rare in female rats and, although more common, not clearly dose related in male rats. In both sexes, however, changes in thyroid hormone concentrations were clearly related to effects of the compound on the thyroid gland.

Liver porphyrin values at the end of the studies had significant, but not dose-related, increases in males exposed at concentrations as low as 33 ppm and were significantly increased in females given diets containing 2,000 ppm pentachlorobenzene.

The percentage of abnormal sperm was significantly increased by 70% or 100% in males that received 330 or 2,000 ppm, respectively; sperm of males that received 1,000 ppm was not examined (Table A2). There were no effects on epididymal sperm motility or density. The length of the estrous cycle was significantly reduced at 33 and 2,000 ppm; females that received 100 or 1,000 ppm were not examined, and the reduction at 330 ppm was not statistically significant. The reduction in the length of the estrous cycle was not dose related and was not clearly related to compound administration.

In male rats, dose-related renal lesions included accumulation of hyaline droplets (cytoplasmic alteration) in the cortical tubular epithelium, tubular dilatation and granular casts at the outer stripe of the outer medulla, and focal mineralization of medullary collecting tubules (Table 8). Cytoplasmic alteration was defined histologically by the study pathologist as an increase relative to controls of large, angular, intracytoplasmic eosinophilic granules (hyaline droplets) present in tubules scattered diffusely throughout the cortex (Figure 2). These large droplets were distinctly different in morphology from the less abundant, smaller, uniformly round eosinophilic droplets seen in the renal tubular cytoplasm of control males (Figure 3). The hyaline droplets were strongly positive with Lee's methanamine blue-basic fuchsin stain for protein (Short et al., 1987).

Dilatation of tubules of the outer stripe of the outer medulla (medullary tubular dilatation) was characterized by distended tubules lined by low cuboidal or flattened epithelium and often containing coarse material (granular casts) (Figure 4). Focal mineralization of the medullary collecting tubules consisted of small plugs of basophilic, sometimes granular, von Kossa stain-positive intraluminal mineralized material (Figure 5). These lesions are considered characteristic of renal toxicity described as "hydrocarbon or hyaline droplet nephropathy" of male rats (Thomas et al., 1985).

Regeneration of cortical tubular epithelium occurred at increased incidences and severity in exposed male and female rats versus controls. This lesion consisted of multiple foci of cortical tubules lined by plump, cuboidal cells that had basophilic cytoplasm and were often supported by thickened, hyalinized basement membranes. Cortical tubular casts occurred in high dose groups of each sex. Composed of homogeneous eosinophilic material, these cortical protein casts were morphologically distinct from the granular casts previously described. The incidences of scattered interstitial foci of mononuclear inflammatory cells (chronic inflammation) with or without fibroplasia, and sometimes associated with clusters of regenerating tubules, were increased in exposed male rats. Tubular regeneration, protein casts, and chronic inflammation are typical features of the spontaneous nephropathy of F344 rats (Peter et al., 1986), which was exacerbated in incidence and severity by pentachlorobenzene exposure.

Small, round, yellow-brown pigment granules were in the cortical renal tubular epithelium of exposed animals of each sex but were most prominent in females. This pigment was negative with the following stains: oil red O (neutral fat), Prussian blue (iron), Hall's bilirubin stain, and Ziehl-Neelsen (acid fast); it was weakly positive by the periodic acid-Schiff (PAS) reaction. The incidence of renal pigmentation correlated well with an increase in liver porphyrin content in rats of each sex (see above). These results indicate that the pigment granules could be composed of porphyrin compounds.

Cortical mineralization observed in exposed and control female rats consisted of scattered foci of basophilic granular material in cortical tubules. This lesion was topographically distinct from the collecting tubule mineralization seen in males. It is a common spontaneous change in female rats and is not considered to be compound related.

Compound-related centrilobular hepatocellular hypertrophy was present at increased incidences in the liver of exposed male (330-2,000 ppm) and female (1,000-2,000 ppm) rats. Hypertrophy was

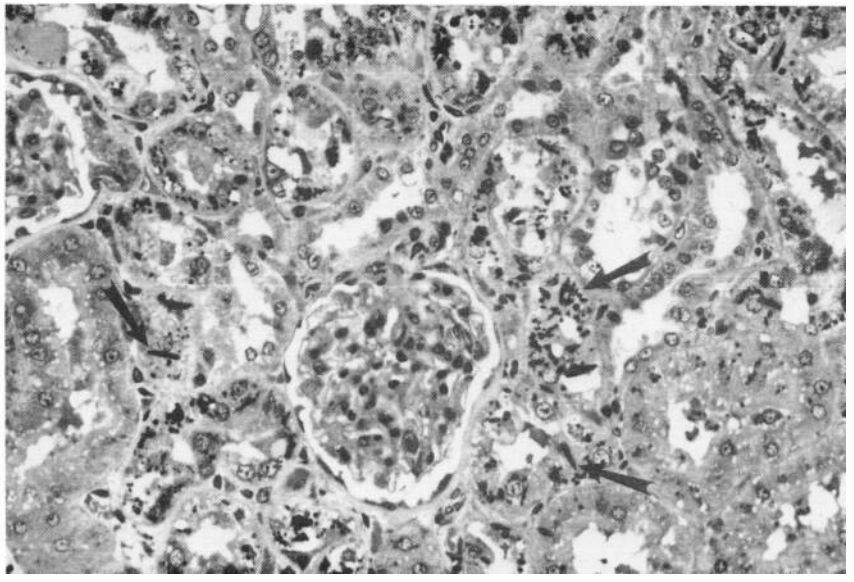


Figure 2. Kidney from male rat given 2,000 ppm pentachlorobenzene in feed for 13 weeks. Accumulation of abnormal hyaline droplets (cytoplasmic alteration) is evident as angular, crystalline inclusions (arrows) in tubular epithelial cells (Lee's methanamine blue-basic fuchsin). Compare with Figure 3 at same magnification.

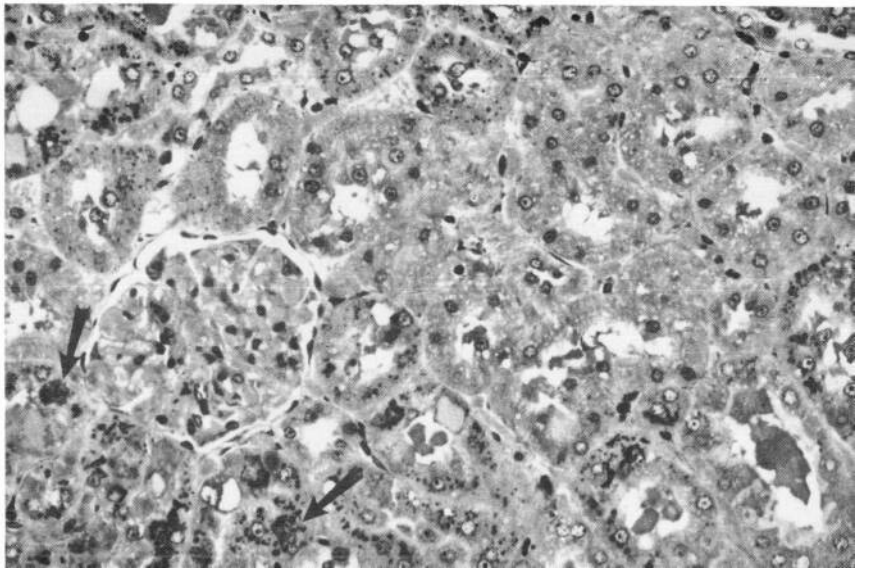


Figure 3. Kidney from control male rat in the 13-week study. Collections of small, uniformly round hyaline droplets are present in the cytoplasm of some tubular epithelial cells (arrows) (Lee's methanamine blue-basic fuchsin). Compare with Figure 2 at same magnification.

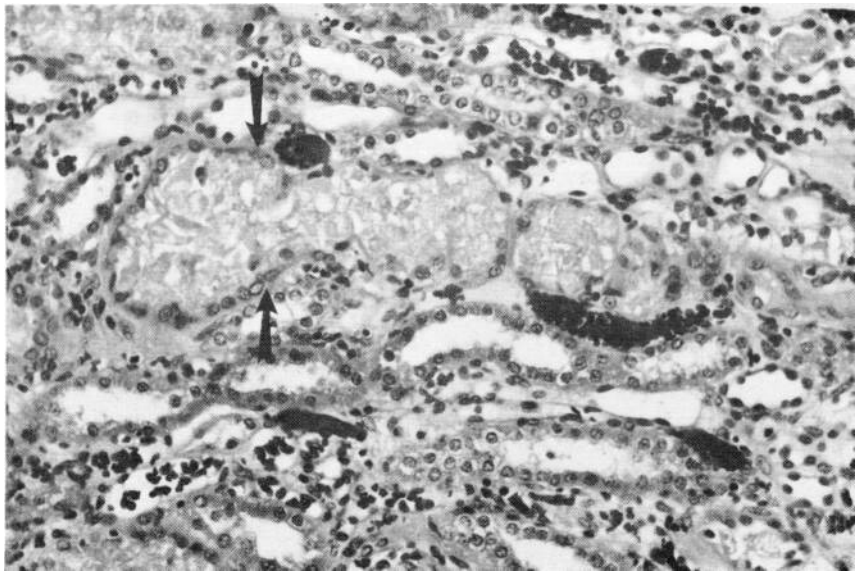


Figure 4. Kidney from male rat given 2,000 ppm pentachlorobenzene in feed for 13 weeks. A dilated medullary tubule (arrows) contains a cast of coarse, granular cellular debris (hematoxylin and eosin).

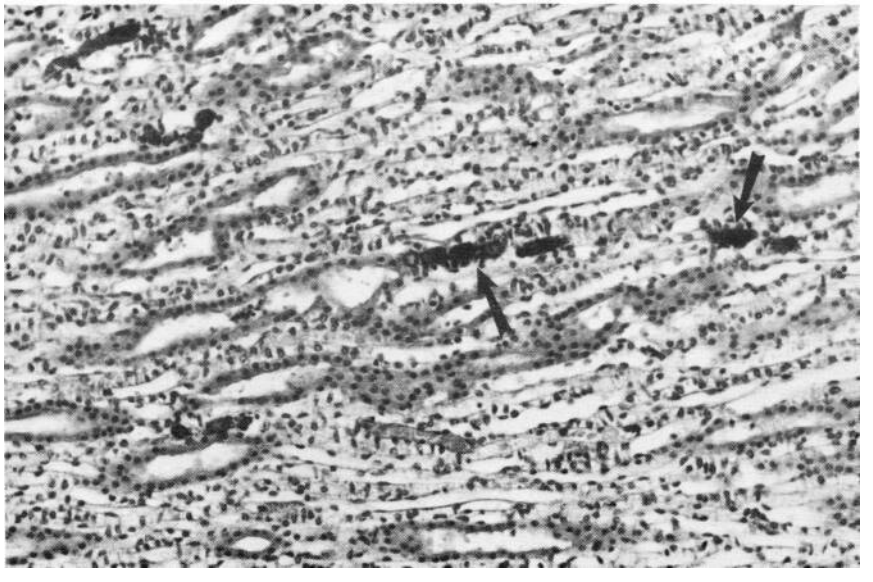


Figure 5. Kidney from male rat given 2,000 ppm pentachlorobenzene in feed for 13 weeks. Mineralization of medullary tubules is present (arrows) (hematoxylin and eosin).

TABLE 8. INCIDENCES OF SELECTED LESIONS IN RATS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Site/Lesion	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE						
Liver						
Centrilobular hypertrophy	0	0	0	10 (1.0)	10 (2.0)	10 (2.1)
Kidney						
Medullary tubular dilatation	0	0	3 (1.0)	10 (2.6)	10 (3.0)	10 (1.8)
Tubular cortex hyaline droplets (b)	0	0	6 (1.0)	10 (2.0)	10 (2.0)	10 (2.0)
Tubular cortex pigmentation	0	0	0	0	0	10 (1.0)
Tubular cortex regeneration	2 (1.0)	0	5 (1.0)	10 (2.0)	10 (2.0)	10 (2.0)
Tubular cortex protein casts	0	0	0	0	0	10 (1.4)
Cortex chronic inflammation	2 (1.0)	0	1 (1.0)	10 (2.0)	10 (3.0)	10 (1.1)
Medullary collecting tubule mineralization	0	0	0	10 (1.2)	10 (1.8)	10 (1.8)
Thyroid Gland						
Follicular cell hypertrophy	0	0	0	0	9	10
FEMALE						
Liver						
Centrilobular hypertrophy	0	0	0	0	10 (1.0)	10 (2.0)
Pigmentation	0	0	0	0	1 (1.0)	10 (1.1)
Periportal cytoplasmic vacuolization	0	0	0	0	1 (1.0)	8 (1.0)
Kidney						
Tubular cortex pigmentation	0	0	0	0	10 (1.0)	10 (2.0)
Tubular cortex regeneration	0	0	0	0	0	9 (1.5)
Tubular cortex protein casts	0	0	0	0	0	10 (1.3)
Cortex mineralization	8 (1.1)	10 (1.4)	10 (1.3)	9 (1.3)	10 (1.2)	10 (1.4)
Thyroid Gland						
Follicular cell hypertrophy	0	0	0	0	10	10

(a) Ten animals were examined from each group; mean severity of animals with the lesion is in parentheses; (1) = minimal; (2) = mild; (3) = moderate, (4) = marked.
(b) Diagnosed as cytoplasmic alteration by laboratory pathologist

characterized histologically as enlargement of hepatocytes with increased cytoplasmic eosinophilia and slightly enlarged nuclei. In the liver of two female rats (one each from the 1,000- and 2,000-ppm groups), pigmentation (small yellow-brown intracytoplasmic granules in hypertrophied hepatocytes) and cytoplasmic vacuolization (multiple clear, round intracytoplasmic vacuoles in periportal hepatocytes) were also noted. The hepatocellular pigment resembled the renal pigment in appearance, and results of special stains for this pigment were the same in both tissues.

Thyroid follicular cell hypertrophy of minimal severity was present in male and female rats in the 1,000- and 2,000-ppm groups. This lesion was characterized histologically by slight enlargement and increased height of thyroid follicular cells, often accompanied by cytoplasmic vacuolation and decreased staining intensity of intraluminal colloid. In some follicles, small papillary projections composed of follicular epithelium extended into the lumen.

STUDIES IN MICE

Fifteen-Day Studies

All mice that received diets containing 3,300 or 10,000 ppm pentachlorobenzene died before the end of the studies (Table 9) The final mean body weights of dosed mice that lived to the end of the studies were similar to those of controls Feed consumption by animals living to the end of the studies was not clearly compound related In mice that received 3,300 ppm, tremors, lethargy, hunched posture, and paralysis were observed on the day or the day before the animals died or were killed in a moribund condition, dyspnea was seen in females given 3,300 ppm. Compound-related clinical signs were not seen in animals that lived to the end of the studies The absolute liver weights and the liver weight to body weight ratios were significantly increased for males and females that received 330 and 1,000 ppm, at 1,000 ppm, the mean absolute liver weight was 1.7 times that of controls for males and 1 5 times that of controls for females

Mild-to-moderate depletion of thymic lymphocytes occurred in males and females that received 3,300 or 10,000 ppm, lymphocyte necrosis was characterized by pyknosis and karyorrhexis, and phagocytized cellular debris was also seen in the thymic macrophages of these animals These lesions are frequently seen in moribund or early-death animals. At lower concentrations, no compound-related lesions were noted in animals that lived to the end of the studies

TABLE 9. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FIFTEEN-DAY FEED STUDIES OF PENTACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Compound Consumption (e)
MALE							
0	5/5	21.3	22.3	+1 0		218	--
100	5/5	21.3	22 3	+1 0	100.0	183	18
330	5/5	21.7	23 3	+1 6	104.5	232	77
1,000	5/5	21.4	24 2	+2 8	108.5	191	191
3,300	(f) 0/5	21.3	(g)	(g)	(g)	(g)	(g)
10,000	(h) 0/5	20.8	(g)	(g)	(g)	(g)	(g)
FEMALE							
0	5/5	18.4	18 7	+0 3		292	-
100	(i) 5/5	18.3	19 0	+0 7	101 6	263	26
330	5/5	18.1	19 2	+1 1	102 7	258	85
1,000	5/5	17.8	19 4	+1 6	103 7	231	231
3,300	(j) 0/5	18.2	(g)	(g)	(g)	(g)	(g)
10,000	(k) 0/5	18.6	(g)	(g)	(g)	(g)	(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 of feed per kilogram body weight per day averaged over the 2-wk period, not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights; not corrected for scatter
(f) Day of death. 4,4,5,5,5
(g) No data are reported due to 100% mortality in this group.
(h) Day of death 3,3,3,3,4
(i) One animal died on day 15.
(j) Day of death: 4,6,6,7,10
(k) Day of death. all 4

Thirteen-Week Studies

No compound-related deaths occurred (Table 10). The final mean body weights were not compound related (Table 11 and Figure 6). Compound-related clinical signs included ventral body swelling and ruffled fur in males and females that received 2,000 ppm.

Compound-related increases in the absolute organ weights and organ weight to body weight ratios were seen for the kidney of males and the liver of males and females (Table 11). The absolute kidney weights were increased in male mice given dietary concentrations as low as 330 ppm; the relative kidney weights for males were increased at concentrations as low as 1,000 ppm. The absolute liver weights were increased at dietary concentrations of 100 ppm and above for males and 330 ppm and above for females. The relative liver weights were increased for male and female mice given diets containing 330 ppm or higher concentrations.

The hemoglobin concentration was significantly decreased and the platelet count was significantly increased for males and females that received 2,000 ppm (Table A3). These values are considered to be within normal variation. Serum sorbitol dehydrogenase activity was significantly increased for males and females that received 1,000 or 2,000 ppm, with a threefold increase at 2,000 ppm. The γ-glutamyl transferase activity was increased fourfold for females at 2,000 ppm.

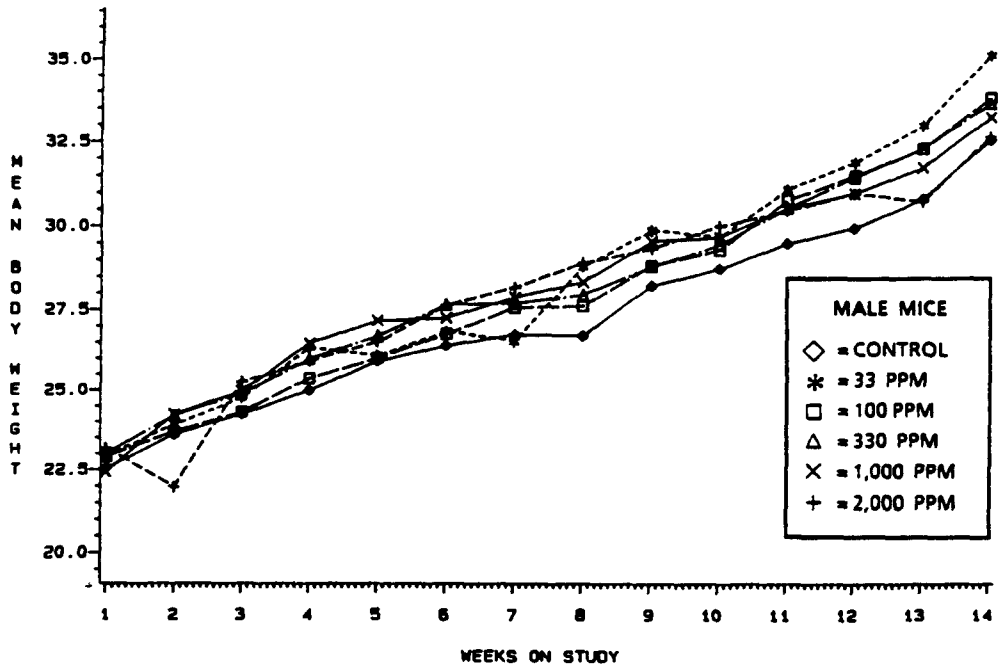
Total thyroxin concentrations were significantly decreased in males and females at pentachlorobenzene concentrations down to 33 ppm. Triiodothyronine concentrations were not dose related. In both control and exposed groups, the thyrotropin concentration either was generally lower than the limit of detection for the analysis or could not be measured because of insufficient serum. Liver porphyrin values at the end of the studies were significantly increased in males and females at 1,000 and 2,000 ppm (nearly twofold for males and threefold to sixfold for females). Sperm morphology endpoints (testicular and epididymal weights and epididymal sperm density, motility, and morphology) and female estrous cycle length were not affected by administration of pentachlorobenzene.

Compound-related increased incidences of minimal-to-moderate centrilobular hepatocellular hypertrophy were present in the liver in male mice in all exposed groups and in female mice in the 330-, 1,000-, and 2,000-ppm groups (Table 12). In exposed animals of each sex, individual cell necrosis of hypertrophied hepatocytes was occasionally present and was considered secondary to the hypertrophy. Necrotic hepatocytes had deeply eosinophilic cytoplasm and pyknotic, often karyorrhectic nuclei. Unlike the individual hepatocellular necrosis seen in dosed mice, the liver lesion in the one control female was a focal lesion and not associated with hypertrophy.

TABLE 10. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE EXAMINED HISTOLOGICALLY IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d)	Compound Consumption (e)
MALE							
0	(f) 9/10	22.5 ± 0.4	32.6 ± 1.0	+9 9 ± 0.9		4 4	-
33	10/10	22.9 ± 0.4	35.2 ± 1.0	+12.3 ± 0.8	108.0	4.6	5 2
100	10/10	22.9 ± 0.3	33.9 ± 0.9	+11.0 ± 0.9	104.0	4 5	16
330	10/10	23.0 ± 0.4	33.7 ± 0.7	+10.7 ± 0.5	103.3	4.5	52
1,000	10/10	22 4 ± 0.4	33.3 ± 0.6	+10.9 ± 0 7	102.1	4.5	162
2,000	10/10	23.2 ± 0.3	32.7 ± 0.4	+9 5 ± 0.4	100.3	4.3	308
FEMALE							
0	10/10	18 6 ± 0.4	27.2 ± 0.6	+8 6 ± 0 8		5.1	--
33	10/10	18.6 ± 0.4	28.2 ± 0.8	+9 5 ± 0 6	103.7	4.9	6.9
100	10/10	18.6 ± 0.4	27.0 ± 0.4	+8 4 ± 0 6	99.3	5.1	22
330	10/10	18 7 ± 0 4	26.9 ± 0.6	+8 2 ± 0 4	98.9	4.7	68
1,000	10/10	18 5 ± 0.4	26.6 ± 0.6	+8 1 ± 0 5	97.8	4.9	217
2,000	10/10	18.9 ± 0.4	26.9 ± 0.3	+8 1 ± 0.4	98.9	4.7	410

(a) Number surviving/number initially in group for animals not bled sequentially
(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 of feed per animal per day averaged over the 13-wk period for both bled and unbled groups (combined); not corrected for scatter.
(e) Milligrams per kilogram per day, based on mean of initial and final body weights; not corrected for scatter.
(f) Death judged to be accidental



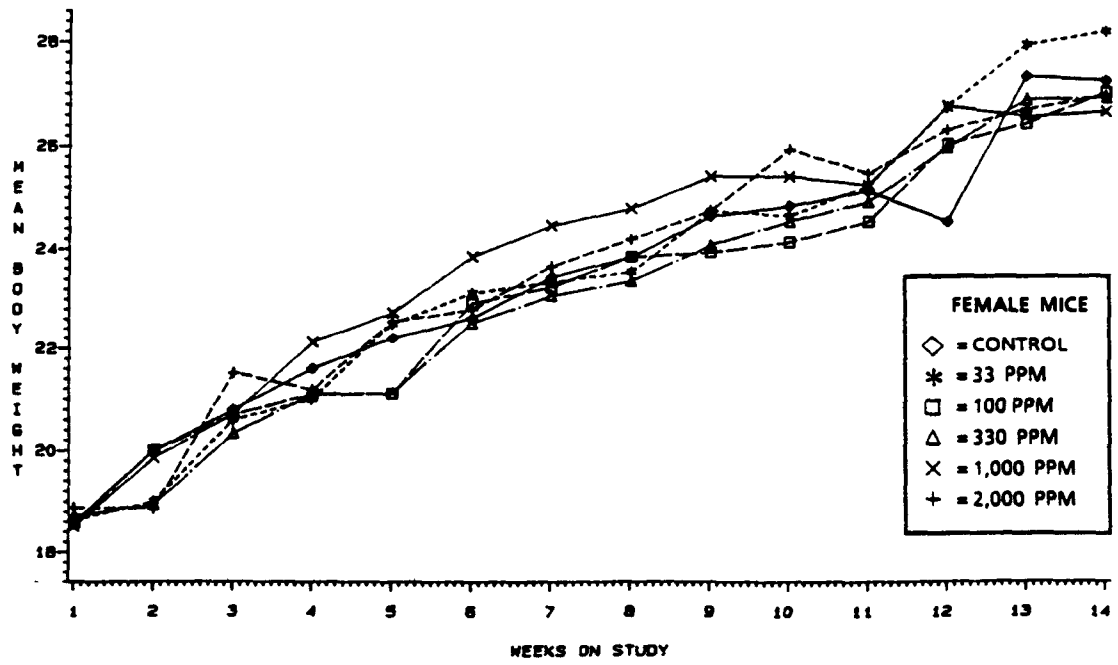


FIGURE 6. GROWTH CURVES FOR MICE FED DIETS CONTAINING PENTACHLOROBENZENE FOR THIRTEEN WEEKS AND EXAMINED HISTOLOGICALLY (NOT BLED)

TABLE 11. ORGAN WEIGHTS FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Organ	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE (b)						
Body weight (grams)	(c) 31.4 ± 0.97	33.2 ± 0.95	32.5 ± 0.80	32.6 ± 0.86	31.8 ± 0.53	31.6 ± 0.53
Right kidney						
Absolute	(c) 257 ± 6.2	269 ± 6.6	270 ± 4.8	*279 ± 6.6	**292 ± 5.5	**301 ± 11 2
Relative	(c) 8.2 ± 0.22	8.1 ± 0.19	8.3 ± 0.16	8.6 ± 0.26	*9.2 ± 0.30	**9.5 ± 0.26
Liver						
Absolute	(c) 1,352 ± 35	1,445 ± 47	*1,471 ± 29	**1,693 ± 72	**2,145 ± 43	**3,585 ± 112
Relative	(c) 43.2 ± 0.73	43.6 ± 0.85	45.5 ± 1.01	**51.9 ± 1.64	**67.5 ± 1 66	**113.3 ± 2.80
FEMALE (b)						
Body weight (grams)	26.6 ± 0.57	27.4 ± 0.88	26.5 ± 0.53	26.1 ± 0.69	27.2 ± 0.61	26.2 ± 0.58
Right kidney						
Absolute	184 ± 4.2	179 ± 3.8	189 ± 4.3	180 ± 4.9	195 ± 3.3	188 ± 4.0
Relative	6.9 ± 0.17	6.6 ± 0.19	7.1 ± 0.18	6.9 ± 0.17	7.2 ± 0.13	7.2 ± 0.12
Liver						
Absolute	1,226 ± 40	1,200 ± 38	1,322 ± 50	*1,345 ± 34	**2,019 ± 60	**3,220 ± 99
Relative	46.1 ± 0.70	44.0 ± 1.00	49.7 ± 1.24	**51.5 ± 0.43	**74.2 ± 1.25	**122.7 ± 1.85

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

TABLE 12. INCIDENCES OF SELECTED LIVER LESIONS IN MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Lesion	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE						
Number examined	10	10	10	10	10	10
Centrilobular hypertrophy	0	3 (1.0)	7 (1.0)	10 (1.7)	10 (2.9)	10 (3.0)
Necrosis	0	1 (1.0)	0	0	0	1 (2.0)
FEMALE						
Number examined	9	10	9	10	10	10
Centrilobular hypertrophy	0	0	0	2 (1.0)	10 (1.8)	10 (2.6)
Necrosis	1 (1.0)	0	0	1 (1.0)	0	4 (2.0)

(a) Mean severity of animals with the lesion is in parentheses; (1) = minimal; (2) = mild; (3) = moderate; (4) = marked.

IV. DISCUSSION AND CONCLUSIONS

In these studies, the organs most affected by the administration of pentachlorobenzene in feed were the kidney and liver in rats and the liver in mice. Minimal thyroid gland effects also occurred in rats. In general, exposure-related lesions were more extensive in rats than in mice.

In 15-day studies, deaths were observed in groups of rats receiving diets containing 10,000 ppm and in mice receiving diets containing 3,300 ppm or higher concentrations of pentachlorobenzene. Compound-related deaths were not observed in rats or mice in the 13-week studies, which used average estimated daily doses of 2-160 mg/kg for rats and 5-400 mg/kg for mice. Actual doses on a milligram per kilogram basis were higher during the early weeks of the studies because young animals tend to consume relatively more diet per kilogram body weight than they do later in the studies. There were no characteristic clinical signs of toxicity observed in these studies, and no clear cause of death could be determined.

In the 15-day studies, compound-related histologic lesions in rats included hyaline droplet accumulation in the renal tubular epithelium of exposed males in the 100- to 3,300-ppm groups. Early death probably precluded development of this lesion in animals in the 10,000-ppm group. The reason for the absence of this lesion in the 10,000-ppm groups and in 3/5 animals in the 3,300-ppm group is unknown. Possibly, there was decreased production of $\alpha_{2\mu}$-globulin by the liver with increased toxicity at these doses.

Other histologic changes were thymic lymphocyte depletion and forestomach hyperkeratosis, seen in the 10,000-ppm rats of each sex. Mild-to-moderate thymic lymphocyte depletion and necrosis were present in higher dose male and female mice. These lesions are often seen in moribund and early-death rodents.

In the 13-week studies, kidney lesions were observed in rats of each sex but were most pronounced in males. Male rats in groups receiving diets containing as little as 100 ppm pentachlorobenzene exhibited hyaline droplet accumulation similar to, but more extensive than, that seen in the exposed male rats in the 15-day study. Hyaline droplet accumulation and tubular dilatation were noted previously in male rats given 125 or 1,000 ppm pentachlorobenzene in the diet for 100 days (Linder et al., 1980).

Other renal lesions observed in male rats in the 13-week studies included tubular dilatation and granular cast formation in the outer stripe of the outer medulla and focal mineralization of medullary collecting ducts. These lesions are consistent with those 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 male androgens and decline in older rats (Roy, 1977), so hyaline nephropathy occurs only in intact, young adult male rats (Alden, 1986) or female rats pretreated with testosterone (Roy, 1977). Administration to rats of various compounds, including light hydrocarbons (Trump et al., 1985), unleaded gasoline components (Olson et al., 1987; Short et al., 1987), jet fuel (Bruner, 1984), *d*-limonene (NTP, 1990b), and others (Dodd et al., 1987; Read et al., 1988), caused increased incidences of hyaline droplet accumulation, hyaline droplet nephropathy, and in some cases, increased incidences of renal epithelial neoplasms (Alden et al., 1984; Busey and Cockrell, 1984; Halder et al., 1984; Kitchen, 1984; Phillips and Cockrell, 1984; Stonard et al., 1986; Dodd et al., 1987).

Accumulation of "eosinophilic inclusions" morphologically compatible with hyaline droplets was reported in male rats fed pentachlorobenzene (Chu et al., 1983). Other chlorinated benzenes have been implicated as a cause of renal toxicity in male rats. Long-term administration of 1,4-dichlorobenzene, but not 1,2-dichlorobenzene, resulted in hyaline droplet accumulation

and epithelial neoplasms in the kidney of F344 rats (NTP, 1985, 1987; Bomhard et al., 1988; Charbonneau et al., 1989). Renal epithelial neoplasms also occurred in rats fed hexachlorobenzene (Lambrecht et al., 1983), of which pentachlorobenzene is a metabolite (Mehendale et al., 1975; Debets et al., 1981; Billi and San Martin de Viale, 1985; Stewart and Smith, 1986).

The exact pathogenesis of hyaline droplet nephropathy is unknown, but 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 epithelial cells (Trump et al., 1985). The resulting phagolyososomes are seen by light microscope as abnormal hyaline droplets in cortical epithelial cells (Short et al., 1987). Cortical epithelial cell necrosis with subsequent tubular regeneration results from hyaline droplet accumulation (Short et al., 1987; Swenberg et al., 1989).

In addition, male rats in the 100- to 2,000-ppm groups and female rats in the 1,000- and 2,000-ppm groups had homogeneous protein tubular casts and focal chronic inflammation in the kidney, which were typical of the spontaneous nephropathy commonly seen in various strains of laboratory rats (Peter et al., 1986). Thus, exposure to pentachlorobenzene was also associated with an exacerbation in severity of nephropathy, especially in male rats.

Urinary glucose and protein concentrations were increased in male and female rats in the higher dose groups. Urinary protein increases in male rats in the 1,000- to 2,000-ppm groups were particularly striking (3 to 10 times greater than controls). Increases in urinary protein and glucose were reported previously in male F344 rats with hyaline droplet nephropathy induced by administration of a light hydrocarbon (C_{10}-C_{11} isoparaffin) (Phillips and Cockrell, 1984).

These findings may result from impaired cortical tubular resorption of glucose and protein, due to pentachlorobenzene-induced hyaline droplet nephropathy in male rats. Granular casts and sloughed tubular epithelial cells could have contributed to the increased urinary protein levels in both sexes. The exacerbated spontaneous nephropathy present in each sex could also have contributed to the observed proteinuria and glucosuria (Peter et al., 1986).

Compound-related histologic liver lesions occurred in both species. Centrilobular hepatocellular hypertrophy was present in higher dose groups of male and female rats in the 15-day and 13-week studies. Centrilobular hepatocellular hypertrophy with minimal necrosis was present in exposed mice of each sex in the 13-week studies. The marked increased liver weight (absolute and relative) at the highest dose may explain the clinical observation of "distended abdomen." There was no evidence of ascites or other lesions to account for this clinical observation.

Hepatocellular hypertrophy was previously seen in the liver of male and female rats given pentachlorobenzene in the diet (Linder et al., 1980; Chu et al., 1983). Hepatocellular hypertrophy was also observed after administration of compounds, such as other chlorinated benzenes and halogenated biphenyls, which induce hepatic microsomal enzymes; ultrastructurally, the light microscopic changes correlate with increased amounts of smooth endoplasmic reticulum (Strik et al., 1980; Kuiper-Goodman and Grant, 1986).

Pentachlorobenzene acts as a "phenobarbital type" inducer of microsomal cytochrome P450 enzymes, as well as of other hepatocellular enzymes (Ariyoshi et al., 1975; Goerz et al., 1978; Goldstein et al., 1982, 1986; Denomme et al., 1983). Therefore, the hepatic changes observed in these studies may be morphologic manifestations of metabolic enzyme induction.

In the 13-week studies, pigment accumulation was present in hepatocytes of a few female rats given 1,000 or 2,000 ppm pentachlorobenzene and in renal tubular epithelial cells of male rats given 2,000 ppm and female rats given 1,000 or 2,000 ppm. The granular, yellow-brown, intracytoplasmic pigment was negative for special stains for hemosiderin iron (Prussian blue), bile (Hall's stain), and ceroid/lipofuchsin (oil red O and Ziehl-Neelsen acid-fast stain). These results imply, but do not unequivocally prove, that the pigment granules consisted of one or more porphyrins.

Significant but not dose-related increases in liver porphyrin concentrations were noted in exposed male rats, whereas only the females receiving diets containing 2,000 ppm pentachlorobenzene had significantly increased liver porphyrin. Similar mild increases in liver porphyrin values were reported in rats (Linder et al., 1980). The results of the current studies indicate that, compared with hexachlorobenzene (San Martin de Viale et al., 1970; Boger et al., 1979; Smith et al., 1985; Carlson and Kosian, 1987), pentachlorobenzene is not highly porphyrinogenic in rats.

Liver porphyrin values were significantly increased in male and female mice in the 1,000- and 2,000-ppm groups. These increases were much more pronounced in females (three to six times the control values) than in males (about twice the control values) (Table A3). Histologically, there was no detectable porphyrinlike pigment in mice of either sex.

Pigment accumulation in liver and/or kidney was more extensive in females than in males of either species. In other studies, female rats have been shown to be much more sensitive than males to the porphyrinogenic effects of hexachlorobenzene and other polyhalogenated aromatic hydrocarbons (San Martin de Viale et al., 1970; Strik et al., 1980; Kuiper-Goodman and Grant, 1986). Estrogenic drugs can increase the susceptibility of male rats to hexachlorobenzene-induced porphyria, perhaps by decreasing the activity of uroporphyrinogen decarboxylase (Smith and Francis, 1981). Possibly, female sex hormones played a potentiating role in porphyrin accumulation in the current studies.

Increased serum sorbitol dehydrogenase activity is a nonspecific indicator of mild hepatocyte damage (Duncan and Prasse, 1977), so the significantly increased activity seen in exposed male and female rats and mice was not surprising. The slight increase in γ-glutamyl transferase (GGT) activity in mice in the 2,000-ppm groups suggests mild cholestasis.

Compound-related histologic changes were present in the thyroid gland in male and female rats in the 13-week studies. Thyroid follicular cell hypertrophy and a decreased staining intensity of intraluminal colloid of minimal severity occurred in rats given 1,000 or 2,000 ppm pentachlorobenzene. Similar lesions have been noted previously in rats fed diets containing 500 ppm pentachlorobenzene for 28 days (Chu et al., 1983).

Induction of thyroid gland proliferative lesions (hyperplasia, hypertrophy and/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), 1,4-dichlorobenzene (NTP, 1987), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (NTP, 1982), and polychlorinated biphenyls (Capen and Martin, 1989).

In the current studies, free thyroxin and total thyroxin concentrations in serum were significantly decreased for male rats in the 33- to 2,000-ppm groups and female rats in the 100- to 2,000-ppm groups (Table A2). Free thyroxin is an important measurement of thyroid function, independent of changes in binding proteins. Thyrotropin concentrations were significantly increased in males and females that received 1,000 and 2,000 ppm; however, the erratic values at different times (Table A2) may reflect laboratory assay performance rather than physiologic changes related to exposure. Overall, the thyroid hormone data in rats are strongly indicative of a moderate primary hypothyroxinemia, especially in males.

In studies with other polyhalogenated aromatic hydrocarbons, similar functional abnormalities have also been noted, frequently in conjunction with morphologic thyroid gland lesions similar to those described above (Collins and Capen, 1980; van den Berg et al., 1988).

Decreased serum thyroxin concentrations 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 were also accelerated, presumably because of chemical-related induction of hepatic thyroxine UDP-glucuronyltransferase (Bastomsky and Murthy, 1976; McClain, 1989). Collins et al. (1977) proposed that the lowered thyroxin concentrations after polychlorinated biphenyl administration

are caused by 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 found in these studies was not determined.

In male mice exposed to 330 or 1,000 ppm pentachlorobenzene, there were significant decreases at all time points in free thyroxine (with the exception of 1,000 ppm at day 15). There were no significant decreases at 2,000 ppm. In the same mice, in dose groups at and above 100 ppm, there were significant decreases in total thyroxine concentrations at all time points. No significant changes occurred, however, in triiodothyronine or thyrotropin concentrations.

In female mice, significant changes (decreases) occurred only in total thyroxine concentrations (all time points, at and above concentrations of 33 ppm). Changes in hormone concentrations in female mice do not provide strong evidence for a primary effect on the thyroid gland. Changes in serum concentrations of binding proteins, for example, can produce decreases in total thyroxine without affecting concentrations of the other hormones (Henry, 1984). In male mice, the decreases in free thyroxine (not dose related) and in total thyroxine do provide some evidence for a primary effect on the thyroid gland.

Hematologic findings for supplemental study rats included significantly decreased hematocrit values, hemoglobin concentration, mean corpuscular hemoglobin, and mean cell volume in males and females that received 1,000 or 2,000 ppm. These findings are suggestive of a microcytic, mildly hypochromic, poorly regenerative anemia. Because the serum albumin and creatinine concentrations indicate possible dehydration, the anemia may have been even more severe than is indicated by the absolute hematologic values. Reticulocyte counts were increased in males that received 2,000 ppm, but these findings are probably attributable to dehydration. Similar changes in hematologic values were noted in male F344/N rats given 1,4-dichlorobenzene by gavage (NTP, 1987).

The exact cause of this anemia was not determined, but the disturbances in porphyrin metabolism may have played a role, since porphyrins are precursors in heme biosynthesis (Bonkowsky, 1982). Decreased renal erythropoietin production, considered an important pathogenic factor in the anemia of renal failure (Anagnostou et al., 1981), could also have contributed to the hematologic derangements observed in the current studies.

The increased percentage of abnormal sperm in male rats that received 330 or 2,000 ppm pentachlorobenzene was considered to be an exposure-related change that may reflect a primary effect on spermatogenesis. The reduced estrous cycle length in exposed female rats was not considered to be exposure related.

The no-observed effect levels (NOELs) for histologic lesions were 33 ppm for male rats and 330 ppm for female rats. The NOEL for histologic lesions in female mice was 100 ppm. A NOEL was not reached for male mice.

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APPENDIX

ORGAN WEIGHTS AND HEMATOLOGIC, SERUM CHEMISTRY, URINALYSIS, REPRODUCTIVE SYSTEM, AND LIVER PORPHYRIN DATA FOR RATS AND MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE

TABLE A1. ORGAN WEIGHTS FOR RATS EXAMINED HISTOLOGICALLY IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Organ	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE						
Body weight (grams)	354 ± 7.1	339 ± 7.9	344 ± 8.5	345 ± 5.6	335 ± 8.3	**299 ± 6 8
Brain						
Absolute	1,905 ± 25	1,936 ± 14	1,933 ± 20	1,936 ± 18	1,905 ± 23	1,891 ± 21
Relative	5.4 ± 0.12	*5.8 ± 0.14	5.6 ± 0.14	5.6 ± 0.08	5.7 ± 0.12	**6.4 ± 0.14
Heart						
Absolute	1,060 ± 36	1,105 ± 47	1,047 ± 35	1,026 ± 21	1,067 ± 42	998 ± 26
Relative	3.0 ± 0.10	3.3 ± 0.12	3.0 ± 0.05	3.0 ± 0.07	3.2 ± 0.09	*3.4 ± 0.12
Right testis						
Absolute	1,489 ± 20	1,462 ± 20	1,481 ± 25	1,468 ± 24	1,506 ± 22	1,495 ± 18
Relative	4.2 ± 0.09	4.3 ± 0.11	4.3 ± 0.06	4.3 ± 0.07	*4.5 ± 0.06	**5.0 ± 0 07
FEMALE						
Body weight (grams)	209 ± 5.3	200 ± 2.9	197 ± 3.1	**189 ± 4.5	*195 ± 2.2	**193 ± 2.2
Brain						
Absolute	1,789 ± 31	1,831 ± 19	1,793 ± 17	1,737 ± 27	1,760 ± 29	1,754 ± 22
Relative	8.6 ± 0.24	9.2 ± 0.17	9.1 ± 0.13	9.2 ± 0.19	9.0 ± 0.17	9.1 ± 0.13
Heart						
Absolute	706 ± 15	661 ± 13	692 ± 15	649 ± 23	717 ± 15	708 ± 11
Relative	3.4 ± 0.08	3.3 ± 0.06	3.5 ± 0.09	3.4 ± 0.09	*3.7 ± 0.09	*3.7 ± 0.07

(a) Mean ± standard error in milligrams (absolute) or milligrams per gram (relative) 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. RESULTS OF SUPPLEMENTAL ANALYSES FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Analysis	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE						
No examined (b)	9	10	10	10	10	10
Hematocrit (percent)	46 1 ± 0 52	45 7 ± 0 43	46 2 ± 0 88	44 9 ± 0 49	**42 0 ± 0 76	**41 9 ± 0 26
Hemoglobin (g/dl)	16 6 ± 0 13	16 5 ± 0 11	16 5 ± 0 23	*16 0 ± 0 16	**14 8 ± 0 21	**14 9 ± 0 12
Mean corpuscular hemoglobin (pg)	18 6 ± 0 22	18 6 ± 0 14	18 4 ± 0 18	18 1 ± 0 07	**17 8 ± 0 10	**17 9 ± 0 13
Mean corpuscular hemoglobin concentration (g/dl)	36 1 ± 0 37	36 1 ± 0 21	35 8 ± 0 38	35 8 ± 0 20	*35 3 ± 0 23	35 5 ± 0 29
Mean cell volume (microns^3)	51 4 ± 0 38	51 7 ± 0 30	51 4 ± 0 27	50 7 ± 0 30	*50 4 ± 0 27	*50 4 ± 0 37
Platelets (10^3/microliter)	558 ± 8 9	579 ± 22 0	547 ± 15 5	550 ± 8 0	*592 ± 9 6	**648 ± 17 2
Erythrocytes (10^6/microliter)	9 0 ± 0 13	8 8 ± 0 06	9 0 ± 0 17	8 9 ± 0 10	**8 3 ± 0 16	**8 3 ± 0 07
Reticulocytes (10^6/microliter)	0 14 ± 0 011	0 12 ± 0 012	0 15 ± 0 012	0 14 ± 0 013	0 16 ± 0 013	*0 19 ± 0 012
Albumin (g/dl)						
Day 3	(c) 4 3 ± 0 05	4 3 ± 0 06	4 4 ± 0 05	4 4 ± 0 07	4 3 ± 0 09	4 3 ± 0 09
Day 15	4 3 ± 0 09	4 3 ± 0 09	(d) 4 4 ± 0 07	4 4 ± 0 08	**4 6 ± 0 05	**5 3 ± 0 09
Day 45	4 5 ± 0 05	4 7 ± 0 06	4 5 ± 0 10	*4 9 ± 0 11	**5 1 ± 0 14	**5 7 ± 0 11
Day 90	5 1 ± 0 07	5 0 ± 0 12	5 3 ± 0 09	5 1 ± 0 06	**5 9 ± 0 08	**6 2 ± 0 06
Creatinine (mg/dl)						
Day 3	(c) 0 36 ± 0 037	0 34 ± 0 037	0 37 ± 0 026	0 34 ± 0 040	0 37 ± 0 033	0 37 ± 0 030
Day 15	0 28 ± 0 015	0 30 ± 0 026	0 29 ± 0 026	0 32 ± 0 025	**0 44 ± 0 034	**0 38 ± 0 025
Day 45	0 39 ± 0 020	0 40 ± 0 015	0 44 ± 0 016	**0 46 ± 0 016	**0 58 ± 0 025	**0 53 ± 0 015
Day 90	0 51 ± 0 035	0 52 ± 0 029	0 57 ± 0 040	0 57 ± 0 026	**0 69 ± 0 023	**0 66 ± 0 016
Sorbitol dehydrogenase (IU/liter)						
Day 3	(e) 8 3 ± 0 49	(d) 8 9 ± 0 64	(f) 9 6 ± 0 51	(g) 8 4 ± 0 53	(h) 8 9 ± 0 98	f) 10 0 ± 0 63
Day 15	7 8 ± 1 30	(h) 9 9 ± 1 01	(h) 9 3 ± 0 71	10 8 ± 1 37	**(h) 13 6 ± 0 53	** h) 11 6 ± 0 87
Day 45	(d) 8 3 ± 0 31	7 0 ± 0 42	8 3 ± 0 30	(d) 9 5 ± 0 57	*(d) 10 8 ± 0 82	**(h) 13 7 ± 0 96
Day 90	12 3 ± 1 07	9 3 ± 0 47	7 6 ± 0 90	11 7 ± 0 33	13 7 ± 1 80	**22 2 ± 1 02
GGT (IU/liter)						
Day 3	(c) 2 7 ± 0 45	2 2 ± 0 47	4 5 ± 1 10	3 8 ± 0 95	4 1 ± 0 74	2 6 ± 0 64
Day 15	0 9 ± 0 68	2 1 ± 0 91	2 6 ± 1 02	1 6 ± 0 43	1 1 ± 0 23	*3 3 ± 1 06
Day 45	0 9 ± 0 68	0 6 ± 0 34	0 3 ± 0 15	0 7 ± 0 40	0 2 ± 0 20	**2 5 ± 0 43
Day 90	0 7 ± 0 33	1 0 ± 0 37	0 4 ± 0 16	0 6 ± 0 27	0 3 ± 0 15	2 0 ± 0 37
Triiodothyronine (ng/dl)						
Day 15	80 7 ± 2 66	76 4 ± 4 92	76 8 ± 3 21	**61 8 ± 2 68	**51 4 ± 2 28	**65 4 ± 3 54
Day 45	(d) 102 9 ± 6 18	114 6 ± 8 24	103 6 ± 7 29	(h) 89 9 ± 8 53	*73 7 ± 4 99	94 5 ± 8 56
Day 90	82 7 ± 5 72	66 0 ± 4 12	76 0 ± 2 72	*64 1 ± 3 70	*64 1 ± 2 57	73 4 ± 3 59
Free thyroxin (ng/dl)						
Day 15	0 28 ± 0 011	**0 22 ± 0 014	**0 21 ± 0 009	**0 10 ± 0 005	**0 08 ± 0 002	**0 10 + 0 005
Day 45	0 23 ± 0 008	0 21 ± 0 007	**0 18 ± 0 009	**0 13 ± 0 005	**0 10 ± 0 004	**0 10 ± 0 004
Day 90	0 22 ± 0 007	**0 18 ± 0 006	**0 19 ± 0 006	**0 13 ± 0 003	**0 09 ± 0 004	**0 10 ± 0 005
Total thyroxin (micrograms/dl)						
Day 15	6 4 ± 0 21	**5 2 ± 0 27	**4 5 ± 0 19	**1 9 ± 0 06	**1 1 ± 0 06	** h) 1 2 ± 0 05
Day 45	6 6 ± 0 25	6 0 ± 0 26	**5 0 ± 0 27	**2 5 ± 0 10	**1 6 ± 0 06	**1 4 ± 0 05
Day 90	6 7 ± 0 19	**5 3 ± 0 20	**5 4 ± 0 15	**3 0 ± 0 10	**1 7 ± 0 03	**1 7 ± 0 04
Thyrotropin (ng/ml)						
Day 15	3 4 ± 0 37	*5 2 ± 0 88	(d) 3 9 ± 0 41	4 5 ± 0 72	**(d) 6 8 ± 0 66	** d) 6 8 ± 1 02
Day 45	(d) 6 1 ± 0 93	(h) 5 9 ± 0 72	(g) 8 2 ± 1 76	(g) 4 7 ± 0 55	(h) 6 0 ± 0 50	h) 5 7 ± 0 77
Day 90	2 9 ± 0 21	3 5 ± 0 59	*3 8 ± 0 33	3 3 ± 0 46	*4 0 ± 0 39	**5 5 ± 0 82
Liver porphyrin (micrograms/g) for nonbled animals						
Day 90	(c) 0 54 ± 0 075	*0 77 ± 0 076	0 62 ± 0 083	*0 86 ± 0 080	*0 92 ± 0 157	*0 80 ± 0 113
Day 90 (ratio) (i)		143	115	159	170	148
Abnormal sperm (percent) for nonbled animals						
Day 90	(c) 0 620 ± 0 76	0 680 ± 0 085		*1 060 ± 0 133		**1 240 ± 0 181
Day 90 (ratio) (i)		110		171		200
Sperm density (× 10^6)						
Day 90	769 ± 42	739 ± 43		754 ± 41		799 ± 37
Day 90 (ratio) (i)		0 96		0 98		1 04
Sperm motility (percent)						
Day 90	95 1 ± 0 74	94 6 ± 0 69		93 0 ± 1 06		92 9 ± 1 31
Day 90 (ratio) (i)		0 99		0 98		0 98
Caudal weight (mg)						
Day 90	178 ± 4	178 ± 6		175 ± 5		168 ± 2
Day 90 (ratio) (i)		1 00		0 98		0 94
Right epididymal weight (mg)						
Day 90	435 ± 5	435 ± 6		432 ± 8		418 ± 4
Day 90 (ratio) (i)		1 00		0 99		0 96
No examined for urinalysis (b)	9	5	4	5	10	10
Urine specific gravity						
Day 3	(e) 1 036 ± 0 004	(j) 1 030 ± 0 003	(k) 1 028 ± 0 009	(d) 1 039 ± 0 004	(g) 1 044 ± 0 007	1 028 ± 0 002
Day 15	(l) 1 030 ± 0 005	1 047 ± 0 010	1 038 ± 0 012	1 039 ± 0 011	(f) 1 032 ± 0 003	(d) 1 044 ± 0 003
Day 90	1 058 ± 0 003	(g) 1 054 ± 0 008	(h) 1 065 ± 0 004	**(h) 1 044 ± 0 001	**1 041 ± 0 001	**1 041 ± 0 001
Urinary glucose (mg/16 h)						
Day 3	1 5 ± 0 75	1 5 ± 0 75	1 7 ± 0 27	1 6 ± 0 19	1 3 ± 0 27	1 8 ± 0 28
Day 15	0 8 ± 0 14	1 5 ± 0 51	0 7 ± 0 17	1 0 ± 0 22	2 0 ± 0 43	**3 6 ± 0 40
Day 90	1 4 ± 0 11	1 3 ± 0 25	1 2 ± 0 13	**3 5 ± 0 28	**5 4 ± 0 24	**6 6 ± 0 40

TABLE A2. RESULTS OF SUPPLEMENTAL ANALYSES FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (Continued)

Analysis	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE (Continued)						
Urine pH						
Day 3	(e) 6 3 ± 0 11	(j) 6 3 ± 0 25	(k) 6 3 ± 0 17	(d) 6 1 ± 0 08	6 2 ± 0 08	6 2 ± 0 08
Day 15	(l) 6 8 ± 0 14	6 4 ± 0 19	6 5 ± 0 20	6 5 ± 0 16	(f) 6 5 ± 0 22	**(d) 6 1 ± 0 06
Day 90	6 4 ± 0 11	(d) 6 4 ± 0 18	(c) 6 4 ± 0 11	(h) 6 2 ± 0 08	6 2 ± 0 08	6 1 ± 0 07
Urinary protein (mg/16 h)						
Day 3	3 0 ± 0 36	2 7 ± 1 07	2 6 ± 0 55	2 8 ± 0 49	1 7 ± 0 39	2 4 ± 0 40
Day 15	2 7 ± 0 57	2 9 ± 0 67	3 0 ± 1 36	3 2 ± 1 41	4 7 ± 0 91	4 9 ± 0 59
Day 90	3 4 ± 0 25	2 9 ± 0 53	3 0 ± 0 39	*6 0 ± 0 56	**11 8 ± 1 73	**34 7 ± 4 06
Urine volume (ml/16 h)						
Day 3	(e) 3 2 ± 0 48	(j) 3 9 ± 2 35	(k) 3 2 ± 0 43	(h) 2 5 ± 0 56	1 3 ± 0 40	3 1 ± 0 43
Day 15	(f) 1 8 ± 0 61	2 1 ± 0 78	2 6 ± 1 42	(e) 2 2 ± 1 13	(f) 2 6 ± 0 49	(d) 2 7 ± 0 28
Day 90	2 2 ± 0 19	(h) 1 6 ± 0 39	(c) 1 3 ± 0 23	(c) 3 6 ± 0 36	**4 5 ± 0 40	**5 5 ± 0 40
FEMALE						
No examined (b)	10	9	10	10	10	9
Hematocrit (percent)	46 1 ± 0 65	46 4 ± 0 61	45 6 ± 0 55	45 9 ± 0 64	**43 5 ± 0 48	**43 3 ± 0 47
Hemoglobin (g/dl)	16 6 ± 0 11	16 6 ± 0 10	16 2 ± 0 13	16 3 ± 0 14	**15 5 ± 0 15	**15 4 ± 0 18
Mean corpuscular hemoglobin (pg)	19 8 ± 0 18	19 6 ± 0 10	19 4 ± 0 16	19 2 ± 0 28	**18 6 ± 0 05	**18 1 ± 0 14
Mean corpuscular hemoglobin concentration (g/dl)	36 0 ± 0 38	35 7 ± 0 33	35 5 ± 0 28	35 6 ± 0 49	35 7 ± 0 30	35 6 ± 0 24
Mean cell volume ($microns^3$)	55 0 ± 0 30	55 0 ± 0 41	54 7 ± 0 30	**53 8 ± 0 25	**52 1 ± 0 35	**50 9 ± 0 20
Platelets (10^3/microliter)	591 ± 12 6	*551 ± 8 4	**528 ± 19 3	**497 ± 7 8	**482 ± 9 7	**547 ± 6 7
Erythrocytes (10^6/microliter)	8 4 ± 0 11	8 4 ± 0 09	8 3 ± 0 11	8 5 ± 0 13	8 3 ± 0 09	8 5 ± 0 07
Reticulocytes (10^6/microliter)	0 14 ± 0 012	0 16 ± 0 018	0 15 ± 0 015	0 13 ± 0 012	0 15 ± 0 011	0 16 ± 0 016
Albumin (g/dl)						
Day 3	4 3 ± 0 12	(c) 4 4 ± 0 09	4 4 ± 0 16	4 5 ± 0 15	4 3 ± 0 13	(c) 4 4 ± 0 14
Day 15	4 5 ± 0 06	**4 9 ± 0 09	*4 8 ± 0 09	**4 9 ± 0 08	**5 3 ± 0 12	**(c) 5 6 ± 0 12
Day 45	4 8 ± 0 25	5 1 ± 0 09	5 2 ± 0 08	**5 4 ± 0 09	**5 8 ± 0 10	**6 4 ± 0 06
Day 90	5 1 ± 0 17	5 4 ± 0 16	5 4 ± 0 22	*5 8 ± 0 24	**6 1 ± 0 19	**6 6 ± 0 29
Creatinine (mg/dl)						
Day 3	0 30 ± 0 021	(c) 0 26 ± 0 016	0 25 ± 0 017	0 33 ± 0 021	0 29 ± 0 018	(c) 0 32 ± 0 020
Day 15	0 41 ± 0 023	0 44 ± 0 041	0 43 ± 0 021	0 42 ± 0 033	0 45 ± 0 022	(c) 0 45 ± 0 034
Day 45	0 47 ± 0 037	0 49 ± 0 035	0 47 ± 0 033	0 48 ± 0 036	0 43 ± 0 033	0 47 ± 0 029
Day 90	0 61 ± 0 057	0 59 ± 0 039	0 59 ± 0 048	0 62 ± 0 042	0 55 ± 0 017	0 60 ± 0 029
Sorbitol dehydrogenase (IU/liter)						
Day 3	8 4 ± 0 37	(c) 8 1 ± 0 41	(h) 10 3 ± 0 90	(h) 9 2 ± 0 83	9 0 ± 0 47	(c) 9 2 ± 0 42
Day 15	10 3 ± 0 78	(d) 10 0 ± 0 96	(d) 12 5 ± 1 12	(h) 12 1 ± 1 34	**(h) 18 4 ± 1 73	**(e) 14 3 ± 1 43
Day 45	(h) 7 2 ± 0 47	(d) 7 5 ± 0 66	*9 5 ± 0 78	(h) 10 7 ± 1 83	**12 7 ± 1 02	**(d) 12 9 ± 1 33
Day 90	8 5 ± 0 85	9 7 ± 0 97	**12 8 ± 0 90	**15 4 ± 1 01	**19 7 ± 1 67	**22 1 ± 1 41
GGT (IU/liter)						
Day 3	2 3 ± 0 76	(e) 0 9 ± 0 28	1 4 ± 0 52	2 7 ± 0 79	1 6 ± 0 45	(e) 2 2 ± 1 03
Day 15	1 5 ± 0 48	2 8 ± 0 74	2 2 ± 0 47	1 8 ± 0 66	2 2 ± 0 51	*(e) 3 8 ± 0 63
Day 45	1 6 ± 0 40	1 3 ± 0 53	1 1 ± 0 31	1 0 ± 0 45	1 6 ± 0 52	3 1 ± 0 68
Day 90	0 5 ± 0 31	0 9 ± 0 39	0 4 ± 0 27	0 4 ± 0 21	0 0 ± 0 00	2 0 ± 0 50
Triiodothyronine (ng/dl)						
Day 15	116 8 ± 3 92	(d) 105 8 ± 5 86	117 3 ± 5 81	115 1 ± 5 78	102 7 ± 3 94	*(c) 98 7 ± 4 36
Day 45	(h) 97 9 ± 4 33	87 6 ± 6 31	85 3 ± 3 83	94 7 ± 6 86	95 9 ± 5 29	87 6 ± 3 69
Day 90	96 3 ± 3 53	102 3 ± 7 23	100 1 ± 4 34	96 0 ± 3 81	104 5 ± 5 93	92 2 ± 4 52
Free thyroxin (ng/dl)						
Day 15	0 20 ± 0 012	*(d) 0 15 ± 0 013	*0 15 ± 0 012	**0 09 ± 0 008	**0 08 ± 0 006	**(c) 0 08 ± 0 004
Day 45	0 16 ± 0 013	0 13 ± 0 013	**0 10 ± 0 009	**0 07 ± 0 006	**0 07 ± 0 006	**0 08 ± 0 004
Day 90	0 20 ± 0 014	0 18 ± 0 010	**0 14 ± 0 009	**0 10 ± 0 005	**0 11 ± 0 003	**0 11 ± 0 007
Total thyroxin (micrograms/dl)						
Day 15	4 6 ± 0 16	*(d) 3 9 ± 0 28	**3 6 ± 0 24	**2 2 ± 0 12	**1 7 ± 0 03	**(c) 1 6 ± 0 05
Day 45	4 7 ± 0 35	3 9 ± 0 35	**3 2 ± 0 21	**2 3 ± 0 09	**1 8 ± 0 07	**1 5 ± 0 03
Day 90	5 0 ± 0 29	4 7 ± 0 25	**3 8 ± 0 24	**2 5 ± 0 09	**2 0 ± 0 08	**1 7 ± 0 06
Thyrotropin (ng/ml)						
Day 15	2 0 ± 0 15	(d) 2 6 ± 0 32	(h) 2 3 ± 0 23	(h) 1 8 ± 0 19	(h) 3 3 ± 0 45	**(d) 4 4 ± 0 55
Day 45	(f) 2 4 ± 0 52	(e) 2 3 ± 0 55	(d) 2 8 ± 0 33	(h) 2 1 ± 0 25	(d) 3 2 ± 0 25	**(d) 4 8 ± 0 36
Day 90	2 7 ± 0 43	3 3 ± 0 29	3 5 ± 0 38	(h) 3 4 ± 0 48	**6 4 ± 1 15	**8 5 ± 1 21
Liver porphyrin (micrograms/g) for nonbled animals						
Day 90	(h) 0 48 ± 0 061	0 48 ± 0 088	0 41 ± 0 089	0 63 ± 0 054	0 64 ± 0 185	*(c) 0 74 ± 0 045
Day 90 (ratio) (i)		101	85	131	134	154
Estrous cycle length for nonbled animals (days)	4 90 ± 0 10	**(c) 4 30 ± 0 15		4 80 ± 0 13		** h) 4 30 ± 0 15
Estrous cycle stage (percent)						
Proestrus	14 3	12 9		21 4		14 3
Estrus	21 4	27 1		22 9		27 1
Metestrus	21 4	20 0		20 0		24 3
Diestrus	42 9	40 0		35 7		34 3

TABLE A2. RESULTS OF SUPPLEMENTAL ANALYSES FOR RATS IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (Continued)

FEMALE (Continued)

No examined for urinalysis (b)	7	8	10	7	7	9
Urine specific gravity						
Day 3	1 034 ± 0 005	1 035 ± 0 002	(f) 1 041 ± 0 005	1 036 ± 0 003	1 040 ± 0 007	(f) 1 028 ± 0 005
Day 90	(d) 1 042 ± 0 006	1 047 ± 0 007	1 044 ± 0 006	*(c) 1 061 ± 0 004	(f) 1 052 ± 0 004	*1 063 ± 0 004
Urinary glucose (mg/16 h)						
Day 3	0 6 ± 0 11	1 0 ± 0 27	0 6 ± 0 11	*1 0 ± 0 16	*1 0 ± 0 21	1 1 ± 0 23
Day 90	0 4 ± 0 11	0 6 ± 0 13	0 5 ± 0 08	0 7 ± 0 09	**1 2 ± 0 18	**2 6 ± 0 20
Urine pH						
Day 3	6 4 ± 0 07	6 3 ± 0 13	(f) 6 2 ± 0 12	6 3 ± 0 10	6 4 ± 0 09	(e) 6 3 ± 0 11
Day 90	(d) 6 7 ± 0 28	(h) 6 4 ± 0 13	6 3 ± 0 21	(c) 6 3 ± 0 15	(f) 6 2 ± 0 12	**6 0 ± 0 00
Urinary protein (mg/16 h)						
Day 3	0 4 ± 0 11	0 7 ± 0 21	0 4 ± 0 08	0 7 ± 0 12	0 7 ± 0 19	0 7 ± 0 10
Day 90	0 4 ± 0 08	0 5 ± 0 10	0 4 ± 0 06	0 6 ± 0 10	**1 1 ± 0 15	**15 5 ± 1 87
Urine volume (ml/16 h)						
Day 3	1 9 ± 0 52	2 5 ± 0 38	(f) 2 1 ± 0 59	2 4 ± 0 33	2 4 ± 0 73	(g) 1 9 ± 0 63
Day 90	(d) 0 9 ± 0 20	(h) 0 9 ± 0 24	1 0 ± 0 18	(c) 0 9 ± 0 17	1 1 ± 0 33	**2 1 ± 0 19

(a) Includes hematologic, serum chemical, urinalysis, reproductive function, and liver porphyrin data Mean ± standard error; GGT = γ glutamyl transpeptidase, hematologic analyses are for animals bled on day 90, except as noted, all analyses were performed on sequentially bled animals P values are vs the controls by Dunn s test (*Dunn, 1964*) or Shirley's test (*Shirley, 1977*)
(b) Unless otherwise specified
(c) Ten animals were examined
(d) Eight animals were examined
(e) Six animals were examined
(f) Five animals were examined
(g) Seven animals were examined
(h) Nine animals were examined
(i) Ratio = (exposed group mean/control group mean) × 100
(j) Two animals were examined
(k) Three animals were examined
(l) Four animals were examined
*P < 0 05
**P < 0 01

TABLE A3. RESULTS OF SUPPLEMENTAL ANALYSES FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (a)

Analysis	Control	33 ppm	100 ppm	330 ppm	1,000 ppm	2,000 ppm
MALE						
Number examined (b)	10	10	10	10	9	9
Hemoglobin (g/dl)	17 8 ± 0 27	17 6 ± 0 24	18 3 ± 0 38	17 9 ± 0 17	17 8 ± 0 18	*16 9 ± 0 21
Mean cell volume (microns^3)	48 9 ± 0 64	48 5 ± 0 43	49 0 ± 0 56	47 8 ± 0 59	47 9 ± 0 45	48 0 ± 0 62
Platelets (10^3/microliter)	(c) 710 ± 24	702 ± 20	624 ± 46	742 ± 38	768 ± 26	**922 ± 26
GGT (IU/liter)	(d) 10 5 ± 4 60	3 9 ± 1 46	(d) 8 0 ± 4 93	(c) 8 0 ± 2 24	(d) 5 4 ± 2 54	(d) 12 5 ± 2 15
Sorbitol dehydrogenase (IU/liter)	(d) 109 ± 15 9	99 ± 9 4	(c) 88 ± 5 9	(c) 122 ± 8 0	**209 ± 15 7	**(d 311 ± 58 2
Free thyroxin (ng/dl)						
Day 15	0 46 ± 0 035	0 43 ± 0 029	0 41 ± 0 016	*0 39 ± 0 017	(e) 0 39 ± 0 026	0 51 ± 0 008
Day 45	0 35 ± 0 031	0 29 ± 0 026	0 28 ± 0 032	**0 21 ± 0 023	**(e) 0 21 ± 0 021	0 35 ± 0 026
Day 90	(c) 0 51 ± 0 014	0 49 ± 0 014	0 46 ± 0 015	**0 36 ± 0 031	**0 41 ± 0 016	(e) 0 52 ± 0 000
Total thyroxin (micrograms/dl)						
Day 15	6 6 ± 0 53	5 9 ± 0 21	**4 3 ± 0 23	**3 4 ± 0 15	**(e) 2 6 ± 0 12	**2 8 ± 0 09
Day 45	5 0 ± 0 25	4 5 ± 0 36	**3 4 ± 0 22	**(c) 2 4 ± 0 17	**(e) 1 9 ± 0 10	**2 4 ± 0 14
Day 90	(c) 6 9 ± 0 56	*5 4 ± 0 34	**(c) 3 8 ± 0 24	**2 5 ± 0 21	**2 4 ± 0 15	**(e) 2 2 ± 0 0~
Triiodothyronine (ng/dl)						
Day 15	(f) 108 4 ± 5 72	(g) 85 1 ± 10 02	(h) 86 9 ± 9 22	78 2 ± 5 63	(i) 76 2 ± 6 10	(i) 108 4 ± 12 44
Day 45	(h) 97 1 ± 13 37	(g) 96 1 ± 10 01	(h) 102 2 ± 8 98	(f) 60 1 ± 14 59	(h) 65 0 ± 8 86	(g) 86 4 ± 13 82
Day 90	(d) 94 6 ± 5 87	(d) 94 1 ± 7 51	(h) 91 6 ± 5 58	(h) 91 7 ± 7 75	(g) 84 0 ± 6 28	(d) 91 6 ± 5 01
Thyrotropin (ng/dl)	(j) 1 3 ± 0 33	(c) 1 2 ± 0 14	(d) 1 5 ± 0 42	(c) 1 0 ± 0 04	(d) 1 2 ± 0 18	(d) 1 5 ± 0 26
Liver porphyrin (micrograms/g) for nonbled animals						
Day 90	(c) 0 51 ± 0 036	0 54 ± 0 064	0 48 ± 0 084	0 57 ± 0 071	**0 93 ± 0 112	**(e) 0 92 ± 0 0~9
Day 90 (ratio) (k)		106	94	112	182	180
FEMALE						
Number examined (b)	8	10	9	10	9	8
Hemoglobin (g/dl)	17 7 ± 0 29	17 2 ± 0 14	17 4 ± 0 16	17 5 ± 0 22	17 3 ± 0 05	**16 7 ± 0 23
Mean cell volume (microns^3)	50 6 ± 0 50	49 4 ± 0 37	50 3 ± 0 17	49 8 ± 0 55	49 9 ± 0 26	**48 8 ± 0 56
Platelets (10^3/microliter)	616 ± 49	646 ± 39	595 ± 27	638 ± 32	728 ± 30	**824 ± 21
GGT (IU/liter)	4 2 ± 1 79	3 0 ± 0 72	(d) 7 4 ± 2 19	6 5 ± 2 56	3 1 ± 1 01	**17 6 ± 1 94
Sorbitol dehydrogenase (IU/liter)	(i) 56 ± 3 9	58 ± 3 4	64 ± 1 8	(c) 64 ± 3 5	**102 ± 3 7	**165 ± 11 5
Free thyroxin (ng/dl)						
Day 15	(e) 0 51 ± 0 008	0 47 ± 0 019	(e) 0 46 ± 0 022	0 49 ± 0 014	(e) 0 51 ± 0 013	(e) 0 52 ± 0 000
Day 45	0 49 ± 0 023	(c) 0 51 ± 0 008	0 50 ± 0 009	0 51 ± 0 008	0 52 ± 0 001	0 52 ± 0 000
Day 90	(e) 0 50 ± 0 013	0 50 ± 0 010	(e) 0 47 ± 0 024	*0 43 ± 0 015	(e) 0 50 ± 0 013	(e) 0 52 ± 0 000
Total thyroxin (micrograms/dl)						
Day 15	(e) 7 6 ± 0 19	**6 4 ± 0 22	**(e) 5 6 ± 0 39	**4 1 ± 0 20	**(e) 2 5 ± 0 10	**(e) 3 1 ± 0 19
Day 45	7 2 ± 0 34	**5 9 ± 0 21	**4 9 ± 0 23	**3 6 ± 0 07	**2 4 ± 0 09	**(c) 2 8 ± 0 09
Day 90	(e) 5 5 ± 0 29	5 0 ± 0 20	**(e) 4 4 ± 0 25	**3 1 ± 0 08	**(e) 2 4 ± 0 09	**(e) 2 6 ± 0 09
Triiodothyronine (ng/dl)						
Day 15	(h) 57 0 ± 7 98	(g) 54 1 ± 9 68	(h) 54 4 ± 6 72	(f) 53 0 ± 7 97	(i) 38 6 ± 7 75	*(c) 104 7 ± 11 15
Day 45	(i) 114 4 ± 8 55	(j) 89 9 ± 6 59	(f) 94 9 ± 4 71	(g) 100 3 ± 3 18	(f) 106 3 ± 4 18	(l) 138 3 ± 29 75
Day 90	(e) 62 3 ± 5 27	(c) 63 7 ± 1 72	(e) 55 2 ± 7 65	53 8 ± 5 12	(e) 57 2 ± 4 60	(e) 72 6 ± 3 62
Thyrotropin (ng/dl)	(g) 1 2 ± 0 15	(c) 1 1 ± 0 12	(e) 1 1 ± 0 05	(d) 1 4 ± 0 37	(d) 1 2 ± 0 15	(d) 1 0 ± 0 00
Liver porphyrin (micrograms/g) for nonbled animals						
Day 90	(i) 0 31 ± 0 066	(h) 0 29 ± 0 056	(i) 0 33 ± 0 064	0 44 ± 0 082	**1 07 ± 0 225	**2 05 ± 0 259
Day 90 ratio (k)		94	106	142	345	661

TABLE A3. RESULTS OF SUPPLEMENTAL ANALYSES FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF PENTACHLOROBENZENE (Continued)

(a) Includes hematologic, serum chemical, and liver porphyrin data. Mean ± standard error; GGT = γ glutamyl transpeptidase; P values are vs the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977). Analyses are for animals bled on d 90 unless otherwise noted; except as noted, all analyses were performed on sequentially bled animals.
(b) Unless otherwise specified
(c) Nine animals were examined.
(d) Eight animals were examined.
(e) Ten animals were examined.
(f) Four animals were examined.
(g) Five animals were examined.
(h) Six animals were examined.
(i) Seven animals were examined
(j) Three animals were examined.
(k) Ratio = (exposed group mean/control group mean) × 100
(l) Two animals were examined.
*P<0.05
**P<0.01