National Toxicology Program
Toxicity Report Series
Number 66

NTP Technical Report
on the Toxicology Studies of

3,3′,4,4′-Tetrachloroazoxybenzene

(CAS No. 21232-47-3)

Administered by Gavage to
F344/N Rats and B6C3F$_1$ Mice

Angélique P.J.M. van Birgelen, Ph.D., Study Scientist
National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

U.S. Department of Health And Human Services
Public Health Service
National Institutes of Health

FOREWORD

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

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

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

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Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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National Toxicology Program
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Research Triangle Park, NC 27709

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CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

A.P.J.M. van Birgelen, Ph.D., Study Scientist
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
C.D. Hébert, Ph.D.
J. Mahler, D.V.M.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems

Microbiological Associates, Inc.

Conducted studies, evaluated pathology findings

M.L. Wenk, Ph.D., Principal Investigator
R.M. Kovatch, D.V.M.
L.L. Pippin, D.V.M.
J.M. Pletcher, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, D.V.M., M.S., Ph.D.

Environmental Health Research and Testing, Inc.

Provided sperm motility and vaginal cytology evaluations

L.K. Grimes, D.V.M., Principal Investigator
T.A. Sexton

NTP Pathology Working Group

Evaluated slides, prepared pathology report on rats and mice (25 September 1996)

D.G. Goodman, V.M.D., Chairperson
PATHCO, Inc.
N. Barlow, D.V.M., Observer
North Carolina State University
S. Botts, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
D. Wolf, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology

Analytical Sciences, Inc.

Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
K.P. McGowan, M.B.A.
M.A. Mauney, M.S.
N.G. Mintz, B.S.
J.T. Scott, M.S.

Biotechnical Services, Inc.

Prepared Toxicity Study Report

S.R. Gunnels, M.A., Principal Investigator
J.R. Carlton, B.A.
A.M. Macri-Hanson, M.A., M.F.A.
W.D. Sharp, B.A., B.S.
S.M. Swift, B.S.

PEER REVIEW

The draft report on the toxicity studies of 3,3′,4,4′-tetrachloroazoxybenzene was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

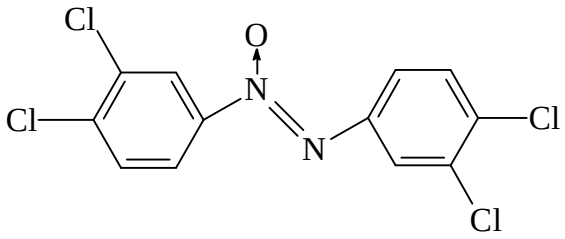
Abraham Brouwer, Ph.D.
Division of Toxicology
Department of Food Technology
and Nutritional Sciences
Agricultural University Wageningen
Wageningen, The Netherlands

John M. Cullen, Ph.D., V.M.D.
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

John P. Geisy, Ph.D.
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI

CONTENTS

ABSTRACT



3,3′,4,4′-TETRACHLOROAZOXYBENZENE

CAS No. 21232-47-3

Chemical Formula: $C_{12}H_6Cl_4N_2O$ Molecular Weight: 336.0

Synonyms: Azoxybenzene, 3,3′,4,4′-tetrachloro-(8CI); diazene, bis(3,4-dichlorophenyl)-1-oxide-(9CI); TCAOB

3,3′,4,4′-Tetrachloroazoxybenzene is not commercially manufactured but is present as a contaminant of 3,4-dichloroaniline and its herbicidal derivative Diuron®. In addition, environmental contamination occurs when 3,3′,4,4′-tetrachloroazoxybenzene is formed by the photolysis and biolysis of 3,4-dichloroaniline. 3,3′,4,4′-Tetrachloroazoxybenzene was nominated by the United States Environmental Protection Agency for toxicity testing based on concerns over the potential for human exposure, the structural resemblance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and the reported dioxin-like effects of 3,3′,4,4′-tetrachloroazoxybenzene. The toxicity of 3,3′,4,4′-tetrachloroazoxybenzene was evaluated in 16-day and 13-week gavage studies in male and female F344/N rats and B6C3F_1 mice. In addition to histopathology, evaluations included hematology (rats only), clinical chemistry, thyroid hormone analyses (rats only), hepatic cell proliferation (rats only), cytochrome P_{450}1A immunohistological staining in the liver (rats only), and assessments of male reproductive endpoints and estrous cycle length. Additional genetic toxicology studies included mutagenicity tests in *Salmonella typhimurium* and the determination of micronuclei in mouse bone marrow and peripheral blood erythrocytes.

In the 16-day studies, groups of five male and five female rats received 3,3′,4,4′-tetrachloroazoxybenzene in corn oil by gavage at doses of 0, 12.5, 32, 80, 200, or 500 mg per kg body weight, 5 days a week. Groups of five male and five female mice received 0, 1, 3.2, 10, 32, or 100 mg/kg in corn oil by gavage, 5 days a week. Major effects in rats included increases in liver and lung weights, and decreases in mean body weights and body

weight gains, heart weights, and thymus weights. Effects in mice included increases in liver weights and decreases in thymus weights. No effects on survival were observed. Treatment-related lesions included cytoplasmic alteration of hepatocytes, splenic hematopoietic cell proliferation, thymic atrophy, and nephropathy in rats and thymic atrophy, splenic hematopoietic cell proliferation, and hepatic foci of inflammation and necrosis in mice.

In the 13-week studies, groups of 10 male and 10 female rats and mice received 3,3′,4,4′-tetrachloroazoxybenzene in corn oil by gavage at doses of 0, 0.1, 1, 3, 10, or 30 mg/kg, 5 days a week.

In the 13-week rat study, all males and seven females in the 30 mg/kg groups died. Decreases in final mean body weights and body weight gains were observed in 3 and 10 mg/kg males and 10 and 30 mg/kg females. Decreased thymus weights, accompanied by thymic atrophy observed microscopically, were observed at doses of 1 mg/kg or greater in males and females. Increased liver weights were observed in males and females administered 1 mg/kg or greater, and hepatic cytochrome P_{450}1A staining was increased in 1 and 3 mg/kg males and 3, 10, and 30 mg/kg females. In addition, a responsive anemia and decreases in platelet counts were observed in dosed male and female rats. A marked decrease in circulating thyroxine concentrations was observed in dosed males and females. In spite of this sharp decrease, thyroid-stimulating hormone concentrations were marginally increased. A decrease in epididymal spermatozoal motility was observed in all dosed groups tested. In 10 mg/kg females, the estrous cycle length was increased. Major effects included increased incidences of hyperplasia of the forestomach in 3, 10, and 30 mg/kg males and 10 and 30 mg/kg females. Increased incidences of centrilobular degeneration and hematopoietic cell proliferation were observed in the liver of dosed males and females. Furthermore, chronic active inflammation of the lung vasculature and hematopoietic cell proliferation in the spleen were observed in dosed males and females. The increased severities of cardiomyopathy and nephropathy in males and the incidences of cardiomyopathy and nephropathy and severity of cardiomyopathy in females were 3,3′,4,4′-tetrachloroazoxybenzene related.

In the 13-week mouse study, the major effects included increases in liver weights in males administered 3 mg/kg or greater and females administered 1 mg/kg or greater. Hyperplasia of the forestomach and dilatation of hair follicles were observed in 10 and 30 mg/kg males and 30 mg/kg females. Furthermore, thymus weights were decreased in males administered 3 mg/kg or greater and in 10 and 30 mg/kg females. Increased incidences of centrilobular hypertrophy of hepatocytes were observed in 10 and 30 mg/kg males and females. Increased incidences of hematopoietic cell proliferation in the spleen were observed in 30 mg/kg males and in 10 and 30 mg/kg females. Increases in the incidences of thymocyte necrosis were observed in 10 mg/kg males and in 10 and 30 mg/kg females. The incidences of splenic pigmentation were increased in all dosed groups of males, and the severity of pigmentation increased with increasing dose in males and females.

3,3′,4,4′-Tetrachloroazoxybenzene was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535 with or without induced S9 metabolic activation enzymes. It did not induce significant increases in micronucleated erythrocytes in a three-exposure male mouse bone marrow micronucleus test up to dose levels of 200 mg/kg, but results of a 13-week peripheral blood micronucleus test conducted in male and female mice were positive.

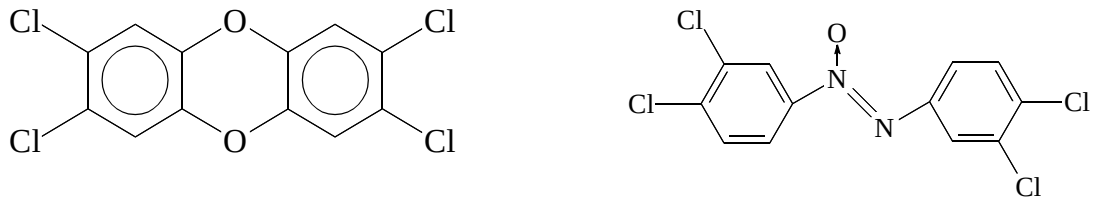
In summary, 3,3′,4,4′-tetrachloroazoxybenzene caused typical dioxin-like effects, including thymic atrophy, increased liver weights, induction of hepatic cytochrome P_{450}1A, and decreased mean body weight gains. Furthermore, a marked decrease in circulating thyroxine concentrations was observed in male and female rats, even at the lowest dose (0.1 mg/kg) in female rats. A decrease in epididymal sperm motility was observed at all doses in rats. Effects on the hematopoietic system occurred at doses including and lower than those that caused histopathologic alterations in the liver. A no-observable-adverse-effect-level (NOAEL) was not reached in rats. In male and female mice, the NOAEL was 1 and 0.1 mg/kg, respectively. Furthermore, treatment-related effects included increased incidences of hyperplasia of the forestomach epithelium in rats and mice, chronic active inflammation of the vasculature of the lung in rats, increased incidences and/or severities of cardiomyopathy and nephropathy in rats, and dilatation of the hair follicles in mice. Comparison of various dioxin-like effects in these studies with those reported in the literature indicate that 3,3′,4,4′-tetrachloroazoxybenzene is six to two orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

3,3′,4,4′-Tetrachloroazoxybenzene is not commercially manufactured but is present as a contaminant of 3,4-dichloroaniline and the anilide herbicide Diuron® (Sundström *et al.*, 1978; Hill *et al.*, 1981). In addition, environmental contamination occurs when 3,3′,4,4′-tetrachloroazoxybenzene is formed by the photolysis and biolysis of 3,4-dichloroaniline (Kaufman *et al.*, 1972; Mansour *et al.*, 1975).

3,3′,4,4′-Tetrachloroazoxybenzene is a yellowish orange, crystalline solid (Hsia and Burant, 1979). In the *trans* configuration, 3,3′,4,4′-tetrachloroazoxybenzene has a molecular shape similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Figure 1; Poland *et al.*, 1976).



2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

3,3′,4,4′-Tetrachloroazoxybenzene

FIGURE 1
Molecular Structures of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and 3,3′,4,4′-Tetrachloroazoxybenzene

PRODUCTION AND HUMAN EXPOSURE

3,3′,4,4′-Tetrachloroazoxybenzene has been found at concentrations of 8 µg/g in 3,4-dichloroaniline and up to 2 µg/g in Diuron (Sundström *et al.*, 1978; Hill *et al.*, 1981). With an estimated production volume of 100,000 to 1,000,000 pounds of 3,4-dichloroaniline per year, the resultant 3,3′,4,4′-tetrachloroazoxybenzene production could be as high as 3.6 kg/year (USEPA, 1985).

3,3′,4,4′-Tetrachloroazoxybenzene was detected in the roots of soybean plants grown in soil treated with 25 ppm 3,3′,4,4′-tetrachloroazobenzene (Worobey, 1984) and also in the soil. Human exposure to 3,3′,4,4′-tetrachloroazoxybenzene has been reported in various manufacturing plants producing 3,4-dichloroaniline or herbicides derived from 3,4-dichloroaniline (Taylor *et al.*, 1977).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Male Sprague-Dawley rats administered a single gavage dose of 10 mg ^{14}C-labeled 3,3′,4,4′-tetrachloroazoxybenzene excreted approximately 20% of the radiolabel in the urine over 5 days (Burant and Hsia, 1984). Male F344 rats administered gavage doses of 3.4 or 34 mg 3,3′,4,4′-tetrachloroazoxybenzene/kg body weight excreted 30% to 35% of the dose in the urine in a 96-hour time period (Ziegler *et al.*, 1996). The oral bioavailability of 3,3′,4,4′-tetrachloroazoxybenzene in this experiment was calculated to be 9%.

The highest concentrations of 3,3′,4,4′-tetrachloroazoxybenzene in male Sprague-Dawley rats administered a single gavage dose of 10 mg were located in the epididymal fat, kidney, lymph nodes, liver, cecum, adrenal gland, pancreas, and lung, based on wet weight (Burant and Hsia, 1984). The liver-to-fat ratio of 3,3′,4,4′-tetrachloroazoxybenzene was 0.40. The lowest concentrations were found in the brain. In a study in which male F344 rats were administered radiolabeled 3,3′,4,4′-tetrachloroazoxybenzene intravenously (3.4 mg/kg) or by gavage (3.4 or 34 mg/kg), radiolabel accumulated in the adipose tissue, kidney, and liver, as shown by tissue-to-blood ratios exceeding 1 (Ziegler *et al.*, 1996). The liver-to-fat ratios of 3,3′,4,4′-tetrachloroazoxybenzene ranged from 0.06 to 0.14 at doses of 34 and 3.4 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene, respectively. Again, the lowest concentrations were found in the brain.

In male Sprague-Dawley rats administered a single gavage dose of 10 mg radiolabeled 3,3′,4,4′-tetrachloroazoxybenzene per animal, 37% of the dose was excreted in the urine and feces after 24 hours (Burant and Hsia, 1984). The pattern in elimination indicated a biphasic elimination, consisting of an early rapid phase with a half-life of 34 hours and a slow terminal phase with a half-life greater than 20 days. The major route of excretion was via the feces (about twice as much as was excreted in the urine). In male F344 rats dosed intravenously with 34 mg/kg radiolabeled 3,3′,4,4′-tetrachloroazoxybenzene, the majority of the radiolabel (52%) was excreted by 48 hours after dosing, and the primary route of elimination was via the feces (Ziegler *et al.*, 1996). This is similar to excretion following oral exposure (55% excreted in the feces). The urinary elimination of 3,3′,4,4′-tetrachloroazoxybenzene-derived radioactivity was 41% after intravenous injection and 30% to 35% after gavage administration in male F344 rats (Ziegler *et al.*, 1996).

In male F344 rats, six major urinary metabolites were characterized after exposure to 3,3′,4,4′-tetrachloroazoxybenzene. These were 3,4-dichloroaniline, a dichloroaniline base with an *N*-acetyl group, a dichloroaniline base with a methylated ring-hydroxyl group, a 3,4-dichloroaniline metabolite with a sulfate-modified hydroxyl group, a 3,4-dichloroaniline metabolite with an *N*-acetyl group, and a 3-chloroaniline metabolite containing an *N*-acetyl group as well as a ring-hydroxyl group that had undergone sulfation (Ziegler *et al.*, 1996). This suggests that azoreduction is necessary for producing metabolites capable of excretion in the urine.

Humans

No absorption, distribution, metabolism, or excretion studies of 3,3′,4,4′-tetrachloroazoxybenzene in humans have been found in a review of the literature.

TOXICITY

Experimental Animals

A 120-day study in male Sprague-Dawley rats exposed to 100 ppm 3,3′,4,4′-tetrachloroazoxybenzene in feed resulted in a 17% decrease in mean body weight compared to the controls at the end of the study (Hsia *et al.*, 1980). The total dietary intake of 3,3′,4,4′-tetrachloroazoxybenzene per animal in this experiment was calculated to be 24 mg. Hematocrit, hemoglobin concentration, and erythrocyte count were decreased. 3,3′,4,4′-Tetrachloroazoxybenzene exposure caused increased liver and spleen weights and decreased testis weight. In addition, the activities of hepatic cytochrome P_{448} and the microsomal aryl hydrocarbon hydroxylase were increased. Furthermore, serum lipid content and serum aspartate aminotransferase activity were increased in exposed rats.

A 60-day study in which six male Sprague-Dawley rats were dosed with 25 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene per week by intraperitoneal injection resulted in two deaths, a decrease in mean body weight, a decrease in thymus weight, and an increase in liver weight (55% above controls; Hsia *et al.*, 1981). In the livers of dosed animals, hepatocyte swelling and cytoplasmic vacuoles were observed. The cortex of the thymus, the outer cortical areas of the mesenteric lymph nodes, and the periarterial lymphatic sheaths of the spleen were atrophied. In addition, the lungs of the treated rats contained thickened alveolar walls and foamy macrophages.

Two intraperitoneal doses of 25 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene per week to weanling male Sprague-Dawley rats resulted in a delayed wasting syndrome characterized by reduced feed consumption, a significant reduction in mean body weight and thymus weight, and death after 6 weeks (Hsia and Kreamer, 1985). In

addition, the animals had atrophy of the thymus and of peripheral lymphoid organs, decreased bone marrow cellularity, and a reduction in splenic plaque-forming cells. Furthermore, serum glucose concentrations were reduced by 20% relative to controls on day 14 and by 44% on day 28. 3,3′,4,4′-Tetrachloroazoxybenzene caused decreases in activities of the following gluconeogenic enzymes in the same study: fructose-1,6-biphosphatase, glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, and pyruvate kinase (Hsia and Kreamer, 1985). Hepatic cytochrome P_{450} activity and malic enzyme activities were induced, whereas alanine aminotransferase activities were decreased by 3,3′,4,4′-tetrachloroazoxybenzene on days 7 and 28 of the study.

Four intraperitoneal doses of 25 mg 3,3′,4,4′-tetrachloroazoxybenzene/kg body weight resulted in greater immunosuppressive effects in weanling animals than in adult Sprague-Dawley rats (Olson *et al.*, 1984). The immunologic parameters affected were thymus weight, splenic plaque-forming cell populations and functions, peritoneal macrophage chemiluminescence, and bone marrow cellularity.

In a study of 3,3′,4,4′-tetrachloroazoxybenzene hepatotoxicity (Schrankel *et al.*, 1980), male Sprague-Dawley rats were administered four daily intraperitoneal injections of 25 mg/kg and were examined on day 5. The hepatocytes were enlarged and had abundant cytoplasmic vacuoles. Proliferation of smooth endoplasmic reticulum was observed; membranous arrays occurred frequently. The hepatic mitotic index was increased in dosed rats compared to the controls. In addition, potential genotoxicity of 3,3′,4,4′-tetrachloroazoxybenzene was suggested by Schrankel *et al.* (1980) because of the occasional appearance of atypical mitotic figures.

Two intraperitoneal injections of 25 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene (on days 1 and 5) to male Sprague-Dawley rats resulted in a decrease in thymus weight to 49% that of the controls and a 32% increase in liver weight relative to the controls 11 days after the initial treatment (Hsia *et al.*, 1982).

In a 28-day feed study in female Swiss-Webster mice, exposure to 40 ppm 3,3′,4,4′-tetrachloroazoxybenzene resulted in a 13% decrease in mean body weight and a 42% decrease in thymus weight at the end of the study (Bleavins *et al.*, 1985a). The daily consumption of 3,3′,4,4′-tetrachloroazoxybenzene was calculated to be 10 mg/kg. Various immune parameters were affected in exposed mice. Treated animals had an increase in the percentage of segmented neutrophils and a decrease in the percentage of lymphocytes. The number of cells recovered from the spleen was significantly lower in exposed mice. The plaque-forming cell response and the hemolysin titer in exposed females were significantly less than the control values (Bleavins *et al.*, 1985a).

Taylor *et al.* (1977) painted the ears of albino rabbits with 0.0001% to 0.1% 3,3′,4,4′-tetrachloroazoxybenzene in acetone daily 5 days per week for 4 weeks; treated rabbits had comedone formation, erythema, edema, and necrosis of the epithelium. The comedone formation was observed in all dosed groups.

3,3′,4,4′-Tetrachloroazoxybenzene was found to be approximately 1,000-fold less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as an inducer of hyperplastic and metaplastic changes in hairless mouse skin after topical exposure (Knutson and Poland, 1982).

Horton and Yeary (1985) studied the chloracnegenic response of five strains of mice (hairless, rhino, rhino+, DBA/2J, and C57BL/6) to topical application of 0.001%, 0.01%, or 0.1% 3,3′,4,4′-tetrachloroazoxybenzene 5 days per week for 3 to 9 weeks. Gross and histologic skin lesions, characteristic of follicular hyperkeratosis, were observed in the rhino and hairless strains administered 0.01% or 0.1% for 3 to 4 weeks.

A dose-dependent decrease was found in the number of thymic lymphoid cells of chicken embryos after exposure to 3,3′,4,4′-tetrachloroazoxybenzene *in ovo* (Nikolaidis *et al.*, 1988). The ED_{50} value was estimated to be 3.6 µg/kg egg.

3,3′,4,4′-Tetrachloroazoxybenzene binds to the aryl hydrocarbon receptor with a specific binding affinity of one-tenth to one-third that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Poland *et al.*, 1976; Schneider *et al.*, 1995). 3,3′,4,4′-Tetrachloroazoxybenzene induces hepatic aryl hydrocarbon hydroxylase activity in chicken embryos, with an ED_{50} of 0.45 nmol/kg (Poland *et al.*, 1976).

Male Sprague-Dawley rats dosed with a single intraperitoneal injection of 100 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene had a 100-fold increase in hepatic microsomal 7-ethoxyresorufin-*O*-deethylase activity, a fourfold increase in 7-pentoxyresorufin-*O*-dependentylase activity, and a 10-fold increase in 7-benzyloxyresorufin alkylase activity (McMillan *et al.*, 1990).

Humans

Within a few months after the beginning of production of the herbicide 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione, over 90% of the 41 workers developed chloracne as a result of exposure to 3,3′,4,4′-tetrachloroazoxybenzene, which is an intermediate formed during the manufacturing process of the herbicide (Taylor *et al.*, 1977). One of the workers had a urinary porphyrin concentration of 120 mEq/L, whereas 36 workers had concentrations below 100 mEq/L. Exposure resulted in chloracne in 30 people in the same factory in 1989 (Dr. A. Smith, University of Leicester, United Kingdom, personal communication).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

3,3′,4,4′-Tetrachloroazoxybenzene is teratogenic in mice and chickens (Schrankel *et al.*, 1982; Hassoun *et al.*, 1984; Bleavins *et al.*, 1985b). The embryotoxicity and tetratogenicity of 3,3′,4,4′-tetrachloroazoxybenzene in chick embryos was studied by Schrankel *et al.* (1982). Doses ranging from 0.00005 to 100 µg 3,3′,4,4′-tetrachloroazoxybenzene per egg were injected into the air cell on day 4 of incubation. In an additional group of eggs, 0.05 µg 3,3′,4,4′-tetrachloroazoxybenzene per egg was injected on days 11, 12, or 13 of incubation. The majority of deaths occurred before day 13 of incubation in groups treated with 0.010 to 100 µg. Eggs that were injected on days 11, 12, or 13 of incubation had a lower incidence of embryo mortality than those injected on day 4. The LD_{50} was calculated to be 12 ng of 3,3′,4,4′-tetrachloroazoxybenzene. Numerous malformations were detected in hatched chicks and in embryos that died prior to hatching. Rump edema was the major abnormality observed in treated embryos. In addition, altered feather pattern, lack of down, hemorrhage, external viscera, reduced body size, failure to withdraw the yolk sac, beak malformation, dilation of blood vessels, and monomicropthalmia were observed (Schrankel *et al.*, 1982).

Intraperitoneal dosing of Ah-responsive (C57CL/6J and NMRI) and nonresponsive (DBA/2J and AKR/2B) pregnant mice to 6 to 8 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene produced cleft palate and hydronephrosis in 50% to 90% of the offspring in the responsive strains (Hassoun *et al.*, 1984). In addition, hydrops were observed in the C57BL offspring. Higher doses (16 mg/kg) resulted in a 40% to 60% fetal death rate in the responsive strains.

Female Swiss-Webster mice received 0.1, 1.0, or 10 ppm 3,3′,4,4′-tetrachloroazoxybenzene in feed beginning 14 days prior to mating and continuing until all pups had been weaned (Bleavins *et al.*, 1985b). No teratogenic effects occurred, and no clinical findings of toxicity were observed in either the exposed dams or pups. At the 10 ppm concentration, a 36% decrease in litter size was observed. In addition, dams and pups exposed to 10 ppm had decreased thymus weights.

Humans

No studies of the reproductive or developmental effects of 3,3′,4,4′-tetrachloroazoxybenzene in humans have been found in a review of the literature.

CARCINOGENICITY

No carcinogenicity studies of 3,3′,4,4′-tetrachloroazoxybenzene in experimental animals nor epidemiologic studies in humans have been found in a review of the literature.

GENETIC TOXICITY

There is little published information on the genotoxicity of 3,3′,4,4′-tetrachloroazoxybenzene. It was not mutagenic in any of several strains of *Salmonella typhimurium*, with or without S9 metabolic activation enzymes, in standard plate incorporation or fluctuation assays (Gilbert *et al.*, 1980; McMillan *et al.*, 1988). No induction of unscheduled DNA synthesis (indicative of DNA damage and subsequent repair) was observed in primary rat hepatocyte cultures exposed to 3,3′,4,4′-tetrachloroazoxybenzene in the absence of animal pretreatment with hepatic mixed-function oxidase inducers (McMillan *et al.*, 1988), but unscheduled DNA synthesis was observed following exposure to 3,3′,4,4′-tetrachloroazoxybenzene of cultured hepatocytes isolated from adult male Sprague-Dawley rats pretreated with the closely related compound, 3,3′,4,4′-tetrachloroazobenzene, a potent cytochrome P_{448} inducer (Shaddock *et al.*, 1989). Negative results were obtained with 3,3′,4,4′-tetrachloroazoxybenzene, with and without S9, in an HGPRT mutation test in cultured Chinese hamster ovary cells (McMillan *et al.*, 1988).

In vivo, increased frequencies of chromatid breaks and rearrangements were reported in splenic lymphocytes of weanling mice fed for 28 days on a diet containing 40 ppm 3,3′,4,4′-tetrachloroazoxybenzene (Bleavins *et al.*, 1985b); no increase in aberrations was seen in splenic lymphocytes of newborn mice of dams fed 10 ppm 3,3′,4,4′-tetrachloroazoxybenzene during gestation and lactation. Neither the weanling mice nor the newborns in this study showed increased frequencies of sister chromatid exchanges. Because this study employed some nonstandard protocols (only 25 to 50 cells were counted per animal for chromosomal aberrations, rather than the usual several hundred, and gaps were scored as breaks), a repeat test is needed to confirm the results.

STUDY RATIONALE AND DESIGN

3,3′,4,4′-Tetrachloroazoxybenzene and the related chemical 3,3′,4,4′-tetrachloroazobenzene were nominated by the United States Environmental Protection Agency (USEPA) for testing based on concerns over the potential for human exposure from consumption of contaminated crops and in occupational settings. In addition, the USEPA was concerned about the structural resemblance of 3,3′,4,4′-tetrachloroazoxybenzene to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as well as the dioxin-like effects observed with 3,3′,4,4′-tetrachloroazoxybenzene exposure.

Because the oral route is the expected route of exposure, 3,3′,4,4′-tetrachloroazoxybenzene was administered by gavage. Male and female F344/N rats and B6C3F_1 mice were dosed for 16 days or 13 weeks. Endpoints evaluated included survival, body and organ weights, clinical findings of toxicity, and gross and microscopic pathology. In the 13-week studies, hematology (rats only), clinical chemistry, sperm motility, and vaginal cytology parameters were also measured. In the rats, plasma thyroid hormone concentrations, hepatic cytochrome P_{450}1A, and hepatic cell proliferation were measured. Alterations in circulating thyroid hormone concentrations and hepatic cytochrome P_{450}1A1 activities are affected by dioxin-like compounds at low levels of exposure. Furthermore, the mutagenicity of 3,3′,4,4′-tetrachloroazoxybenzene was tested in *S. typhimurium*, and the induction of micronuclei in bone marrow cells and frequency of micronuclei in peripheral blood erythrocytes of mice were evaluated.

The doses used in the 16-day studies were based on a 3,3′,4,4′-tetrachloroazobenzene study that reported slightly decreased mean body weights in rats administered 100 ppm in feed (Hsia *et al.*, 1980), equivalent to 0.5 mg/kg per day for rats consuming 17 grams of feed per day, and on 3,3′,4,4′-tetrachloroazoxybenzene studies that reported decreased mean body and thymus weights in mice administered 10 mg/kg per day (Bleavins *et al.*, 1985a,b). Two higher and two lower doses were chosen for each study. Doses for the 13-week studies were based on the results of the 16-day studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 3,3′,4,4′-TETRACHLOROAZOXYBENZENE

3,3′,4,4′-Tetrachloroazoxybenzene was obtained from AccuStandard, Inc. (New Haven, CT) in one lot (G920331B). Information on identity, purity, and stability was provided by the manufacturer; identity was confirmed by the study laboratory. Reports on analyses performed in support of the 3,3′,4,4′-tetrachloroazoxybenzene studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a yellowish orange, crystalline solid, was identified as 3,3′,4,4′-tetrachloroazoxybenzene by infrared spectroscopy. The spectrum was consistent with a literature reference (Hsia and Burant, 1979). Gas chromatography indicated a purity greater than 98%.

Information supplied by the manufacturer indicated that 3,3′,4,4′-tetrachloroazoxybenzene is stable as a bulk chemical when stored at room temperature. Throughout the studies, the bulk chemical was stored at room temperature in a well-ventilated area.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared every 2 to 3 weeks by mixing 3,3′,4,4′-tetrachloroazoxybenzene with corn oil. Homogeneity studies of 0.1, 2.5 and 100 mg/mL formulations and stability studies of 0.1 and 2.5 mg/mL formulations were performed by the study laboratory using high-performance liquid chromatography. Homogeneity was confirmed and the stability of the dose formulations was confirmed for up to 28 days at room temperature when stored in dosing bottles. Dose formulations were stored for no longer than 3 weeks.

Periodic analyses of the dose formulations of 3,3′,4,4′-tetrachloroazoxybenzene were conducted at the study laboratory using high-performance liquid chromatography and ultraviolet spectroscopy. All dose formulations administered to rats and mice and all animal room samples were within 10% of the target concentrations.

16-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). Upon receipt, the rats and mice were 4 weeks old. Rats were quarantined for 13 days and mice for 14 days; rats and mice were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice received 3,3′,4,4′-tetrachloroazoxybenzene in corn oil by gavage 5 days a week at doses of 0, 12.5, 32, 80, 200, or 500 mg/kg (rats) or 0, 1, 3.2, 10, 32, or 100 mg/kg (mice). Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded and animals were weighed initially, on day 8, and at the end of the studies. At the beginning of the studies, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Details of the study design and animal maintenance are summarized in Table 1.

A necropsy was performed on all rats and mice. The heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus were weighed. Histopathologic examinations of selected tissues were performed on all vehicle control rats and mice, all rats and mice in the highest dose groups with at least 60% survivors, and all higher dose groups. Table 1 lists the tissues and organs examined.

13-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). Upon receipt, the rats and mice were 4 weeks old. Rats were quarantined for 13 days and mice for 14 days; rats and mice were 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood samples were collected from five male and five female rats and mice at the beginning of the 13-week studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Groups of 10 male and 10 female rats and mice received 3,3′,4,4′-tetrachloroazoxybenzene in corn oil by gavage at doses of 0, 0.1, 1, 3, 10, or 30 mg/kg. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded and animals were weighed initially, weekly, and at the end of the studies. Special study groups of 10 male and 10 female rats were included in the 13-week rat study and were designated for interim clinical pathology testing. Special study rats were housed with the core-study animals. Details of the study design and animal maintenance are summarized in Table 1.

Hematology and clinical chemistry studies were performed on special study rats on days 3 and 21 and on all core study rats at study termination. At the end of the 13-week study, clinical chemistry analyses were performed on all mice. At all time points, rats and mice were anesthetized with a CO_2/O_2 mixture, and blood was drawn from the retroorbital sinus. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Manual hematocrit determinations were performed using an Adams CT2900 Microhematocrit centrifuge (Clay Adams, Sparks, MD). All other hematology parameters were measured using a Serono-Baker 9000 automated cell counter (Serono-Baker Diagnostics, Allentown, PA). Leukocyte differentials, nucleated erythrocyte counts, and morphological evaluation of blood cells were determined by light microscopic examination of blood films stained with a modified Wright's stain using an Ames Hema-Tek II slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Smears made from preparations of equal volumes of new methylene blue (Sigma Chemical Company, St. Louis, MO) and whole blood were incubated for at least 20 minutes at room temperature and examined microscopically for the quantitative determination of reticulocytes.

Blood for clinical chemistry determinations was placed in tubes with no anticoagulant and allowed to clot at room temperature, and the serum was separated. All clinical chemistry endpoints except total triiodothyronine, total thyroxine, and thyroid-stimulating hormone concentrations were determined using a Hitachi® 717 chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) with reagents obtained from the manufacturer; reagents obtained from Sigma Chemical Company (St. Louis, MO) were used for the determination of sorbitol dehydrogenase and bile acids. Total triiodothyronine, total thyroxine, and thyroid-stimulating hormone concentrations were measured by radioimmunoassay techniques. DPC Coat-A-Count reagent kits (Diagnostic Products Corporation, Los Angeles, CA) were used for the total triiodothyronine and total thyroxine assays. Thyroid-stimulating hormone concentrations were measured using a double-antibody technique and rat-specific reagents obtained from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (Bethesda, MD). The parameters measured for clinical pathology determinations are listed in Table 1.

On days 31 and 87 of the 13-week study, the special study male and female rats used for clinical pathology evaluations were tested for hepatocyte cell proliferation. An Alzet® 1003D infusion pump (Alza Corporation, Palo Alto, CA) containing 30 mg/mL bromodeoxyuridine (BrdU) (Sigma Chemical Company) and 0.01N sodium hydroxide in sterile deionized water was implanted subcutaneously between the scapula of up to five anesthetized male and female rats per dose group. Animals were not dosed with 3,3′4,4′-tetrachloroazoxybenzene on the day of the surgery. After 3 days the animals were killed, and the liver and duodenum were removed, fixed in neutral buffered formalin, and sectioned at 5 to 6 µm. Duplicate slides of the left, median, and right liver lobes and duodenum were prepared; one was stained with hematoxylin and eosin and the other with anti-BrdU antibodies. BrdU-labeled tissues were mounted on slides, deparaffinized, passed through a Tris and detergent buffer, and treated with 2 N hydrochloric acid for 30 minutes at 37 C to denature double-stranded DNA, because the anti-

BrdU antibodies only bind to single-stranded DNA. Slides were rinsed with borate buffer for 3 minutes; then a trypsin solution was applied for 3 minutes at 37 C to expose antigenic sites. Slides were rinsed with distilled water, then an automation buffer, and a 3% hydrogen peroxide block was applied for 10 minutes, followed by two 5-minute rinses with automation buffer. The blocking serum was applied, and the slides were incubated for 20 minutes at room temperature. Excess fluid was shaken from the slides. Slides were treated with anti-BrdU primary antibody for 60 minutes at room temperature and rinsed with automation buffer, and biotinylated secondary antibody was applied for 30 minutes at room temperature, followed by two automation buffer rinses. An avidin-biotin complex was applied to the tissue sections and incubated for 30 minutes at room temperature. After two rinses with automation buffer, 3,3′-diaminobenzidinetetrahydrochloride substrate was applied, and the slides were incubated for 6 minutes at room temperature, then rinsed with tap water and counterstained with Mayer's hematoxylin. The slides were rinsed in ammoniated 70% alcohol, washed, and dehydrated through graded alcohols to xylene. Coverslips were then applied. Cell proliferation in the median lobe of the liver was evaluated in all animals that demonstrated immunodetectable incorporation of BrdU in the duodenum. The number of labeled and unlabeled hepatocyte nuclei per eight randomly selected high-power fields was counted for each animal, and the labeling index was calculated.

The left liver lobe was collected from core study rats for immunohistochemical determination of hepatic cytochrome P_{450}1A. The tissues were fixed in 4% paraformaldehyde for 19 to 23 hours at 4 C . The tissues were then washed in cold phosphate-buffered saline for 6 hours at 4 C with a change at 3 hours and were stored in 70% ethanol until processed into paraffin blocks and sectioned, and slides were prepared. Liver tissue samples were stained with anti-P_{450}1A antibodies (Oxford Biomedical, Oxford, MI). Sections of a liver from a rat exposed to 3,3′,4,4′-tetrachloroazoxybenzene served as the quality control in cytochrome P_{450}1A determinations. The presence and intensity of cytochrome P_{450}1A staining in the liver lobe were then rated.

At the end of the 13-week studies, samples were collected for sperm motility and vaginal cytology evaluations from rats administered 0, 1, 3, or 10 mg/kg and mice administered 0, 3, 10, or 30 mg/kg. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1991). For 12 consecutive days prior to the scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for

five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65 C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

A necropsy was performed on all core study animals. The heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all vehicle control animals, all animals in the highest dose groups with at least 60% survivors, and all higher dose groups at the end of the studies. Target organs were examined in all lower dose groups. Table 1 lists the tissues and organs examined.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 3,3′,4,4′-Tetrachloroazoxybenzene

16-Day Studies	13-Week Studies
Study Laboratory Microbiological Associates, Inc. (Bethesda, MD)	Microbiological Associates, Inc. (Bethesda, MD)
Strain and Species Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$
Animal Source Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Studies Rats: 13 days Mice: 14 days	Rats: 13 days Mice: 14 days
Average Age When Studies Began 6 weeks	Rats: 6 weeks Mice: 6 weeks (males) or 7 weeks (females)
Date of First Dose Rats: 19 October 1992 Mice: 20 October 1992	Rats: 12 (males) or 13 (females) January 1993 Mice: 14 (males) or 15 (females) January 1993
Duration of Dosing 16 days (5 days/week)	91 days (5 days/week)
Date of Last Dose Rats: 3 November 1992 Mice: 4 November 1992	Rats: 12 (males) or 13 (females) April 1993 Mice: 14 (males) or 15 (females) April 1993
Necropsy Dates Rats: 4 November 1992 Mice: 5 November 1992	Core study rats: 13 (males) or 14 (females) April 1993 Mice: 15 (males) or 16 (females) April 1993
Average Age at Necropsy 8 weeks	Rats: 19 weeks Mice: 19 weeks (males) or 20 weeks (females)
Size of Study Groups 5 males and 5 females	10 males and 10 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 16-day studies
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 16-day studies
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Laboratories, Waterford, WI), available *ad libitum*	Same as 16-day studies

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 3,3′,4,4′-Tetrachloroazoxybenzene

16-Day Studies	13-Week Studies
Cages Polycarbonate (Lab Products, Maywood, NJ), changed twice weekly for rats and female mice and weekly for male mice	Same as 16-day studies
Bedding Sani-Chips® (P.J. Murphy Forest Products, Montville, NJ), changed twice weekly for rats and female mice and weekly for male mice	Same as 16-day studies
Racks Stainless steel (Lab Products, Rochelle Park, NJ), changed and rotated every 2 weeks	Same as 16-day studies
Animal Room Environment Temperature: 72 ± 3 F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72 ± 3 F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses Rats: 0, 12.5, 32, 80, 200, or 500 mg/kg in corn oil by gavage (dosing volume = 5 mL/kg body weight) Mice: 0, 1, 3.2, 10, 32, or 100 mg/kg in corn oil by gavage (dosing volume = 10 mL/kg body weight)	0, 0.1, 1, 3, 10, or 30 mg/kg in corn oil by gavage (dosing volume = 5 mL for rats or 10 mL for mice per kg body weight)
Type and Frequency of Observation Observed twice daily; animals were weighed and clinical findings were recorded initially, on day 8, and at the end of the studies.	Observed twice daily; animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies.
Method of Sacrifice 70%:30% CO_2:O_2	Same as 16-day studies
Necropsy Necropsy was performed on all animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus.	Necropsy was performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus.
Clinical Pathology None	Blood was collected from the retroorbital sinus of all special study rats on days 3 and 21 and all core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of all mice surviving to the end of the study for clinical chemistry. ***Hematology:*** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and total leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, bile acids, thyroid-stimulating hormone (rats), total triiodothyronine (rats), and total thyroxine (rats)

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 3,3′,4,4′-Tetrachloroazoxybenzene

16-Day Studies	13-Week Studies
Histopathology	
Histopathology was performed on all vehicle control animals, all animals in the highest dose groups with at least 60% survivors, and all higher dose groups. In addition to gross lesions and tissue masses, the following tissues were examined: liver, kidney, mesenteric lymph nodes, spleen, stomach (forestomach and glandular), and thymus.	Complete histopathology was performed on all vehicle control animals, all animals in the highest dose groups with at least 60% survivors, and all higher dose groups at the end of the studies. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone (including marrow), brain, clitoral gland, esophagus, gallbladder (mice), heart, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Target organs examined in all lower dose groups of rats were forestomach, heart, kidney, liver, lung, mesenteric lymph nodes (females only), spleen, and thymus. Target organs examined in all lower dose groups of mice were liver, stomach (forestomach and glandular), skin, spleen, and thymus.
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from all core study male animals in the 0, 1, 3, and 10 mg/kg groups (rats) and 0, 3, 10, and 30 mg/kg groups (mice) for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from all core study females administered 0, 1, 3, or 10 mg/kg (rats) and 0, 3, 10, or 30 mg/kg (mice) for vaginal cytology evaluations. The parameters evaluated were the percentage of cycle spent in the various estrous stages and estrous cycle length.
Hepatocyte Proliferation Analyses	
None	On days 31 and 87, up to five male and five female special study rats per dose group were implanted with bromodeoxyuridine infusion pumps for 3 days for the determination of hepatocyte proliferation in the liver and duodenum.
Hepatic Cytochrome P_{450}1A Staining	
None	Cytochrome P_{450}1A presence and staining intensity was determined in core study rats at the end of the study.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions as presented in Appendix A are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Hepatic cytochrome P_{450}1A staining presence values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

QUALITY ASSURANCE METHODS

The 13-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al*. (1987). 3,3′,4,4′-Tetrachloroazoxybenzene was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37 C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37 C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of 3,3′,4,4′-tetrachloroazoxybenzene. In the absence of toxicity, 10,000 µg/plate was selected as the high dose.

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

Mouse Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 3,3′,4,4′-tetrachloroazoxybenzene exposure. The standard three-exposure protocol is described in detail by Shelby *et al*. (1993). Groups of five male B6C3F$_1$ mice were injected intraperitoneally three times at 24-hour intervals with 3,3′,4,4′-tetrachloroazoxybenzene dissolved in corn oil at dose levels up to 200 mg/kg; the total dosing volume was 0.4 mL. Solvent control animals were injected with 0.4 mL of corn oil only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage

trend test, followed by pairwise comparisons between each dosed group and the vehicle control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented in MacGregor *et al*. (1990). At the end of the 13-week toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes in each of five animals per dose group. Results were analyzed by the same methods described in the mouse bone marrow micronucleus test protocol.

RESULTS

RATS

16-DAY STUDY

All rats survived to the end of the study (Table 2). The final mean body weights of males and females in the 80, 200, and 500 mg/kg groups were significantly less than those of the vehicle controls. The mean body weight gains of all groups of dosed males and 80, 200, and 500 mg/kg females were significantly less than those of the vehicle controls. No chemical-related clinical findings were observed during the study.

TABLE 2
Survival and Body Weights of Rats in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	129 ± 6	217 ± 8	88 ± 3	
12.5	5/5	130 ± 2	205 ± 3	75 ± 1*	95
32	5/5	129 ± 4	201 ± 5	72 ± 2**	93
80	5/5	130 ± 4	189 ± 7**	60 ± 3**	87
200	5/5	130 ± 4	176 ± 5**	46 ± 3**	81
500	5/5	128 ± 5	163 ± 10**	35 ± 6**	75
Female					
0	5/5	109 ± 2	144 ± 5	36 ± 3	
12.5	5/5	107 ± 2	140 ± 2	33 ± 2	97
32	5/5	108 ± 3	141 ± 4	33 ± 1	98
80	5/5	106 ± 1	132 ± 3*	26 ± 2*	92
200	5/5	107 ± 2	127 ± 4**	20 ± 2**	88
500	5/5	105 ± 4	121 ± 2**	15 ± 4**	84

* Significantly different (P 0.05) from the vehicle control group by Williams' test
** P 0.01
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

The absolute and relative liver weights of all dosed groups of males and females, except the absolute liver weight of the 500 mg/kg male group, were significantly greater than those of the vehicle controls (Tables 3 and C1). Absolute right testis weights were significantly less and the relative right testis weights were significantly greater in 200 and 500 mg/kg males than in the vehicle controls. The absolute and relative thymus weights of all dosed groups of male and female rats were significantly less than those of the vehicle controls. Absolute and relative uterus weights of females administered 500 mg/kg were significantly less than those of the vehicle controls. The increases in liver and relative right testis weights and the decreases in thymus, uterus, and absolute right testis weights generally occurred with significant trends. Absolute heart weights of males in the 200 and 500 mg/kg groups and females in the 500 mg/kg group were significantly less than those of the vehicle controls. Generally, the relative lung weights of dosed groups of males and the absolute and relative lung weights of all dosed groups of females were greater than those of the vehicle controls. Increases in relative kidney and spleen weights in males and females and relative lung weights in females were dose dependent or occurred with a positive trend.

No treatment-related gross lesions were observed at necropsy. Microscopically, cytoplasmic alteration of hepatocytes in the liver, hematopoietic cell proliferation of the spleen, and thymic atrophy were observed and considered treatment related. The incidences, but not the severities, of nephropathy were slightly increased in dosed males and females.

The dose selection for the 13-week study in rats was based on the lower thymus weights observed in male and female rats administered 12.5 mg/kg or greater. Because 3,3′,4,4′-tetrachloroazoxybenzene is expected to bioaccumulate, although to a lesser extent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the doses for the 13-week study in rats were chosen to be minimally immunotoxic. Because it was unknown whether the animals would develop a tolerance to 3,3′,4,4′-tetrachloroazoxybenzene (i.e., by increased metabolism), the doses chosen for the 13-week study in rats were 0, 0.1, 1, 3, 10, and 30 mg/kg.

TABLE 3
Selected Organ Weight Data for Rats in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	12.5 mg/kg	32 mg/kg	80 mg/kg	200 mg/kg	500 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	217 ± 8	205 ± 3	201 ± 5	189 ± 7**	176 ± 5**	163 ± 10**
Liver						
Absolute	9.976 ± 0.449	12.389 ± 0.327*	12.353 ± 0.352*	13.630 ± 0.455**	13.318 ± 0.505**	11.710 ± 0.801
Relative	45.94 ± 0.71	60.34 ± 1.40**	61.53 ± 0.77**	72.03 ± 0.86**	75.71 ± 1.54**	71.87 ± 0.84**
Lung						
Absolute	1.119 ± 0.056	1.232 ± 0.0.89	1.456 ± 0.455**	1.456 ± 0.017**	1.420 ± 0.041	1.103 ± 0.082
Relative	5.15 ± 0.12	5.99 ± 0.39	7.27 ± 0.25**	7.53 ± 0.44**	7.11 ± 0.08**	6.77 ± 0.19**
R. Testis						
Absolute	1.256 ± 0.041	1.207 ± 0.033	1.195 ± 0.044	1.195 ± 0.028	1.146 ± 0.017*	1.102 ± 0.022**
Relative	5.80 ± 0.07	5.88 ± 0.16	5.94 ± 0.09	6.32 ± 0.13	6.53 ± 0.11**	6.86 ± 0.36**
Thymus						
Absolute	0.480 ± 0.029	0.249 ± 0.014**	0.194 ± 0.011**	0.158 ± 0.011**	0.130 ± 0.008**	0.126 ± 0.015**
Relative	2.21 ± 0.10	1.21 ± 0.07**	0.97 ± 0.05**	0.83 ± 0.03**	0.74 ± 0.04**	0.77 ± 0.06**
Female						
Necropsy body wt	144 ± 5	140 ± 2	141 ± 4	132 ± 3*	127 ± 4**	121 ± 2**
Liver						
Absolute	6.438 ± 0.325	7.535 ± 0.237*	9.100 ± 0.363**	9.232 ± 0.227**	9.430 ± 0.371**	8.536 ± 0.249**
Relative	44.57 ± 1.31	53.94 ± 0.99**	64.49 ± 1.95**	69.86 ± 0.93**	73.92 ± 0.77**	70.79 ± 1.76**
Lung						
Absolute	0.850 ± 0.030	1.016 ± 0.041*	1.012 ± 0.045*	0.992 ± 0.038*	1.010 ± 0.032*	0.914 ± 0.027
Relative	5.89 ± 0.08	7.27 ± 0.21**	7.17 ± 0.26**	7.53 ± 0.41**	7.93 ± 0.17**	7.58 ± 0.14**
Thymus						
Absolute	0.381 ± 0.016	0.210 ± 0.012**	0.207 ± 0.017**	0.107 ± 0.019**	0.116 ± 0.011**	0.103 ± 0.007**
Relative	2.64 ± 0.09	1.50 ± 0.08**	1.46 ± 0.09**	0.81 ± 0.14**	0.91 ± 0.07**	0.85 ± 0.06**
Uterus						
Absolute	0.376 ± 0.025	0.313 ± 0.048	0.361 ± 0.035	0.271 ± 0.027	0.291 ± 0.046	0.194 ± 0.019**
Relative	2.61 ± 0.15	2.24 ± 0.33	2.55 ± 0.21	2.05 ± 0.18	2.31 ± 0.40	1.61 ± 0.16*

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

13-WEEK STUDY

All male rats and seven female rats in the 30 mg/kg groups died before the end of the study (Table 4). The final mean body weights and body weights gains were significantly decreased in 3 and 10 mg/kg males and 10 and 30 mg/kg females compared to the vehicle controls (Table 4; Figure 2); these decreases were dose dependent. Males that received 30 mg/kg and that died before the end of the study also had decreased body weights, with five males losing an average of 35 g of body weight during week 6. Clinical findings included pale extremities and eyes, ruffled fur, thinness, and lethargy in male and female rats in the 30 mg/kg groups; the incidences were greater in males.

TABLE 4
Survival and Body Weights of Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	124 ± 2	366 ± 5	241 ± 4	
0.1	10/10	123 ± 3	357 ± 8	234 ± 6	98
1	10/10	127 ± 2	351 ± 6	224 ± 5	96
3	10/10	125 ± 3	332 ± 5**	207 ± 4**	91
10	10/10	125 ± 3	301 ± 13**	176 ± 10**	82
30	0/10[c]	126 ± 2	—	—	—
Female					
0	10/10	105 ± 2	197 ± 3	91 ± 2	
0.1	10/10	108 ± 2	192 ± 3	84 ± 2*	98
1	10/10	107 ± 1	191 ± 2	85 ± 3	97
3	10/10	106 ± 2	191 ± 3	84 ± 2	97
10	10/10	105 ± 2	185 ± 4**	80 ± 3**	94
30	3/10[d]	105 ± 2	175 ± 2**	71 ± 1**	89

* Significantly different (P 0.05) from the vehicle control group by Williams' test
** P 0.01
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No data were calculated for groups with 100% mortality.
[c] Week of deaths: 6, 7, 7, 7, 7, 7, 8, 8, 9, 9
[d] Week of deaths: 8, 9, 10, 10, 11, 12, 12

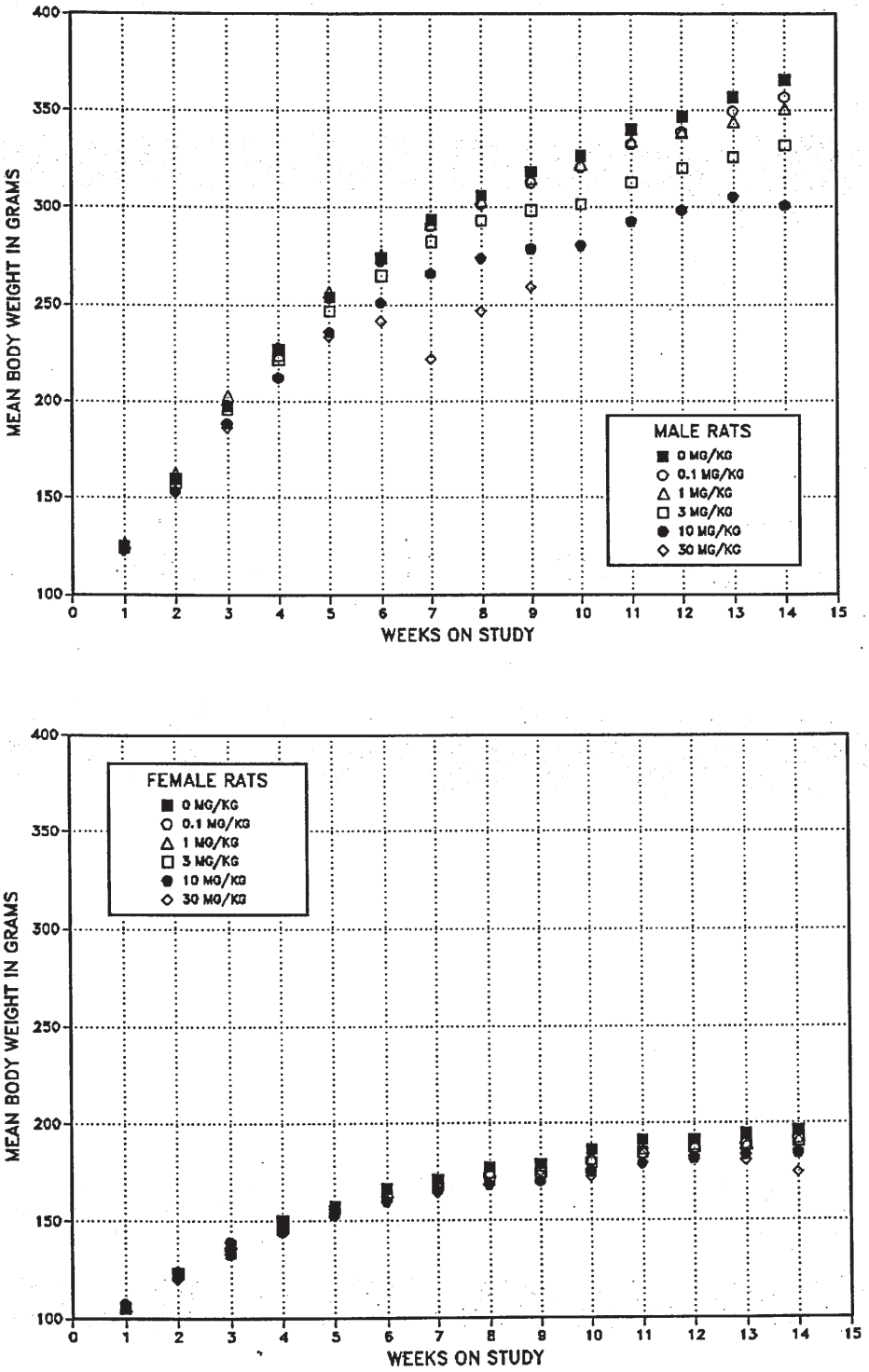


FIGURE 2
Body Weights of Rats Administered 3,3′,4,4′-Tetrachloroazoxybenzene by Gavage for 13 Weeks

The hematology and clinical chemistry data are listed in Tables 5 and B1. At week 13, a treatment-related anemia occurred in 1, 3, and 10 mg/kg males and 10 and 30 mg/kg females. The anemia was evidenced by dose-dependent decreases in erythrocyte counts, hemoglobin concentrations, and hematocrit values. The anemia was characterized as macrocytic, normochromic, and responsive. Evidence of a macrocytosis was demonstrated by a dose-dependent increase in the mean cell volumes; the difference from vehicle controls was significant in the 10 mg/kg males. A minimal increase in mean cell hemoglobin accompanied the increases in mean cell volume and probably reflects the increased erythrocyte size in males. Normochromic erythrocytes were evidenced by the absence of change in the mean cell hemoglobin concentrations in dosed males. An erythropoietic response was demonstrated by increased reticulocyte counts, which occurred with a positive trend in males and females. On day 21, there was some evidence of a responsive anemia in the 30 mg/kg males and 1, 10, and 30 mg/kg females, suggesting the anemia developed over time. On day 21, a thrombocytopenia, evidenced by decreases in platelet counts, was observed in the 3, 10, and 30 mg/kg male and female rats and occurred with a negative trend; at study termination, a thrombocytopenia was evident in 3 and 10 mg/kg males and in all female groups. On day 21 and at study termination, minimal decreases in leukocyte counts and differentials, characterized by decreases in lymphocyte and eosinophil counts, occurred in males that received 1 mg/kg or greater and 30 mg/kg females; this would be consistent with a stress-related (steroid-like) leukocyte response (Jain, 1986a).

At week 13, dose-dependent decreases in total thyroxine concentrations occurred in males that received 1 mg/kg or greater and in all dosed female groups; decreased total triiodothyronine concentrations occurred only in 10 and 30 mg/kg females. In an apparent response to decreased thyroid hormone concentrations, thyroid-stimulating hormone concentrations were moderately increased in the 1, 3, and 10 mg/kg males and occurred with a significant dose-dependent trend.

On day 3, increases in total protein and albumin concentrations occurred in the 30 mg/kg females; by day 21, these changes were evident in the 3 mg/kg males and the 10 and 30 mg/kg males and females. At study termination, increases in albumin concentration occurred in the 1, 3, and 10 mg/kg males, and the increase in the 10 mg/kg group was accompanied by an increase in protein concentration.

On day 21 in males and at week 13 in males and females, serum alanine aminotransferase activities were decreased in groups that received 1 mg/kg or greater. In contrast, on day 21, activity of sorbitol dehydrogenase, another marker of hepatocellular leakage, was dose-dependently increased in all groups of dosed females. At 13 weeks, activities of serum alkaline phosphatase were increased in males that received 1 mg/kg or greater and in 1 and 3 mg/kg females, suggesting a cholestatic event. Also at week 13, bile acid concentration, a marker of cholestasis and/or altered hepatocellular function, was significantly increased in 3, 10, and 30 mg/kg females, and the increases occurred with a positive trend.

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	8	0[b]
Hematology						
Automated hematocrit (%)						
Day 3	38.2 ± 0.4	38.6 ± 0.5	39.1 ± 0.5	38.8 ± 0.3	39.8 ± 0.6	37.8 ± 1.3
Day 21	41.1 ± 0.7	40.6 ± 0.5	39.9 ± 0.4	40.1 ± 0.5	41.1 ± 0.6	39.3 ± 0.3
Week 13	42.9 ± 0.4	42.0 ± 0.5	41.6 ± 0.6	39.0 ± 0.4**	37.2 ± 0.3**	—
Manual hematocrit (%)						
Day 3	44.1 ± 0.4	44.5 ± 0.6	45.4 ± 0.6	45.2 ± 0.4	46.3 ± 0.7*	45.2 ± 0.4
Day 21	46.6 ± 0.7	46.9 ± 0.7	45.4 ± 0.4	46.0 ± 0.6	46.6 ± 0.6	44.1 ± 0.5*
Week 13	46.6 ± 0.4	45.7 ± 0.5	44.7 ± 0.5**	42.9 ± 0.3**	40.4 ± 0.3**	—
Hemoglobin (g/dL)						
Day 3	14.2 ± 0.1	14.1 ± 0.1	14.2 ± 0.1	14.2 ± 0.1	14.6 ± 0.2	13.8 ± 0.4
Day 21	15.2 ± 0.2	14.8 ± 0.2	14.6 ± 0.1	14.8 ± 0.2	15.0 ± 0.1	14.2 ± 0.2**
Week 13	15.4 ± 0.1	15.1 ± 0.1	14.7 ± 0.2**	13.9 ± 0.1**	13.1 ± 0.2**	—
Erythrocytes (10^6/μL)						
Day 3	6.34 ± 0.07	6.37 ± 0.10	6.45 ± 0.08	6.48 ± 0.05	6.63 ± 0.09	6.26 ± 0.21
Day 21	6.83 ± 0.12	6.75 ± 0.08	6.73 ± 0.07	6.81 ± 0.07	7.11 ± 0.10*	6.85 ± 0.05
Week 13	8.59 ± 0.09	8.39 ± 0.10	8.35 ± 0.12	7.73 ± 0.09**	6.96 ± 0.11**	—
Reticulocyte (10^6/μL)						
Day 3	0.29 ± 0.04	0.21 ± 0.02	0.25 ± 0.04	0.25 ± 0.03	0.23 ± 0.02	0.22 ± 0.02
Day 21	0.12 ± 0.01	0.11 ± 0.01	0.17 ± 0.03	0.16 ± 0.02	0.13 ± 0.01	0.23 ± 0.03**
Week 13	0.07 ± 0.01	0.09 ± 0.02	0.11 ± 0.01**	0.09 ± 0.01*	0.14 ± 0.01**	—
Mean cell volume (fL)						
Day 3	60.2 ± 0.2	60.6 ± 0.3	60.7 ± 0.1	59.9 ± 0.4	60.0 ± 0.3	60.4 ± 0.3
Day 21	60.1 ± 0.3	60.2 ± 0.3	59.3 ± 0.2*	58.9 ± 0.3**	57.9 ± 0.3**	57.3 ± 0.3**
Week 13	49.9 ± 0.1	50.0 ± 0.2	49.8 ± 0.1	50.4 ± 0.3	53.6 ± 0.4**	—
Mean cell hemoglobin (pg)						
Day 3	22.3 ± 0.1	22.1 ± 0.2	22.0 ± 0.1	22.0 ± 0.1	22.0 ± 0.1	22.0 ± 0.1
Day 21	22.3 ± 0.1	22.0 ± 0.1	21.7 ± 0.1*	21.8 ± 0.1*	21.1 ± 0.2**	20.7 ± 0.2**
Week 13	17.9 ± 0.1	18.0 ± 0.1	17.6 ± 0.1	18.0 ± 0.1	18.8 ± 0.1**	—
Mean cell hemoglobin concentration (g/dL)						
Day 3	37.1 ± 0.1	36.5 ± 0.3	36.3 ± 0.2*	36.7 ± 0.3	36.7 ± 0.2	36.4 ± 0.2
Day 21	37.1 ± 0.2	36.5 ± 0.2	36.6 ± 0.3	37.0 ± 0.2	36.5 ± 0.3	36.0 ± 0.2*
Week 13	35.8 ± 0.2	36.0 ± 0.2	35.4 ± 0.2	35.7 ± 0.3	35.2 ± 0.2	—
Platelets (10^3/μL)						
Day 3	962.7 ± 9.4	996.4 ± 20.9	1,009.6 ± 13.1	959.7 ± 19.9	954.2 ± 17.8	914.3 ± 35.5
Day 21	841.3 ± 9.7	839.8 ± 13.4	860.6 ± 15.0	790.4 ± 16.3*	760.1 ± 15.4**	574.6 ± 14.5**
Week 13	716.8 ± 26.0	753.3 ± 21.7	681.8 ± 12.3	617.0 ± 17.6**	291.3 ± 22.5**	—

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	8	0
Clinical Chemistry						
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.5 ± 0.1
Day 21	6.4 ± 0.1	6.4 ± 0.0	6.5 ± 0.1	6.8 ± 0.1**	6.8 ± 0.2**	6.6 ± 0.1*
Week 13	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.2	7.5 ± 0.1	7.6 ± 0.1**	—
Albumin (g/dL)						
Day 3	4.1 ± 0.0	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1
Day 21	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.1**	4.7 ± 0.1*	4.7 ± 0.1
Week 13	4.7 ± 0.0	4.8 ± 0.1	4.9 ± 0.1*	5.1 ± 0.1**	5.1 ± 0.1**	—
Alanine aminotransferase (IU/L)						
Day 3	48 ± 2	45 ± 2	43 ± 1	45 ± 2	42 ± 1	48 ± 2
Day 21	41 ± 1	39 ± 1	34 ± 1**	36 ± 1**	32 ± 1**	30 ± 1**
Week 13	60 ± 2	57 ± 3	50 ± 3*	47 ± 1**	48 ± 2**	—
Alkaline phosphatase (IU/L)						
Day 3	787 ± 19	772 ± 11	807 ± 23	809 ± 23	810 ± 21	833 ± 23
Day 21	523 ± 17	486 ± 10	485 ± 16	479 ± 6**	481 ± 9*	464 ± 11**
Week 13	288 ± 8	301 ± 6	318 ± 15*	318 ± 11*	329 ± 11**	—
Sorbitol dehydrogenase (IU/L)						
Day 3	20 ± 1	18 ± 1	16 ± 1	18 ± 1	21 ± 2	19 ± 1
Day 21	18 ± 1	18 ± 1	20 ± 1	19 ± 1	20 ± 1	20 ± 1
Week 13	19 ± 1	16 ± 1	18 ± 1	16 ± 2	21 ± 2	—
Bile acids (µmol/L)						
Day 3	39.1 ± 6.6	37.3 ± 4.3	41.1 ± 3.9	42.0 ± 5.0	42.0 ± 3.6	43.8 ± 4.6
Day 21	28.8 ± 5.4	38.9 ± 5.0	33.2 ± 4.6	29.9 ± 4.2	29.3 ± 2.3	33.6 ± 3.6
Week 13	40.9 ± 3.8	34.3 ± 4.1	37.8 ± 4.6	45.8 ± 4.7	56.0 ± 6.7	—
Thyroid-stimulating hormone (ng/mL)						
Week 13	1.6 ± 0.2	2.6 ± 0.2*	2.9 ± 0.5*	3.1 ± 0.5*	3.5 ± 0.6**	—
Total Triiodothyronine (ng/dL)						
Week 13	98 ± 5	118 ± 7	102 ± 6	117 ± 8	99 ± 7	—
Total thyroxine (µg/dL)						
Week 13	2.9 ± 0.1	3.0 ± 0.1	1.9 ± 0.1**	0.9 ± 0.1**	0.4 ± 0.1**	—
Female						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Hematology						
Automated hematocrit (%)						
Day 3	41.8 ± 0.6	41.1 ± 0.7	41.5 ± 0.5	41.1 ± 0.5	42.0 ± 0.6	42.6 ± 0.5
Day 21	42.7 ± 0.5	42.0 ± 0.6	41.2 ± 0.5	42.9 ± 0.3	41.3 ± 0.5	40.9 ± 1.0
Week 13	41.0 ± 0.6	41.9 ± 0.7	40.6 ± 0.5	41.0 ± 0.3	38.8 ± 0.5*	18.5 ± 1.4**

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Hematology (continued)						
Manual hematocrit (%)						
Day 3	45.4 ± 0.8	45.2 ± 0.7	45.0 ± 0.6	45.3 ± 0.7	45.7 ± 0.6	46.2 ± 0.8
Day 21	45.4 ± 0.4	44.9 ± 0.5	43.8 ± 0.6	45.9 ± 0.5	44.3 ± 0.6	43.9 ± 1.0
Week 13	44.7 ± 0.5	45.8 ± 0.7	44.1 ± 0.6	44.8 ± 0.3	42.8 ± 0.5*	20.3 ± 1.2**
Hemoglobin (g/dL)						
Day 3	14.8 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	15.0 ± 0.2	15.1 ± 0.2
Day 21	15.4 ± 0.1	15.2 ± 0.2	14.6 ± 0.1**	15.3 ± 0.2	14.6 ± 0.1**	14.4 ± 0.3**
Week 13	14.9 ± 0.1	15.1 ± 0.2	14.6 ± 0.2	14.5 ± 0.1	13.6 ± 0.1**	6.9 ± 0.5**
Erythrocytes (10^6/μL)						
Day 3	6.90 ± 0.09	6.80 ± 0.12	6.82 ± 0.09	6.81 ± 0.08	6.91 ± 0.10	7.01 ± 0.11
Day 21	7.04 ± 0.09	6.94 ± 0.11	6.83 ± 0.09	7.21 ± 0.07	6.97 ± 0.09	7.11 ± 0.19
Week 13	7.45 ± 0.09	7.58 ± 0.14	7.42 ± 0.09	7.51 ± 0.04	7.06 ± 0.08*	2.92 ± 0.26**
Reticulocyte (10^6/μL)						
Day 3	0.17 ± 0.01	0.23 ± 0.01*	0.21 ± 0.02	0.21 ± 0.02	0.24 ± 0.03	0.20 ± 0.01
Day 21	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01*	0.15 ± 0.01*	0.17 ± 0.01**	0.19 ± 0.01**
Week 13	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.12 ± 0.01**	0.19 ± 0.02**
Mean cell volume (fL)						
Day 3	60.5 ± 0.3	60.5 ± 0.3	60.9 ± 0.4	60.4 ± 0.2	60.8 ± 0.3	60.8 ± 0.3
Day 21	60.7 ± 0.2	60.6 ± 0.2	60.4 ± 0.2	59.6 ± 0.2**	59.3 ± 0.3**	57.6 ± 0.2**
Week 13	55.0 ± 0.3	55.3 ± 0.3	54.7 ± 0.2	54.5 ± 0.2	55.0 ± 0.2	63.5 ± 1.0
Mean cell hemoglobin (pg)						
Day 3	21.5 ± 0.1	21.7 ± 0.2	21.6 ± 0.1	21.6 ± 0.1	21.7 ± 0.1	21.6 ± 0.2
Day 21	21.8 ± 0.2	21.9 ± 0.1	21.4 ± 0.1	21.3 ± 0.1*	21.0 ± 0.2**	20.2 ± 0.1**
Week 13	20.0 ± 0.1	20.0 ± 0.2	19.7 ± 0.1	19.3 ± 0.1**	19.3 ± 0.1**	23.7 ± 0.8
Mean cell hemoglobin concentration (g/dL)						
Day 3	35.5 ± 0.3	35.8 ± 0.3	35.5 ± 0.2	35.8 ± 0.2	35.7 ± 0.2	35.5 ± 0.2
Day 21	36.0 ± 0.2	36.2 ± 0.2	35.4 ± 0.2*	35.7 ± 0.2	35.5 ± 0.3	35.1 ± 0.2**
Week 13	36.4 ± 0.2	36.1 ± 0.2	36.1 ± 0.3	35.4 ± 0.2	35.2 ± 0.2*	37.4 ± 0.6
Platelets (10^3/μL)						
Day 3	953.6 ± 20.3	910.8 ± 16.1	936.8 ± 14.6	877.7 ± 13.2*	925.9 ± 19.4	947.8 ± 21.8
Day 21	819.1 ± 20.3	814.5 ± 16.2	817.1 ± 12.9	713.0 ± 9.8**	727.2 ± 11.3**	613.0 ± 6.8**
Week 13	700.8 ± 21.8	637.0 ± 14.9*	591.0 ± 12.6**	525.2 ± 7.0**	475.9 ± 20.1**	65.7 ± 13.7**
Clinical Chemistry						
Total protein (g/dL)						
Day 3	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.6 ± 0.1*
Day 21	6.2 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.1**	6.7 ± 0.1**
Week 13	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.0	7.0 ± 0.1	6.6 ± 0.3
Albumin (g/dL)						
Day 3	4.0 ± 0.0	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.0*
Day 21	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.6 ± 0.1	4.8 ± 0.1**	4.8 ± 0.1**
Week 13	5.1 ± 0.0	5.1 ± 0.1	5.2 ± 0.0	5.3 ± 0.0	5.1 ± 0.1	5.1 ± 0.2

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Clinical chemistry (continued)						
Alanine aminotransferase (IU/L)						
Day 3	39 ± 2	38 ± 1	35 ± 2	37 ± 1	36 ± 2	37 ± 2
Day 21	35 ± 2	36 ± 1	30 ± 1	35 ± 1	34 ± 1	33 ± 2
Week 13	54 ± 5	48 ± 3	43 ± 3**	46 ± 3*	36 ± 2**	42 ± 5*
Alkaline phosphatase (IU/L)						
Day 3	596 ± 15	634 ± 13	656 ± 18*	659 ± 9**	691 ± 14**	712 ± 19**
Day 21	396 ± 6	406 ± 8	395 ± 6	384 ± 7	373 ± 10	347 ± 9**
Week 13	250 ± 8	279 ± 12	298 ± 10*	309 ± 11**	288 ± 6	241 ± 8
Sorbitol dehydrogenase (IU/L)						
Day 3	17 ± 1	17 ± 1	20 ± 1	17 ± 1	18 ± 2	17 ± 1
Day 21	15 ± 1	21 ± 1**	20 ± 1**	26 ± 2**	32 ± 2**	49 ± 4**
Week 13	16 ± 2	18 ± 1	16 ± 2	21 ± 1	15 ± 2	38 ± 20
Bile acids (µmol/L)						
Day 3	32.0 ± 3.4	42.7 ± 4.4	34.0 ± 3.4	39.1 ± 3.0	43.1 ± 3.4	42.0 ± 4.0
Day 21	36.4 ± 4.1	28.8 ± 2.5	37.4 ± 4.7	43.8 ± 4.0	58.1 ± 4.1**	49.8 ± 8.0*
Week 13	28.9 ± 3.9	31.6 ± 2.9	33.2 ± 2.6	36.9 ± 3.2*	52.4 ± 4.4**	117.2 ± 19.6**
Thyroid-stimulating hormone (ng/mL)						
Week 13	1.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.2
Total triiodothyronine (ng/dL)						
Week 13	97 ± 6	90 ± 7	89 ± 5	83 ± 3	64 ± 4**	83 ± 6*
Total thyroxine (µg/dL)						
Week 13	2.5 ± 0.2	1.7 ± 0.2*	1.2 ± 0.1**	0.3 ± 0.1**	0.1 ± 0.0**	0.0 ± 0.0**

* Significantly different (P 0.05) from the vehicle control group by Dunn's or Shirley's test
** P 0.01
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] No data available due to 100% mortality.

Generally, absolute and relative liver weights were significantly increased and absolute and relative thymus weights were significantly decreased in males and females administered 1 mg/kg or greater (Tables 6 and C2). The absolute and relative heart and right kidney weights of 30 mg/kg female rats and the absolute and relative lung and spleen weights of 10 and 30 mg/kg females were significantly greater than those of the vehicle controls. These organ weight changes occurred with significant trends. The relative heart, right kidney, lung, spleen, and right testis weights of males also occurred with positive trends.

TABLE 6
Selected Organ Weight Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n	10	10	10	10	10	0[b]
Necropsy body wt	366 ± 5	357 ± 8	351 ± 6	332 ± 5**	301 ± 13**	
Liver						
Absolute	12.854 ± 0.218	13.433 ± 0.285	14.563 ± 0.419*	14.068 ± 0.251*	14.039 ± 0.625*	
Relative	35.14 ± 0.35	37.67 ± 0.45**	41.46 ± 0.63**	42.37 ± 0.29**	46.59 ± 0.28**	
Thymus						
Absolute	0.409 ± 0.016	0.384 ± 0.017	0.279 ± 0.006**	0.301 ± 0.011**	0.249 ± 0.030**	
Relative	1.12 ± 0.04	1.08 ± 0.04	0.80 ± 0.02**	0.91 ± 0.03**	0.81 ± 0.08**	
Female						
n	10	10	10	10	10	3
Necropsy body wt	197 ± 3	192 ± 3	191 ± 2	191 ± 3	185 ± 4*	159 ± 5**
Heart						
Absolute	0.689 ± 0.018	0.668 ± 0.015	0.647 ± 0.005	0.673 ± 0.011	0.735 ± 0.026	0.892 ± 0.074**
Relative	3.51 ± 0.06	3.48 ± 0.06	3.39 ± 0.04	3.53 ± 0.05	3.97 ± 0.08**	5.10 ± 0.35**
R. Kidney						
Absolute	0.681 ± 0.013	0.671 ± 0.016	0.674 ± 0.010	0.690 ± 0.011	0.699 ± 0.019	0.797 ± 0.028**
Relative	3.47 ± 0.05	3.50 ± 0.07	3.52 ± 0.05	3.62 ± 0.05	3.78 ± 0.05**	4.56 ± 0.10**
Liver						
Absolute	6.266 ± 0.190	6.489 ± 0.196	6.713 ± 0.159	6.980 ± 0.193*	8.160 ± 0.314**	9.923 ± 0.110**
Relative	31.91 ± 0.90	33.78 ± 0.83	35.08 ± 0.70*	36.57 ± 0.57**	44.09 ± 1.16**	56.85 ± 1.34**
Lung						
Absolute	1.154 ± 0.058	1.210 ± 0.054	1.155 ± 0.042	1.211 ± 0.025	1.659 ± 0.179**	1.390 ± 0.023*
Relative	5.88 ± 0.29	6.31 ± 0.29	6.03 ± 0.19	6.36 ± 0.17	8.99 ± 0.98**	7.96 ± 0.22**
Spleen						
Absolute	0.447 ± 0.017	0.449 ± 0.019	0.457 ± 0.017	0.468 ± 0.017	0.545 ± 0.037**	0.565 ± 0.033*
Relative	2.28 ± 0.09	2.34 ± 0.08	2.38 ± 0.07	2.45 ± 0.06	2.95 ± 0.19**	3.23 ± 0.18**
Thymus						
Absolute	0.274 ± 0.010	0.264 ± 0.014	0.212 ± 0.007**	0.201 ± 0.009**	0.166 ± 0.011**	0.101 ± 0.006**
Relative	1.40 ± 0.05	1.37 ± 0.06	1.11 ± 0.03**	1.05 ± 0.04**	0.90 ± 0.05**	0.58 ± 0.03**

* Significantly different (P 0.05) from the vehicle control group by Williams' test
** Significantly different (P 0.01) from the vehicle control group by Williams' or Dunnett's test
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No data available due to 100% mortality.

Epididymal spermatozoal motility was dose-dependently reduced, and the difference was significant in all groups tested (Table D1). Females in the 10 mg/kg group had a significantly longer estrous cycle than the vehicle controls.

No increase in the hepatic cell proliferation was observed in male or female rats treated with 3,3′,4,4′-tetrachloroazoxybenzene for 31 or 87 days (Table E1).

The presence and intensity of hepatic cytochrome P_{450}1A staining were significantly increased in 1 and 3 mg/kg males and 3, 10, and 30 mg/kg females (Table F1).

Gross lesions were observed at necropsy in 10 and 30 mg/kg males and females and included foci in the liver, small thymuses, and thin blood. Microscopically, treatment effects of 3,3′,4,4′-tetrachloroazoxybenzene administration were observed in the thymus, spleen, kidney, heart, lung, liver, and forestomach (Tables 7, A1, and A2).

Reduced thymus weights in males and females corresponded microscopically to thymic lymphoid atrophy, characterized by diminished cortical thickness due to a reduction in the number of cortical lymphocytes. This effect was significant in males that received 3 mg/kg or greater and in 10 and 30 mg/kg females. The incidence and severities increased with increasing dose.

Hyperplasia of the forestomach epithelium was a treatment-related effect in the 10 and 30 mg/kg male and female groups as well as the 3 mg/kg males. This change was characterized by a minimal to mild increase in the thickness of the squamous epithelium at the limiting ridge, often accompanied by increased keratin (hyperkeratosis). Severities in males increased with dose.

In male F344/N rats, minimal chronic nephropathy is a common spontaneous lesion characterized by one or more scattered foci of regenerative tubules in the cortex. The severity of nephropathy was increased in a dose-dependent manner with 3,3′,4,4′-tetrachloroazoxybenzene administration as determined by an increased number of foci. In female rats, the incidences of nephropathy were significantly increased at 3, 10, and 30 mg/kg, and nephropathy was also observed in the 0.1 and 1 mg/kg groups. This effect likely accounts for the increases in kidney weights.

TABLE 7
Incidence of Selected Nonneoplastic Lesions in Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control		0.1 mg/kg		1 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
Male												
Thymus[a]	10		10		10		10		10		10	
Thymocyte, Atrophy[b]	0		0		0		9**	(1.2)[c]	10**	(3.6)	10**	(3.7)
Stomach, Forestomach	10		10		10		10		10		10	
Epithelium, Hyperplasia, Focal	0		0		1	(1.0)	6*	(1.0)	8**	(1.5)	9**	(1.8)
Kidney	10		10		10		10		10		9	
Nephropathy	10	(1.0)	10	(1.1)	10	(1.3)	10	(1.5)*	10	(1.9)**	9	(2.8)**
Spleen	10		10		10		10		10		9	
Hematopoietic Cell Proliferation	0		0		0		0		0		9**	(1.7)
Lung	10		10		10		10		10		10	
Artery, Inflammation, Chronic Active	0		0		0		0		4*	(1.5)	10**	(2.3)
Interstitium, Inflammation, Chronic	10	(1.6)	10	(1.0)*	10	(1.0)*	10	(1.5)	8	(1.6)	9	(2.2)*
Heart	10		9		10		10		10		10	
Myocardium, Cardiomyopathy	10	(1.6)	9	(1.4)	9	(1.3)	8	(1.4)	6	(1.3)	10	(2.7)**
Liver	10		10		10		10		10		10	
Hematopoietic Cell Proliferation	0		0		0		0		0		6**	(1.3)
Bile Duct, Hyperplasia	0		0		0		0		0		3	(1.0)
Hepatocyte, Centrilobular, Degeneration	0		0		0		0		5*	(1.2)	10**	(3.3)

TABLE 7
Incidence of Selected Nonneoplastic Lesions in Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female						
Thymus	10	10	10	10	10	10
Thymocyte, Atrophy	0	0	0	1 (1.0)	10** (1.9)	10** (3.4)
Stomach, Forestomach	10	10	10	10	10	10
Epithelium, Hyperplasia, Focal	0	0	0	0	10** (2.0)	8** (1.8)
Kidney	10	10	10	10	10	10
Nephropathy	0	1 (1.0)	2 (1.0)	6** (1.0)	7** (1.0)	9** (1.0)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	0	0	0	5* (1.2)	7** (2.0)
Lung	10	10	10	10	10	10
Artery, Inflammation, Chronic Active	0	0	0	0	0	8** (2.0)
Interstitium, Inflammation, Chronic	5 (1.0)	10* (1.0)	10* (1.0)	9 (1.0)	10* (1.8)**	9 (2.4)**
Heart	10	10	10	10	10	10
Myocardium, Cardiomyopathy	5 (1.2)	8 (1.0)	7 (1.0)	9 (1.0)	3 (1.0)	10* (2.4)**
Liver	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	0	0	0	1 (1.0)	6** (1.0)
Bile Duct, Hyperplasia	0	0	0	0	3 (1.0)	3 (1.0)
Hepatocyte, Centrilobular, Degeneration	0	0	0	0	2 (2.5)	9** (2.3)

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

Increased incidences of hematopoietic cell proliferation in the red pulp of the spleen were a treatment-related effect in males and females. The increase in blood cell precursors was primarily in the erythroid series. Minimal to mild hematopoietic cell proliferation occurred in the 30 mg/kg males and the 10 and 30 mg/kg females. The increased spleen weights in females were attributed to this lesion; spleen weights of 30 mg/kg males were not recorded due to early deaths in this group.

Cardiopulmonary lesions were related to treatment with 3,3′,4,4′-tetrachloroazoxybenzene in male and female rats. In the heart, the change was an exacerbation of cardiomyopathy, which may be found as a spontaneous lesion in control F344/N rats of this age and is more common in males than in females. The typical lesion is focal interstitial inflammatory cell infiltration with myocyte loss and occasionally slight fibrosis, primarily occurring in the ventricular septum and left ventricle. The increased severities in 30 mg/kg male and female rats was manifested by an increased number of degenerative foci (Plate 1) and more widespread distribution, with involvement of the right atrium and ventricle, focal myocardial hemorrhage, acute inflammation, and endocardial

proliferation. In 30 mg/kg females, both incidence and severity were increased and likely accounted for increased heart weights. The severity of chronic inflammation of the lung interstitium was significantly lower than that of the vehicle controls in 0.1 and 1 mg/kg males; however, the severities in 30 mg/kg males and 10 and 30 mg/kg females were significantly greater than the vehicle controls. Spectra of inflammatory and degenerative changes of the lung vasculature, present in 10 and 30 mg/kg males and 30 mg/kg females, were diagnosed collectively as chronic active inflammation (vasculitis). This change involved the smaller arteries, arterioles, and alveolar wall capillaries and consisted of vessels with thickened walls (Plate 2), endothelial proliferation, medial hypertrophy or fibrinoid necrosis (Plate 3), and perivascular infiltration of fibrin, macrophages, and other leukocytes. Fibrin thrombi with partial or complete occlusion of the vascular lumen were observed in some animals (Plate 4), and in others there was concentric periarteriolar fibrosis. There was focal necrosis of alveolar capillaries and septae, with exudation into alveolar spaces, and generalized pulmonary vascular dilatation and congestion.

The incidences of a complex of lesions involving the centrilobular hepatocytes of the liver were increased in 10 and 30 mg/kg males and 30 mg/kg females. The complex included cytoplasmic vacuolar degeneration of centrilobular cells, hepatocellular necrosis ranging from that of individual cells to focal coagulation necrosis, and atrophy of centrilobular cords with associated sinusoidal dilatation and congestion (Plate 5). Small sinusoidal foci of erythroid hematopoietic cell proliferation were present in the livers of 30 mg/kg males and females. Minimal hyperplasia of the bile ducts was present in a few 30 mg/kg males and 10 and 30 mg/kg females. Collectively, these changes accounted for increased liver weights at the higher doses.

Several tissues were microscopically noted to be markedly diminished in size, including the preputial gland and seminal vesicles in males and the clitoral gland in females. These changes were considered secondary to inanition and not a direct chemical effect.

MICE

16-DAY STUDY

All mice survived to the end of the study (Table 8). No significant differences in final mean body weights or body weight gains relative to vehicle controls were observed. No clinical findings of toxicity were observed during the study.

TABLE 8
Survival and Body Weights of Mice in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	23.6 ± 1.1	25.4 ± 1.0	1.8 ± 0.5	
1	5/5	23.5 ± 0.9	26.2 ± 0.7	2.7 ± 0.2	103
3.2	5/5	23.8 ± 0.5	25.7 ± 0.4	1.9 ± 0.2	101
10	5/5	23.8 ± 0.7	25.9 ± 0.4	2.2 ± 0.4	102
32	5/5	23.6 ± 0.8	26.1 ± 0.4	2.5 ± 0.6	103
100	5/5	23.6 ± 0.3	26.6 ± 0.3	3.0 ± 0.2	105
Female					
0	5/5	19.3 ± 0.6	20.3 ± 0.5	1.0 ± 0.5	
1	5/5	18.6 ± 0.4	20.6 ± 0.4	2.0 ± 0.3	101
3.2	5/5	18.8 ± 0.3	20.4 ± 0.3	1.6 ± 0.4	101
10	5/5	18.7 ± 0.2	20.5 ± 0.4	1.7 ± 0.3	101
32	5/5	19.3 ± 0.7	20.4 ± 0.7	1.1 ± 0.1	100
100	5/5	18.5 ± 0.4	20.2 ± 0.6	1.7 ± 0.3	99

[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

Absolute and relative liver weights of males in the 3.2, 10, 32, and 100 mg/kg groups and all dosed groups of females were significantly greater than those of the vehicle controls, and the increased weights were dose-dependent (Tables 9 and C3). The absolute and relative thymus weights of males and females administered 3.2 mg/kg or greater were significantly less than those of the vehicle controls, and the decreased weights were dose dependent. A dose-dependent trend was observed for the increase in relative heart weights in females that received 10 mg/kg or greater.

TABLE 9
Selected Organ Weight Data for Mice in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	1 mg/kg	3.2 mg/kg	10 mg/kg	32 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.4 ± 1.0	26.2 ± 0.7	25.7 ± 0.4	25.9 ± 0.4	26.1 ± 0.4	26.6 ± 0.3
Liver						
Absolute	1.307 ± 0.042	1.435 ± 0.075	1.582 ± 0.052**	1.635 ± 0.029**	1.778 ± 0.035**	1.912 ± 0.025**
Relative	51.56 ± 0.42	54.66 ± 2.23	61.47 ± 1.57**	63.11 ± 0.83**	68.33 ± 2.06**	72.00 ± 0.98**
Thymus						
Absolute	0.050 ± 0.003	0.044 ± 0.003	0.040 ± 0.002**	0.030 ± 0.001**	0.031 ± 0.002**	0.021 ± 0.002**
Relative	1.97 ± 0.05	1.67 ± 0.08**	1.54 ± 0.08**	1.17 ± 0.04**	1.17 ± 0.05**	0.80 ± 0.07**
Female						
Necropsy body wt	20.3 ± 0.5	20.6 ± 0.4	20.4 ± 0.3	20.5 ± 0.4	20.4 ± 0.7	20.2 ± 0.6
Liver						
Absolute	1.065 ± 0.058	1.193 ± 0.022*	1.219 ± 0.026*	1.293 ± 0.037**	1.345 ± 0.042**	1.498 ± 0.047**
Relative	52.36 ± 2.27	58.00 ± 0.78**	59.64 ± 1.20**	63.12 ± 0.69**	66.01 ± 0.37**	74.14 ± 1.06**
Thymus						
Absolute	0.069 ± 0.003	0.066 ± 0.004	0.057 ± 0.001*	0.049 ± 0.003**	0.039 ± 0.003**	0.032 ± 0.004**
Relative	3.40 ± 0.16	3.19 ± 0.17	2.78 ± 0.07*	2.40 ± 0.12**	1.91 ± 0.18**	1.60 ± 0.20**

* Significantly different (P 0.05) from the vehicle control group by Williams' test
** P 0.01
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

No gross lesions observed at necropsy were considered to be treatment related. Microscopically, increases in the incidences of atrophy of the thymus were noted in the male and female 100 mg/kg groups. The incidences of hematopoietic cell proliferation in the spleen and hepatic foci of inflammation and necrosis in males and females administered 100 mg/kg were greater than those in the vehicle controls.

The dose selection for the 13-week study was based on lower thymus weights observed in males and females administered 3.2 mg/kg or greater. Because 3,3′,4,4′-tetrachloroazoxybenzene is expected to bioaccumulate, although to a lesser extent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the doses for the 13-week study were chosen to be minimally immunotoxic. Because it was not known whether the animals would develop a tolerance to 3,3′,4,4′-tetrachloroazoxybenzene (i.e., by increased metabolism), the doses for the 13-week mouse study were set at 0, 0.1, 1, 3, 10, and 30 mg/kg per day. These were the same doses used for the 13-week rat study.

13-WEEK STUDY

One female each in the 10 and 30 mg/kg groups died before the end of the study; both deaths were due to gavage accidents (Table 10). The final mean body weights and body weight gains were similar between all dosed groups and the vehicle controls (Table 10 and Figure 3). No treatment-related clinical findings of toxicity were observed.

TABLE 10
Survival and Body Weights of Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	24.4 ± 0.5	36.7 ± 0.9	12.3 ± 0.8	
0.1	10/10	24.5 ± 0.4	37.1 ± 1.0	12.6 ± 0.9	101
1	10/10	24.6 ± 0.6	36.7 ± 1.3	12.1 ± 0.9	100
3	10/10	24.5 ± 0.5	37.5 ± 1.1	13.0 ± 1.0	102
10	10/10	24.2 ± 0.4	35.5 ± 0.8	11.3 ± 0.6	97
30	10/10	24.4 ± 0.4	38.1 ± 0.9	13.7 ± 1.0	104
Female					
0	10/10	19.6 ± 0.2	27.7 ± 0.8	8.1 ± 0.6	
0.1	10/10	19.5 ± 0.3	27.9 ± 0.5	8.4 ± 0.5	101
1	10/10	19.4 ± 0.3	27.0 ± 0.8	7.7 ± 0.7	98
3	10/10	19.6 ± 0.3	26.7 ± 0.7	7.1 ± 0.6	96
10	9/10[c]	19.1 ± 0.4	25.9 ± 0.6	6.5 ± 0.4	93
30	9/10[d]	19.4 ± 0.3	26.1 ± 0.6	6.6 ± 0.4	94

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the vehicle control group are not significant by Dunnett's test.
[c] Week of death: 2 (gavage accident)
[d] Week of death: 4 (gavage accident)

The clinical chemistry data for mice are listed in Table B2. There were decreases in total protein concentration of 10 and 30 mg/kg males. This occurred without a concomitant alteration in albumin concentration. In contrast, there was a minimal increase in albumin concentration without a concomitant increase in total protein concentration in 10 and 30 mg/kg females. The albumin/globulin ratios of males administered 1 mg/kg or greater and of females administered 3 mg/kg or greater were significantly increased and occurred with positive trends.

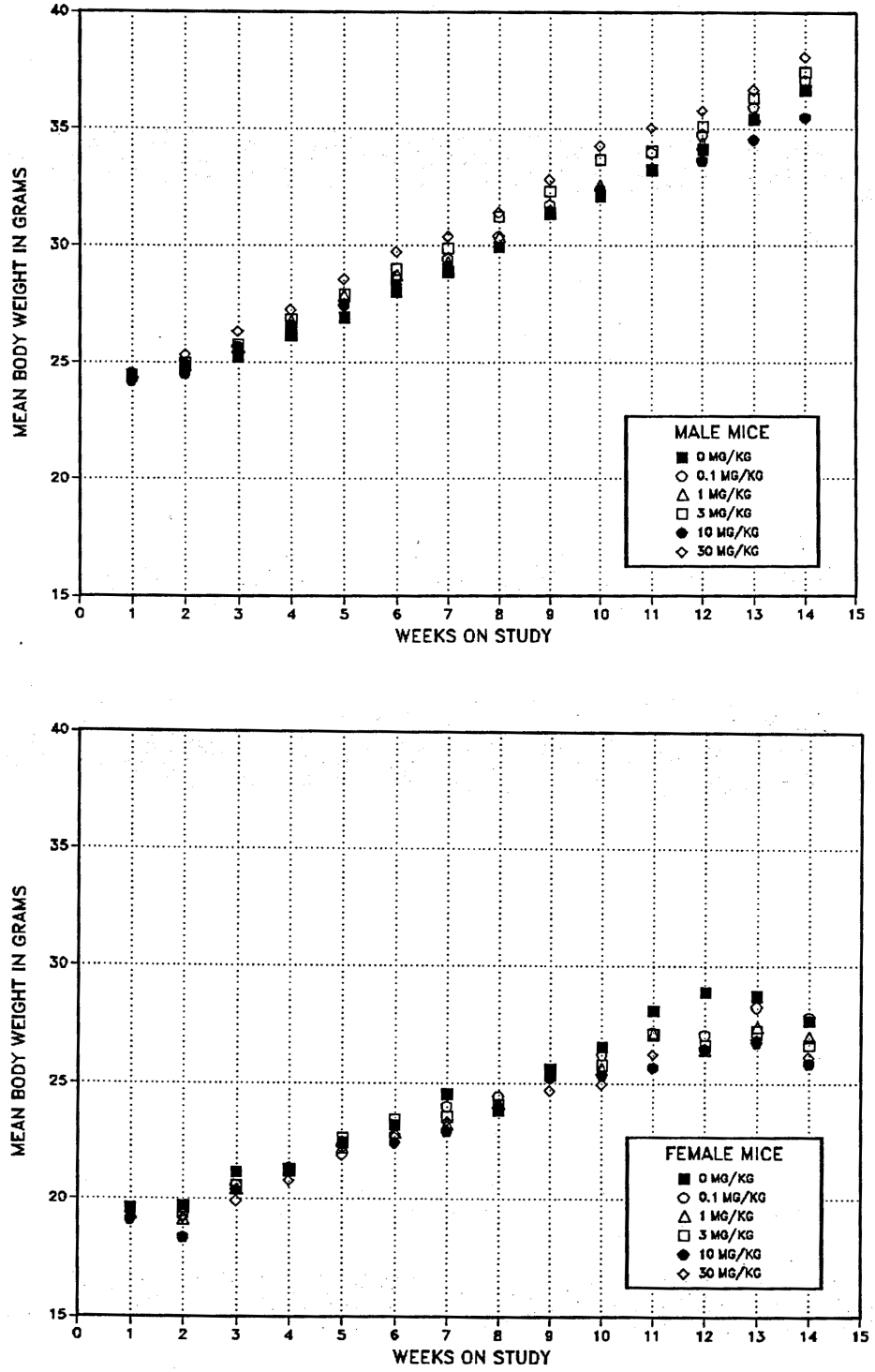


FIGURE 3
Body Weights of Mice Administered 3,3′,4,4′-Tetrachloroazoxybenzene by Gavage for 13 Weeks

The absolute and relative liver weights of males in the 3, 10, and 30 mg/kg groups and females in the 1, 3, 10, and 30 mg/kg groups were significantly greater than those of the vehicle controls (Tables 11 and C4). The absolute kidney weight of 30 mg/kg males and the absolute and relative kidney weights of 10 and 30 mg/kg females were significantly greater than those of the vehicle controls. Absolute and relative thymus weights were significantly less than those of the vehicle controls in the 3, 10, and 30 mg/kg males and in the 10 and 30 mg/kg females. Males in the 30 mg/kg group had a significantly greater absolute heart weight than the vehicle controls. These changes generally occurred with significant trends. The relative heart weights of females increased with increasing dose.

TABLE 11
Selected Organ Weight Data for Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	36.7 ± 0.9	37.1 ± 1.0	36.7 ± 1.3	37.5 ± 1.1	35.5 ± 0.8	38.1 ± 0.9
Liver						
Absolute	1.742 ± 0.041	1.827 ± 0.062	1.899 ± 0.067	2.046 ± 0.086**	2.011 ± 0.071**	2.307 ± 0.071**
Relative	47.68 ± 1.42	49.30 ± 1.35	51.96 ± 1.59	54.68 ± 1.93**	56.60 ± 1.40**	60.55 ± 1.24**
Thymus						
Absolute	0.051 ± 0.003	0.046 ± 0.004	0.043 ± 0.004	0.037 ± 0.003**	0.032 ± 0.004**	0.029 ± 0.002**
Relative	1.41 ± 0.10	1.25 ± 0.11	1.16 ± 0.09	1.01 ± 0.07**	0.90 ± 0.09**	0.78 ± 0.06**
Female						
n	10	10	10	10	9	9
Necropsy body wt	27.7 ± 0.8	27.9 ± 0.5	27.0 ± 0.8	26.7 ± 0.7	25.9 ± 0.6	26.1 ± 0.6
R. Kidney						
Absolute	0.172 ± 0.003	0.181 ± 0.005	0.181 ± 0.002	0.182 ± 0.003	0.188 ± 0.004**	0.188 ± 0.005**
Relative	6.23 ± 0.12	6.52 ± 0.16	6.76 ± 0.19*	6.84 ± 0.14**	7.27 ± 0.16**	7.20 ± 0.08**
Liver						
Absolute	1.157 ± 0.021	1.264 ± 0.050	1.355 ± 0.057**	1.367 ± 0.035**	1.456 ± 0.048**	1.600 ± 0.045**
Relative	41.93 ± 0.86	45.29 ± 1.25*	50.08 ± 1.31**	51.28 ± 0.93**	56.16 ± 0.91**	61.21 ± 0.96**
Thymus						
Absolute	0.047 ± 0.004	0.043 ± 0.002[b]	0.041 ± 0.001	0.042 ± 0.002	0.034 ± 0.001**	0.032 ± 0.002**
Relative	1.66 ± 0.09	1.55 ± 0.08[b]	1.53 ± 0.05	1.58 ± 0.07	1.31 ± 0.06**	1.22 ± 0.09**

* Significantly different (P 0.05) from the vehicle control group by Williams' test
** P 0.01
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n = 9

The left cauda epididymis weights were increased in male mice in the 10 and 30 mg/kg groups (Table D3). No differences in vaginal cytology parameters were observed between dosed and vehicle control female mice (Table D4).

No treatment-related gross lesions were observed. Microscopically, effects of 3,3′,4,4′-tetrachloroazoxybenzene administration to mice were found in the spleen, liver, forestomach, thymus, and skin (Tables 12, A3, and A4).

Splenic changes consisted of increased incidences of hematopoietic cell proliferation and pigmentation in 30 mg/kg males and 10 and 30 mg/kg females relative to the vehicle controls. Hematopoiesis was primarily of the erythroid cells and was evidenced by only a slight increase in incidence and/or severity. The severity of pigmentation was significantly greater than the vehicle controls in females that received 1 mg/kg or greater. Pigmentation was characterized by yellow-brown cytoplasmic granules within macrophages; these granules were interpreted to be hemosiderin. The increased incidences of pigmentation were more apparent than the proliferation, particularly in males, for which a no-effect level was not observed. These changes were not associated with increased mean spleen weights.

Centrilobular hypertrophy of hepatocytes occurred in the liver of treated mice. This was a minimal change characterized by enlarged hepatocytes with increased amounts of eosinophilic cytoplasm surrounding the central veins of the liver lobule. The incidences of this change increased with increasing dose and were significant in the 10 and 30 mg/kg groups; these lesions likely accounted for increased liver weights in these groups.

Hyperplasia of the forestomach epithelium at the limiting ridge was a compound effect in males and females. The incidences of this lesion were significantly increased in 10 and 30 mg/kg males and 30 mg/kg females.

At higher doses, there was a slight increase in the number of apoptotic lymphocytes in the thymus (thymocyte necrosis) compared to the vehicle controls. However, thymus weights suggested a more atrophic effect than was reflected microscopically by the degree of necrotic cells.

Minimal to mild dilatation of the hair follicles was found in sections of skin of treated males and females (Plate 6). Affected follicles were often missing the hair shaft and contained keratin debris. The incidences of this change were significantly increased in 10 and 30 mg/kg males and in 30 mg/kg females.

TABLE 12
Incidence of Selected Nonneoplastic Lesions in Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control		0.1 mg/kg		1 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
Male												
Spleen[a]	10		10		10		10		10		10	
Red Pulp, Hematopoietic Cell Proliferation, Diffuse[b]	2	(1.0)[c]	2	(1.0)	2	(1.0)	4	(1.0)	6	(1.2)	8*	(1.6)
Red Pulp, Pigmentation, Diffuse	0		4*	(1.0)	9**	(1.2)	10**	(1.6)	10**	(1.9)	10**	(2.2)
Liver	10		10		10		10		10		10	
Hepatocyte, Centrilobular Hypertrophy	0		0		0		3	(1.0)	6**	(1.0)	10**	(1.0)
Stomach, Forestomach	10		10		10		10		10		10	
Epithelium, Hyperplasia	0		0		1	(2.0)	1	(1.0)	7**	(1.0)	10**	(1.8)
Thymus	10		10		9		10		10		9	
Thymocyte, Necrosis	2	(1.0)	0		0		0		8*	(1.1)	6	(1.5)
Skin	10		10		10		10		10		10	
Hair Follicle, Dilation	0		1	(1.0)	1	(1.0)	3	(1.3)	10**	(1.0)	10**	(1.6)
Female												
Spleen	10		10		10		10		10		9	
Red Pulp, Hematopoietic Cell Proliferation, Diffuse	2	(1.0)	6	(1.0)	6	(1.2)	4	(1.0)	7*	(1.1)	9**	(1.8)
Red Pulp, Pigmentation, Diffuse	10	(1.4)	10	(1.5)	10	(2.0)*	10	(2.4)**	9	(2.4)**	9	(2.9)**
Liver	10		10		10		10		10		10	
Hepatocyte, Centrilobular, Hypertrophy	0		0		0		1	(1.0)	4*	(1.0)	6**	(1.0)
Stomach, Forestomach	10		10		10		10		10		9	
Epithelium, Hyperplasia	0		0		0		0		3	(1.3)	7**	(2.0)
Thymus	10		10		9		10		8		8	
Thymocyte, Necrosis	1	(1.0)	1	(1.0)	0		2	(1.0)	5*	(1.0)	8**	(1.5)
Skin	10		10		10		10		10		10	
Hair Follicle, Dilation	0		0		0		0		1	(1.0)	8**	(1.0)

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

GENETIC TOXICOLOGY

3,3′,4,4′-Tetrachloroazoxybenzene was not mutagenic in *Salmonella typhimurium* strain TA97, TA98, TA100, or TA1535 with or without induced S9 metabolic activation enzymes (Table G1), and no significant increase in the frequency of micronuclei was noted in bone marrow erythrocytes of male mice treated with 3,3′,4,4′-tetrachloroazoxybenzene by intraperitoneal injection three times at 24-hour intervals (Table G2). However, results of a peripheral blood micronucleus test, in which up to 30 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene was administered to mice once daily, 5 days per week, for 13 weeks by gavage, were positive for male and female mice (Table G3). The micronucleus frequencies observed in female mice in this latter test increased with increasing dose of 3,3′,4,4′-tetrachloroazoxybenzene (trend test, $P = 0.001$); the two highest doses tested, 10 and 30 mg/kg, produced responses that were significantly different from the vehicle control value. The responses seen in the peripheral blood micronucleus test in male mice were not as strong as those seen in female mice, but because the 10 mg/kg dose produced a significant increase in micronucleus frequency ($P = 0.0003$) and because calculating the trend over the dose range of 0.1 to 10 mg/kg yielded a significant P value of 0.001, the test results were concluded to be positive.

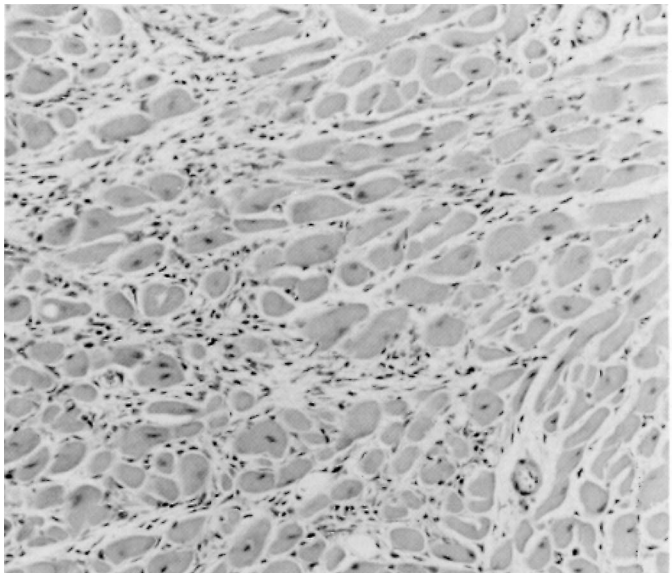


PLATE 1
Moderate cardiomyopathy characterized by widespread separation of myofibers by chronic inflammatory cells of a male F344/N rat administered 30 mg/kg 3,3′,4,4′ -tetrachloroazoxybenzene by gavage for 13 weeks. H&E; 145×

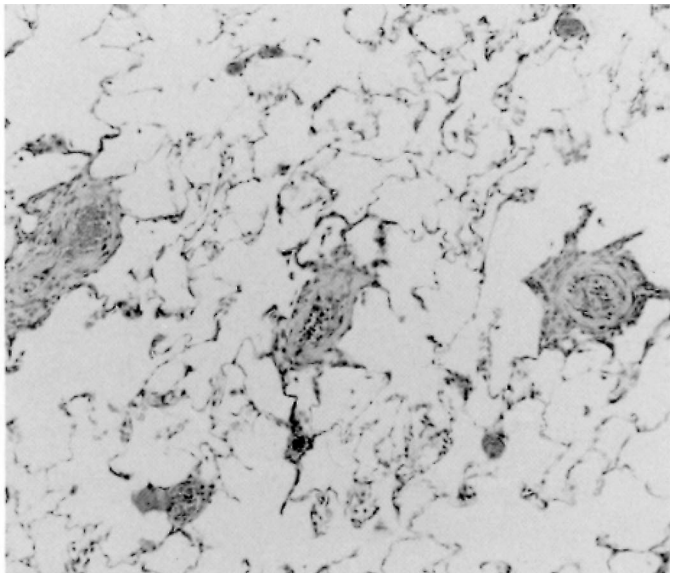


PLATE 2
Parenchymal arterioles are prominent due to thickened walls in a male F344/N rat administered 30 mg/kg 3,3′,4,4′ -tetrachloroazoxybenzene by gavage for 13 weeks. H&E; 90×

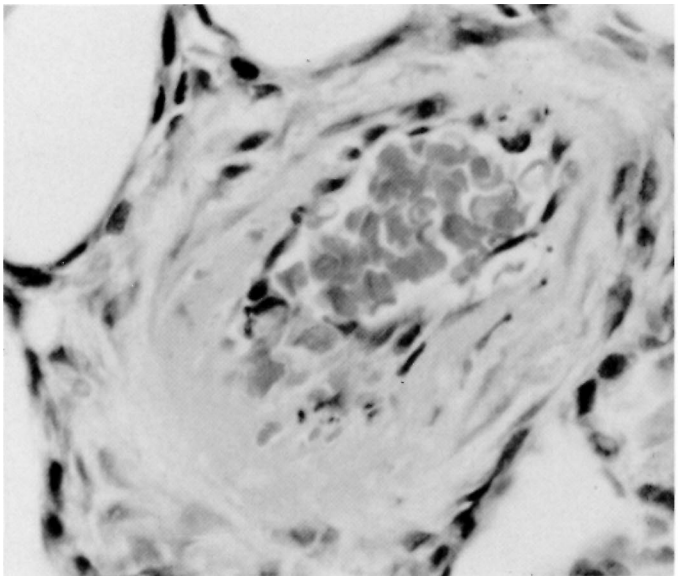


PLATE 3
Higher magnification of a pulmonary arteriole exhibiting endothelial cell proliferation and fibrinoid necrosis of the media of a male F344/N rat administered 30 mg/kg 3,3′,4,4′ -tetrachloroazoxybenzene by gavage for 13 weeks. H&E; 580×

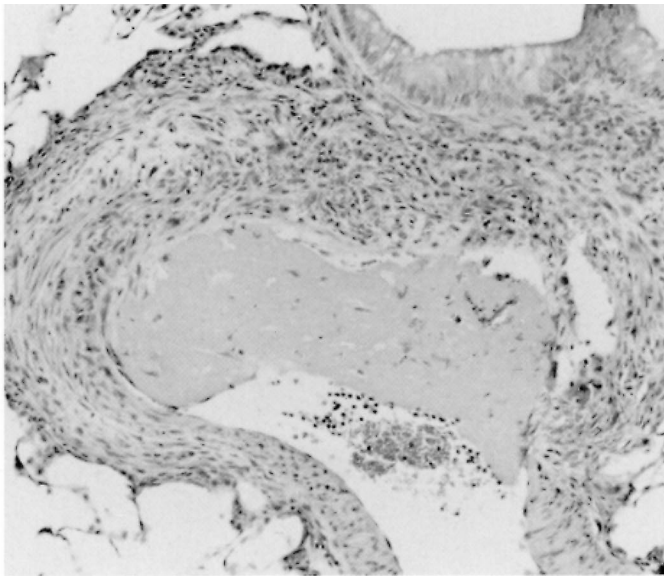


PLATE 4
A thrombus partially occludes the lumen or a larger pulmonary artery with a thickened hypercellular wall of a male F344/N rat administered 30 mg/kg 3,3′,4,4′ -tetrachloroazoxybenzene by gavage for 13 weeks. H&E; 120×

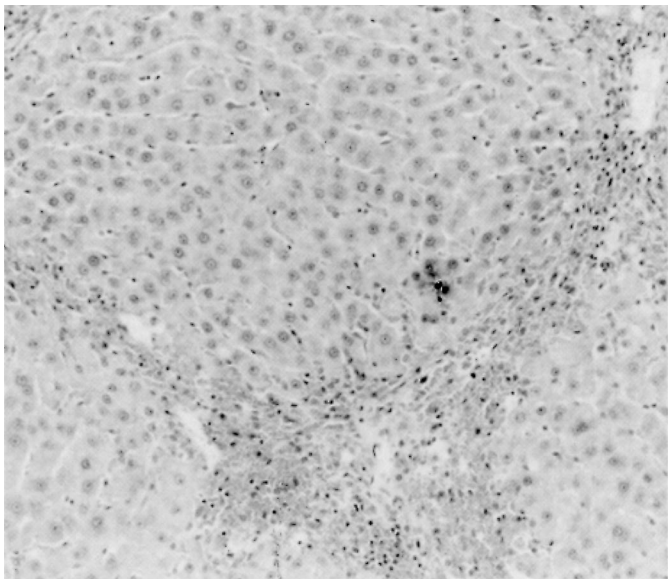


PLATE 5
Bridging centrilobular atrophy of hepatic cords with sinusoidal dilatation and congestion of a male F344/N rat administered 3,3′,4,4′ -tetrachloroazoxybenzene by gavage for 13 weeks. H&E; 120×

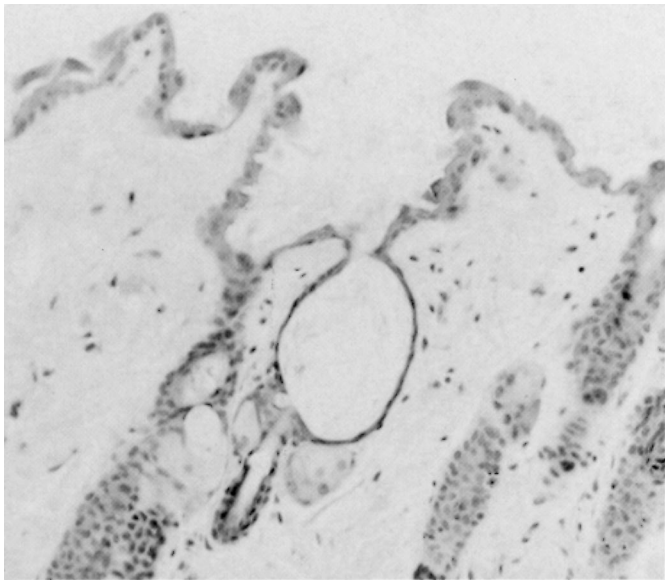


PLATE 6
Ballooning dilatation of an upper hair follicle of a male B6C3F_1 mouse administered 30 mg/kg 3,3′,4,4′ -tetrachloroazoxybenzene by gavage for 13 weeks. H&E; 180×

DISCUSSION

3,3′,4,4′-Tetrachloroazoxybenzene was nominated by the United States Environmental Protection Agency for toxicologic evaluation based on concerns over the potential for human exposure, the structural resemblance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and the dioxin-like effects of 3,3′,4,4′-tetrachloroazoxybenzene. Typical dioxin-like effects were observed in the 16-day and 13-week studies in rats and mice. A summary of the effects in the 13-week studies is given in Table 13.

The common mechanism of action of dioxin-like compounds, such as polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and naphthalenes, involves an initial binding to the aryl hydrocarbon (Ah) receptor. Binding to this receptor is a necessary but not sufficient step in the cascade of effects that occurs after exposure to dioxin-like compounds (Birnbaum, 1994). 3,3′,4,4′-Tetrachloroazoxybenzene binds to the aryl hydrocarbon receptor with an affinity of about one-tenth to one-third that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Poland *et al.*, 1976; Schneider *et al.*, 1995). This is in the same order of magnitude as most potent dioxin-like compounds (Safe, 1990; Kafafi *et al.*, 1993; Schneider *et al.*, 1995). Typical dioxin-like effects in rodents include dermal lesions, body weight loss, thymic atrophy, impairment of immune responses, hepatotoxicity, reproductive and development toxicity, endocrine responses, induction of cytochrome P_{450}1A1, tissue-specific hypo- and hyperplastic responses, and carcinogenesis (Poland and Knutson, 1982; Safe, 1990).

The effects of 3,3′,4,4′-tetrachloroazoxybenzene, which are not entirely consistent with dioxin-like compounds, included the magnitude of the decreased circulating thyroid hormone concentrations of male and female rats, even at the lowest dose (0.1 mg/kg) in female rats in the 13-week study. A decrease in epididymal spermatozoal motility in rats was observed at the lowest dose tested (1 mg/kg). Effects on the hematopoietic system occurred at doses that induced no histopathologic alterations in the liver. Liver lesions typically occur at lower doses than hematopoietic changes with dioxin-like compounds. Additional effects included hyperplasia of the forestomach epithelium in rats and mice, chronic active inflammation of the vasculature of the lung in rats, cardiomyopathy and kidney nephropathy in rats, and dilatation of the hair follicles in mice.

TABLE 13
Summary of Selected Treatment-Related Effects in the 13-Week Gavage Studies of 3,3′,4,4′-Tetrachloroazoxybenzene in F344/N Rats and B6C3F$_1$ Mice

	Affected Dose Groups (mg/kg)			
Endpoint	**Male Rats**	**Female Rats**	**Male Mice**	**Female Mice**
Deaths	30	30	NO[a]	NO
Terminal body weight (decrease)	3↑	10↑	NS[b]	NS
Body weight gain (decrease)	3↑	10↑	NS	NS
Lung				
Weight (increase)	NO	10↑	NO	NO
Chronic active inflammation of vasculature (increased incidence)	10↑	30	NO	NO
Heart				
Weight (increase)	NO	30	NO	NO
Severity of cardiomyopathy (increased severity)	30	30	NO	NO
Skin				
Dilatation of hair follicles (increased incidence)	—[c]	—	10↑	30
Liver				
Weight (increase)	1↑	3↑	3↑	1↑
Centrilobular hypertrophy of hepatocytes (increased incidence)	NO	NO	10↑	10↑
Hematopoietic cell proliferation in liver (increased incidence)	30	30	NO	NO
Centrilobular degeneration of hepatocytes (increased incidence)	10↑	30	NO	NO
Hepatic cytochrome P_{450}1A (increase)	1 and 3	3↑	—	—
Thymus				
Weight (decrease)	1↑	1↑	3↑	10↑
Atrophy (increased incidence)	3↑	10↑	NS	NS
Kidney				
Weight (increase)	NO	30	NS	10↑
Nephropathy (increased incidence)	NS	3↑	NO	—
Forestomach				
Hyperplasia (increased incidence)	3↑	10↑	10↑	30
Spleen				
Weights (increase)	NO	10↑	NO	NO
Hematopoietic cell proliferation (increased incidence)	30	10↑	30	10↑
Responsive anemia	1↑	10↑	—	—
Platelet counts (decrease)	3↑	0.1↑	—	—
Total T_3 concentration (decrease)[d]	NS	10↑	—	—
Total T_4 concentration (decrease)[e]	1↑	0.1↑	—	—
Epididymal spermatozoal motility (decrease)	1, 3, and 10[f]	—	NO	—
Estrous cycle length (longer)	—	10	—	NO

↑ All higher doses affected
[a] NO = not observed
[b] NS = not significantly affected
[c] Not applicable or not analyzed
[d] T_3 = triiodothyronine
[e] T_4 = thyroxine
[f] Only doses tested for this effect

A decrease in mean body weight gain after exposure to 3,3′,4,4′-tetrachloroazoxybenzene in rats has been observed in a 120-day feed study in male Sprague-Dawley rats (Hsia *et al.*, 1980). Assuming an average body weight of 400 grams, the daily intake in the Hsia *et al.*, study was estimated to be about 0.5 mg 3,3′,4,4′-tetrachloroazoxybenzene/kg body weight per day. At this exposure concentration, the decrease in mean body weight gain was about the same as in the current 13-week gavage study in male rats that received 10 mg/kg per day. This suggests that male Sprague-Dawley rats are more sensitive to body weight loss induced by 3,3′,4,4′-tetrachloroazoxybenzene than male F344/N rats. This was also observed for 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). The higher sensitivity of male Sprague-Dawley rats is further demonstrated by the death of male weanling Sprague-Dawley rats administered two 25 mg/kg intraperitoneal injections per week for up to 6 weeks (Hsia and Kreamer, 1985) and by the death of two of six male Sprague-Dawley rats dosed intraperitoneally with 25 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene per week in a 60-day study (Hsia *et al.*, 1981). In the current 13-week gavage study, 6 of 10 male F344/N rats died after 6 weeks of dosing with 30 mg 3,3′,4,4′-tetrachloroazoxybenzene/kg body weight per day. One of the mechanisms that may play a role in body weight loss after exposure to dioxin-like compounds is a decreased feed consumption, regulated by inhibition of key enzymes of gluconeogenesis in the liver (Weber *et al.*, 1991). These authors demonstrated that the induction of appetite suppression was preceded by the inhibition of hepatic phosphoenolpyruvate carboxylase, which caused a reduction in gluconeogenesis followed by a progressive increase in plasma tryptophan concentrations. Tryptophan effectively decreases food consumption (Fernstrom, 1983, 1985). Decreases in mean body weight gains and final body weights were not observed in the present 16-day or 13-week mouse studies. This contrasts with a 28-day feed study with female Swiss-Webster mice that received 10 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene per day (Bleavins *et al.*, 1985a).

The chronic active inflammation of the lung vasculature observed in the 13-week study in male and female rats consisted of vessels with thickened walls, endothelial proliferation, medial hypertrophy, and fibrinoid necrosis. In addition, fibrin thrombi were observed in some animals. These lesions differ significantly from those observed in studies of other dioxin-like compounds, in which changes in bronchiolar and alveolar tissues have been observed to resemble the pathologic picture of chronic bronchitis in humans (Allen *et al.*, 1977; Van Miller *et al.*, 1977; Kociba *et al.*, 1978; NTP, 1982). However, exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in feed for 13 weeks caused a possible thickening of the blood vessel walls in one of five female Sprague-Dawley rats (Kociba *et al.*, 1976); these authors also observed periarteriolar mineralization of the lung of one of five male Sprague-Dawley rats. Exposure of rats to dioxin-like compounds resulted in vascular congestion, pulmonary edema, pleural effusion, and hemorrhage (Boyd, 1982).

The histopathologic changes in the lung induced by 3,3′,4,4′-tetrachloroazoxybenzene resemble those observed in rats exposed to monocrotaline or its metabolite monocrotaline pyrrole (Lalich and Ehrhart, 1962; Meyrick *et al.*, 1980; Hayashi *et al.*, 1984; Reindel *et al.*, 1990). After exposure to these compounds, the walls of the

pulmonary arteries were thickened and had hypertrophy of medial smooth muscle cells, the pulmonary arterial pressure was elevated, and hypertrophy of the right ventricle was observed. Likewise, the cardiomyopathy observed in rats administered 3,3′,4,4′-tetrachloroazoxybenzene by gavage was interpreted to be due to changes in the pulmonary vasculature with resulting pulmonary hypertension and increased work load on the right side of the heart. Although the mechanism for the 3,3′,4,4′-tetrachloroazoxybenzene-induced cardiopulmonary injuries is not known, it can be suggested that as for monocrotaline, metabolites are involved. Once produced in the liver, these metabolites pass to the lung where they can affect the vascular interior. After an intravenous dose of radiolabeled 3,3′,4,4′-tetrachloroazoxybenzene to male Fischer rats, the lung-to-blood ratio of radiolabel was 4.2, indicative of a selective pulmonary retention of 3,3′,4,4′-tetrachloroazoxybenzene or its metabolites (Ziegler *et al.*, 1996). For the structural analogue 3,3′,4,4′-tetrachloroazobenzene, the lung-to-blood ratio of radiolabel was 0.9 in male Fischer rats exposed intravenously (Pillai *et al.*, 1996). No cardiopulmonary effects were observed after administration of 3,3′,4,4′-tetrachloroazobenzene by gavage (NTP, 1998). The pattern of metabolites found in the urine by Ziegler *et al.* (1996) suggests that sulfone-like structures might be involved in these lung lesions, because methyl sulfones of polychlorinated biphenyls are selectively retained in rodent and human pulmonary tissue (Bergman *et al.*, 1979; Brandt and Bergman, 1987; Weistrand and Norén, 1997). The cardiopulmonary effects observed probably accounted for the deaths and the increases in lung and heart weights in rats. No cardiopulmonary effects were observed in mice after administration of 3,3′,4,4′-tetrachloroazoxybenzene for 13 weeks.

In mice, dilatation of keratin-containing hair follicles resembles chloracne-like lesions. These skin lesions have been observed in rabbits, rhino mice (which develop spontaneous follicular hyperkeratosis), and hairless mice exposed dermally to 3,3′,4,4′-tetrachloroazoxybenzene (Taylor *et al.*, 1977; Poland and Knutson, 1982; Horton and Yeary, 1985). The chloracne-like lesions were also observed with dioxin-like compounds in rabbits, monkeys, and hairless mice, but not in haired mice, rats, or guinea pigs (Adams *et al.*, 1941; Von Wedel *et al.*, 1943; Miller, 1944; Vos and Beems, 1971; Allen *et al.*, 1977; Poland and Knutson, 1982; Poland *et al.*, 1982; Vos *et al.*, 1982; Hébert *et al.*, 1990). The present 13-week study is the first report describing chloracne-like lesions in mice other than hairless or rhino mice after exposure to a dioxin-like compound.

3,3′,4,4′-Tetrachloroazoxybenzene caused decreased platelet counts and induced a responsive anemia in the 13-week rat study. Hematopoietic cell proliferation, observed microscopically in the spleen and liver, was consistent with an erythropoietic response. Increased incidences of hematopoietic cell proliferation occurred in the same groups as the increases in spleen weights in female rats. These effects were also observed with 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). Hematopoietic cell proliferation in the liver has not been reported for other dioxin-like compounds. Anemia also occurred in rats exposed to 3,3′,4,4′-tetrachloroazoxybenzene in feed (Hsia *et al.*, 1980). The authors postulated that the hematologic changes were evidence of an aplastic

anemia. This is in contrast to the present study, in which a responsive anemia was observed. Dioxin-like compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran also caused a responsive anemia and decreased platelet counts (Weissberg and Zinkl, 1973; Kociba *et al.*, 1976; Plüss *et al.*, 1988).

The etiology of the anemia in the current 13-week studies is unknown. Increased incidences of hemosiderin accumulation in the spleen were observed in mice, suggesting an increase in erythrocyte injury. A golden-brown pigment was observed in the liver, kidney, and lungs of rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Kociba *et al.*, 1976). A decrease in the erythrocyte activity of enzymes of carbohydrate metabolism might explain the development of a responsive anemia, because this decrease can result in increased susceptibility of erythrocytes to oxidative stress or chemical-induced injury (Dhur *et al.*, 1989; Grossman *et al.*, 1995; Kanno *et al.*, 1995). 3,3′,4,4′-Tetrachloroazoxybenzene has been shown to decrease hepatic enzyme activities involved in carbohydrate metabolism (Hsia and Kreamer, 1985).

The anemia might also be related to the sulfate conjugates of mono- or dichloroaniline, urinary metabolites of 3,3′,4,4′-tetrachloroazoxybenzene in male rats (Pillai *et al.*, 1996). Two metabolites of dichloroaniline, 6-hydroxy-3,4-dichloroaniline and *N*-hydroxy-3,4-dichloroaniline, have been shown to cause a hemolytic anemia (McMillan *et al.*, 1991). The increases in protein and albumin concentrations of rats would be consistent with dehydration and hemoconcentration, suggesting that the anemia was tempered by hemoconcentration. Hemoconcentration has been reported for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Zinkl *et al.*, 1973). The decrease in platelet counts was minimal to mild in severity except for the 30 mg/kg female rats, consistent with the absence of gross or microscopic evidence of hemorrhage. The mechanism by which 3,3′,4,4′-tetrachloroazoxybenzene causes thrombocytopenia is unknown. Dioxin-like compounds are known to cause decreased platelet counts (Zinkl *et al.*,1973; Kociba *et al.*, 1976; Plüss *et al.*, 1988; Viluksela *et al.*, 1997). It has been suggested that an immune-mediated etiology be explored as a possible mechanism (Weissberg and Zinkl, 1973). An immune-mediated erythrocyte injury or production of erythrocytes with a shortened life-span could also be involved in the anemia (Jain, 1986b). Silverstone *et al.* (1996) have shown an increase in lupus-like nephritis in a mouse model after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In addition, an increase in serum immunoglobulin has been reported after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Burns *et al.*, 1996). This strengthens the basis for an immunologic component in the observed anemia and thromboctyopenia in rats.

Histopathologic changes were observed in the liver of dosed rats and mice in all except the 16-day rat study. In dosed rats, cytoplasmic vacuolar degeneration of centrilobular cells, hepatocellular necrosis, and atrophy of centrilobular cords with associated sinusoidal dilatation and congestion were observed. These effects may account for the increased liver weights. The etiology of the centrilobular hepatic degeneration is unknown, but

it might be related to the observed lung lesions. In rats, the histopathologic lesions in the liver were confirmed by the minimal effects on various clinical chemistry parameters. In dosed mice in the 13-week study, centrilobular hypertrophy of hepatocytes was observed. This lesion also occurred in male mice exposed to the structural analogue 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998) and in rats exposed to a highly lipophilic nondioxin-like polychlorinated biphenyl, 2,2′,4,4′,5,5′-hexachlorobiphenyl (Chu *et al.*, 1996; Peng *et al.*, 1997). Centrilobular hypertrophy is often a reflection of enzyme induction and proliferation of smooth endoplasmic reticulum (Butler, 1996). In mice, no alterations were observed in clinical parameters associated with hepatic effects; because only centrilobular hypertrophy was observed, it was not necessarily expected that these clinical parameters would be affected. 3,3′,4,4′-Tetrachloroazoxybenzene caused an increase in hepatic cytochrome P_{450}1A protein concentrations in rats in the 13-week study as determined by immunohistologic staining. An increase in cytochrome P_{450}1A1 and P_{450}1A2 activities, often measured as ethoxyresorufin-*O*-deethylase and methoxyresorufin-*O*-deethylase activities, is a typical dioxin-like effect and is mediated through the Ah receptor (Safe, 1990, 1994).

In the 16-day and 13-week studies, decreased thymus weights were observed in rats and mice and occurred at doses that caused no changes in survival rates or mean body weight gains, suggesting a direct lymphocytotoxic effect and not a stress mechanism. Thymic atrophy has been observed in Sprague-Dawley rats and Swiss-Webster mice administered 3,3′,4,4′-tetrachloroazoxybenzene (Hsia *et al.*, 1981, 1982; Olson *et al.*, 1984; Bleavins *et al.*, 1985a; Hsia and Kreamer, 1985) and is one of the hallmarks of dioxin-like chemical toxicity (Safe, 1990; De Waal *et al.*, 1997). The thymic atrophy in the current studies consisted of thinning of the cortex due to reduced numbers of cortical lymphocytes. An important feature in dioxin-mediated thymus toxicity is the disruption of epithelial cells in the cortex. Histologically, exposure to dioxin-like compounds results in depletion of the cortical area and a loss of demarcation between the cortex and medulla. Thymic atrophy was also observed in the 3,3′,4,4′-tetrachloroazobenzene studies (NTP, 1998).

The immunotoxicity of dioxin-like compounds in mice has been associated with an Ah receptor-dependent mechanism (Vecchi *et al.*, 1983; Davis and Safe, 1988, 1990). In rats, thymic atrophy has been associated with an Ah receptor-mediated mechanism, because the rank order of dioxin-like compounds for thymic atrophy is the same as that based on other toxic and biochemical endpoints (Ahlborg *et al.*, 1992, 1994). It has been suggested that thymic atrophy is mainly due to inhibition of development of the prethymic and early intrathymic stem cell compartment, primarily mediated by activation of hemopoietic cells (Silverstone *et al.*, 1997).

In rats in the 13-week study, thymic atrophy occurred at about the same doses as did slight decreases in leukocyte, lymphocyte, and eosinophil counts. This was also observed for 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). In a 13-week feed study with 2,3,4,7,8-pentachlorodibenzofuran, the decreases in leukocyte

counts and increased incidences of thymic atrophy were equally sensitive endpoints (Plüss *et al.*, 1988), which suggests that the lymphocytotoxic effects are interrelated and are not specific to one organ. Another possible involvement in thymic atrophy could be autoimmunity, as suggested for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Holladay *et al.*, 1991; Vos and Van Loveren, 1995; De Waal *et al.*, 1997). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin has been found to increase lupus-like nephritis in a mouse model, suggesting an association between autoimmunity and exposure to dioxin-like compounds (Silverstone *et al.*, 1996). Hexachlorobenzene, a compound with dioxin-like and nondioxin-like activities, resulted in autoimmunity (Schielen *et al.*, 1993). In most autoimmune-related cases, an estrogen-dependent factor is involved (Ahmed *et al.*, 1985). The thymic atrophy, however, generally occurred at the same doses in male and female rats and mice in the 16-day and 13-week studies. This was also observed for 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998).

Marked decreases in circulating thyroid hormone concentrations occurred in rats in the 13-week study. This effect has also been observed after exposure to other dioxin-like compounds (Bastomsky, 1977; Brouwer and van den Berg, 1986; Henry and Gasiewicz, 1986; Morse *et al.*, 1993; Van Birgelen *et al.*, 1995a). The same magnitudes of decrease in total thyroxine and triiodothyrinone concentrations was also observed after exposure to 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). The apparent magnitude of the thyroxine concentration decrease and relatively weak thyroid-stimulating hormone concentration response, however, is unusual. A weak thyroid-stimulating hormone concentration response has been observed previously after exposure to dioxin-like compounds (Barter and Klaassen, 1994; Morse *et al.*, 1996).

Decreases in circulating thyroid hormone concentrations are associated with induction of hepatic thyroid hormone glucuronyl transferase (Bock, 1991; Schrenk *et al.*, 1991; Barter and Klaassen, 1992; Van Birgelen *et al.*, 1995a). Coinduction of the dioxin-inducible cytochrome P_{450}1A1 and glucuronyl transferase has been observed for various dioxin-like compounds (Bock, 1991; Schrenk *et al.*, 1991; Van Birgelen *et al.*, 1995a), indicating that 3,3′,4,4′-tetrachloroazoxybenzene induces glucuronyl transferase. Furthermore, a destabilization of the complex of thyroxine and transthyretin by metabolites of polychlorinated biphenyls has been proposed to be involved in the decrease in circulating thyroxine concentrations (Brouwer and van den Berg, 1986; Brouwer *et al.*, 1988). Another theory is that changes in thyroid gland function and morphology result in an interference with the synthesis and excretion of thyroxine (Collins and Capen, 1980; Chu *et al.*, 1994).

The pattern of metabolites found in the urine by Ziegler *et al*. (1996) suggests that sulfone-like structures and hydroxylated metabolites might be involved in the decrease of circulating thyroxine. Methyl sulfones of polychlorinated biphenyls decrease thyroid hormone concentrations in mink and rats (Lund *et al.*, 1997, Kato *et al.*, 1998). Hydroxylated metabolites of polychlorinated biphenyls bind to transthyretin, thereby destabilizing the transthyretin-thyroxine complex, and eventually decreasing circulating thyroxine levels (Brouwer and van den Berg, 1986; Lans *et al.*, 1993).

Pre- and postnatal exposures to dioxin-like compounds in humans have been correlated with alterations in circulating thyroid hormone concentrations and (neuro)developmental effects (Jacobson *et al.*, 1990a,b; Koopman-Esseboom *et al.*, 1994; Huisman *et al.*, 1995; Jacobson and Jacobson, 1996; Lonky *et al.*, 1996; Nagayama *et al.*, 1997; Patandin *et al.*, 1997a,b). Decreased thyroid hormone concentrations are associated with permanent alterations in behavior and brain maturation in the offspring (Porterfield and Hendrich, 1993). The developmental studies with 3,3′,4,4′-tetrachloroazoxybenzene reported in the literature did not investigate neurobehavioral effects.

No histopathologic changes were observed in the thyroid gland after exposure to 3,3′,4,4′-tetrachloroazoxybenzene or 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). This is unusual in light of the severity of the decrease in circulating thyroid hormone concentrations. Although thyroid-stimulating hormone concentrations were slightly increased, this increase was insufficient to alter the morphology of the thyroid gland. A possible explanation might be that 3,3′,4,4′-tetrachloroazoxybenzene or its metabolites mimic thyroxine, suppressing the feedback mechanism to stimulate thyroid-stimulating hormone production and release. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin has been shown to mimic the action of thyroxine in the development of tadpoles (McKinney *et al.*, 1985). Of interest for this hypothesis is the high accumulation of azoxybenzene in the thyroid gland in rats (Kujawa *et al.*, 1989).

Chronic nephropathy is a common spontaneous disease in F344/N rats, and exacerbation of this nephropathy is a common effect of chemical administration, particularly in male rats (Montgomery and Seely, 1990). However, in female rats in the 13-week study, the incidences of nephropathy were significantly increased at doses of 3 mg/kg or greater. These increased incidences of nephropathy in dosed female rats are probably related to the increase in kidney weights. Although the etiology of this nephropathy is unknown, it is notable that after an intravenous dose of radiolabeled 3,3′,4,4′-tetrachloroazoxybenzene to male Fischer rats, the kidney-to-blood ratio of radiolabel was 4.1, indicative of a selective retention of 3,3′,4,4′-tetrachloroazoxybenzene or its metabolites (Ziegler *et al.*, 1996). For 3,3′,4,4′-tetrachloroazobenzene, the kidney-to-blood ratio of radiolabel was 1.8 in male Fischer rats exposed intravenously (Pillai *et al.*, 1996). No 3,3′,4,4′-tetrachloroazobenzene-related nephropathy was observed in rats (NTP, 1998). The pattern of metabolites as found in the urine by Ziegler *et al.* (1996) suggests that sulfone-like structures might be involved in the renal retention of 3,3′,4,4′-tetrachloroazoxybenzene-derived radiolabel, because methyl sulfones of polychlorinated biphenyls are selectively retained in the kidney and lung of rodents (Brandt and Bergman, 1987). In the kidney, the target cells of these sulfones are in the middle segment of the proximal tubules (Brandt and Bergman, 1987). The minimal effect of 3,3′,4,4′-tetrachloroazoxybenzene on thyroid-stimulating hormone concentrations in rats in conjunction with the sharply decreased thyroxine concentrations suggests the mimicking of thyroxine by

3,3′,4,4′-tetrachloroazoxybenzene or its metabolites. A selective binding protein of thyroxine has been detected in the kidney of humans (Vié *et al.*, 1996).

Incidences of hyperplasia of the forestomach were increased in dosed rats and mice in the current 13-week studies and in rats and mice administered 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). This effect has been associated with irritation of the forestomach in rat and mouse gavage studies (Brown and Hardisty, 1990). Most 13-week studies with dioxin-like compounds in which histopathologic evaluations were performed were feed studies. Gastric ulcers were observed in *Macaca mulatta* monkeys given "toxic fat" which contained 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in feed (Allen and Carstens, 1967). Albino rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by gavage also had an increased incidence of stomach ulcers (Gupta *et al.*, 1973). In addition, hypertrophy, hyperplasia, and metaplasia were observed in the gastric epithelium in *Macaca mulatta* monkeys exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Allen *et al.*, 1977; McConnell *et al.*, 1978).

3,3′,4,4′-Tetrachloroazoxybenzene administration reduced epididymal spermatozoal motility in male rats in the 13-week study. Most 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-related studies in male animals have found the greatest effects after perinatal exposure (Mably *et al.*, 1992; Gray *et al.*, 1993, 1997; Waalkens-Berendsen *et al.*, 1994, 1996; Sommer *et al.*, 1996). These authors found reduced numbers of epididymal sperm in rats after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, Aroclor 1254, or 3,3′,4,4′,5,5′-hexachlorobiphenyl, and effects on motility after exposure to 2,3,4,7,8-pentachlorodibenzofuran. In mice, a decrease in epididymal spermatozoal concentration was observed in the 13-week 3,3′,4,4′-tetrachloroazobenzene study (NTP, 1998).

An increase in estrous cycle length was observed in 10 mg/kg female rats in the current 13-week study. An irregularity of the estrous cycle, characterized as a prolonged period of diestrus, was also observed in Sprague-Dawley rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Li *et al.*, 1995). Changes in menstrual patterns were observed in humans accidentally exposed to dioxin-like compounds in Japan in 1968 (Kuratsune, 1989). Because estrous cycle length is correlated with fertility, this suggests that fertility would be reduced by exposure to 3,3′,4,4′-tetrachloroazoxybenzene (Chapin *et al.*, 1997). The combined effects on males and females suggest a lower fertility in rats, but not in mice.

3,3′,4,4′-Tetrachloroazoxybenzene was negative in the *Salmonella typhimurium* gene mutation tests, negative in the short-term male mouse bone marrow micronucleus tests, and positive in the peripheral blood micronucleus tests in male and female mice. A similar discordance between short- and long-term micronucleus test results has also been observed with other chemicals, including phenolphthalein, salicylazosulfapyridine, diisopropylcarbodiimide, and 3,3′,4,4′-tetrachloroazobenzene (Bishop *et al.*, 1990; Dietz *et al.*, 1992; Witt *et al.*, 1995, 1996, 1998; NTP, 1998). It has been postulated that, in these cases, the positive results seen in the long-term studies resulted from certain metabolism requirements or total accumulated doses that were difficult

to achieve with short-term exposures. Another factor that may influence the interpretation of the positive results in the peripheral blood micronucleus test is the observed erythropoietic stimulation seen in the spleen of mice treated with 3,3′,4,4′-tetrachloroazoxybenzene. Several reports have described the enhancement of micronucleus assay sensitivity by artificially stimulated erythropoiesis (via bleeding or administration of erythropoietin, for example) (Suzuki *et al.*, 1989, 1994; Hirai *et al.*, 1991). These authors have postulated that increased incidences of hematopoietic cell proliferation can produce an increase in chemical-induced mitotic errors resulting in elevated micronucleus frequencies. However, no alterations in the percentage of polychromatic erythrocytes was noted in peripheral blood of 3,3′,4,4′-tetrachloroazoxybenzene-treated mice; thus, the hematopoietic cell proliferation noted in the spleen may not have been sufficient to influence the response observed in the peripheral blood micronucleus test. Unscheduled DNA synthesis was observed in hepatocytes of rats administered 3,3′,4,4′-tetrachloroazoxybenzene after pretreatment with 3,3′,4,4′-tetrachloroazoxybenzene (Shaddock *et al.*, 1989). Together, the peripheral blood micronucleus test results and the unscheduled DNA synthesis data suggest that an enhanced metabolism is necessary for 3,3′,4,4′-tetrachloroazoxybenzene-induced genetic damage.

For dioxin-like compounds such as 3,3′,4,4′-tetrachloroazoxybenzene, a relative potency value can be estimated for each compound that acts through the Ah receptor. This value expresses the potency of that specific compound compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxic Equivalency Factor (TEF) values are consensus relative potency values derived from all available studies that compare the TEF chemical to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the most potent dioxin-like compound, which is assigned a TEF value of one (USEPA, 1989; Van Zorge *et al.*, 1989; Kutz *et al.*, 1990; Safe, 1990, 1994; Ahlborg *et al.*, 1992, 1994; Feeley and Grant, 1993; Birnbaum and DeVito, 1995). TEF values are based on repeat-dose *in vivo* studies, single-exposure studies, structure-activity considerations, and data from *in vitro* studies, with preference for repeat-dose studies (Ahlborg *et al.*, 1994). Multiplying the TEF value of a specific compound by the concentration of that compound in a mixture results in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) of that compound. The sum of all TEQs for every dioxin-like compound in a mixture gives the total TEQ of that specific mixture. Table 14 summarizes a range of relative potency values for 3,3′,4,4′-tetrachloroazoxybenzene, based on *in vitro* and *in ovo* experiments, short-term, single-dose *in vivo* studies, a 13-week gavage study in female B6C3F$_1$ mice, and comparisons of data from the current rat study with the literature.

TABLE 14
Relative Potency Estimates for 3,3′,4,4′-Tetrachloroazoxybenzene Based on *In Vitro* and *In Ovo* Experiments, Short-Term Single-Dose *In Vivo* Studies, a 13-Week Gavage Study in Female B6C3F$_1$ Mice, and Comparison of Data from the Current Rat Study with the Literature[a]

Effect	Concentration Inducing Effect		Relative Potency for TCAOB	Reference
	TCDD	TCAOB		
***In Vitro* Experiments**				
Binding affinity to the Ah receptor (nM)	0.27	0.93	0.3	Poland *et al.*, 1976
EC_{50} for binding to the mouse hepatic Ah receptor (nM)	1.22	8.78	0.1	Schneider *et al.*, 1995
***In Ovo* Experiments**				
ED_{50} (nmol/kg) for induction of aryl hydrocarbon hydroxylase in chicken embryos	0.31	0.45	0.7	Poland *et al.*, 1976
LD_{50} (ng/egg) in chicken embryos[b]	0.2	12	0.02	Higginbotham *et al.*, 1968; Schrankel *et al.*, 1982
***In Vivo* Studies**				
ED_{50} (nmol/kg) for induction of hepatic aryl hydrocarbon hydroxylase in C57BL/6J mice	0.9	8,200	0.0001	Poland *et al.*, 1976
ED_{50} (µg/kg) for teratogenicity in C57BL mice	20	6,000	0.003	Dencker, 1985
EC_{50} (nM) for *in vivo* thymic toxicity	1	10	0.1	Dencker, 1985
Epidermal hyperplasia, nmol required for a response of 2+ in hr/hr mice	0.36	356	0.001	Knutson and Poland, 1982
Mouse Study				
Cytochrome P_{450}1A1 induction in the skin in a 13-week gavage study in female B6C3F$_1$ mice with TCDD and TCAOB			0.000003–0.00001	Hébert *et al.*, 1993
Rat Studies				
Decreased thyroxine concentrations in 13-week gavage studies[c]			About 2 to 3 orders of magnitude less than TCDD	Van Birgelen *et al.*, 1995b and current study
Thymic atrophy in 13-week gavage studies[c]			About 5 to 6 orders of magnitude less than TCDD	Van Birgelen *et al.*, 1995b and current study

[a] Data are presented as toxic equivalents. TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCAOB = 3,3′,4,4′-tetrachloroazoxybenzene
[b] Experiments were not performed at the same laboratory.
[c] The TCDD feed study used female Sprague-Dawley rats, and the TCAOB study data are from the current gavage study.

3,3′,4,4′-Tetrachloroazoxybenzene has a high binding affinity to the Ah receptor and therefore a great likelihood of having a potency on the same order of magnitude as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Once administered *in vivo* however, the apparent relative potency decreases considerably, ranging from 0.001 to 0.0001 with epidermal hyperplasia, teratogenicity, and hepatic aryl hydrocarbon hydroxylase induction as endpoints (Poland *et al.*, 1976; Poland and Knutson, 1982; Dencker, 1985). 3,3′,4,4′-Tetrachloroazoxybenzene has a short half-life in comparison to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Van den Berg *et al.*, 1994; Zeigler *et al.*, 1996). In repeat-dose experiments, 3,3′,4,4′-tetrachloroazoxybenzene will reach steady-state concentrations fairly quickly, whereas 2,3,7,8-tetrachlorodibenzo-*p*-dioxin will still accumulate. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin will thus appear more potent over time in comparison to 3,3′,4,4′-tetrachloroazoxybenzene. This has been shown with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3,3′,4,4′-tetrachlorobiphenyl (Ahlborg *et al.*, 1994; De Vito and Birnbaum, 1995). Comparing the results of the 13-week gavage study with 3,3′,4,4′-tetrachloroazoxybenzene in F344/N rats to a 13-week feed study with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague-Dawley rats shows again that 3,3′,4,4′-tetrachloroazoxybenzene is of lower potency (Table 14 and Figures 4 and 5). Although a comparison between different strains of rats in different laboratories is not optimal for estimating a relative potency value (Ahlborg *et al.*, 1992, 1994), 3,3′,4,4′-tetrachloroazoxybenzene is about four to five orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using thymic atrophy as the endpoint (Figure 4; Van Birgelen *et al.*, 1995b). This is in the same order of magnitude as the range of 3×10^{-6} to 10^{-5} for the relative potency value based on dermal cytochrome P_{450}1A1 induction in the 13-week gavage study in female $B6C3F_1$ mice (Hébert *et al.*, 1993). In this latter study, 3,3′,4,4′-tetrachloroazoxybenzene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were tested in parallel. Figure 5 shows that the decrease in thyroxine concentrations in F344/N rats in the current gavage study at doses of 0.1 to 1 mg/kg 3,3′,4,4′-tetrachloroazoxybenzene per day is similar to that induced by 1 µg/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in feed per day in female Sprague-Dawley rats (Van Birgelen *et al.*, 1995b), indicating that 3,3′,4,4′-tetrachloroazoxybenzene is about two to three orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

This difference in potency for inducing a decrease in circulating thyroxine concentrations can very likely be explained by the involvement of multiple mechanisms, as has been found with a mixture of dioxin-like compounds (van Birgelen *et al.*, 1997). This mixture caused a marked decrease in circulating thyroid hormone concentrations when compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This synergistic effect has been attributed to the involvement of metabolites of polychlorinated biphenyls and the multiple isoenzymes of thyroxine glucuronidation enzymes (van Birgelen *et al.*, 1997; Birnbaum *et al.*, 1998). Because 3,3′,4,4′-tetrachloroazoxybenzene is quickly metabolized and induces dioxin-like effects, it is likely that metabolites of 3,3′,4,4′-tetrachloroazoxybenzene and Ah receptor-associated glucuronidation of thyroxine are involved in the decrease in circulating thyroid hormone concentrations.

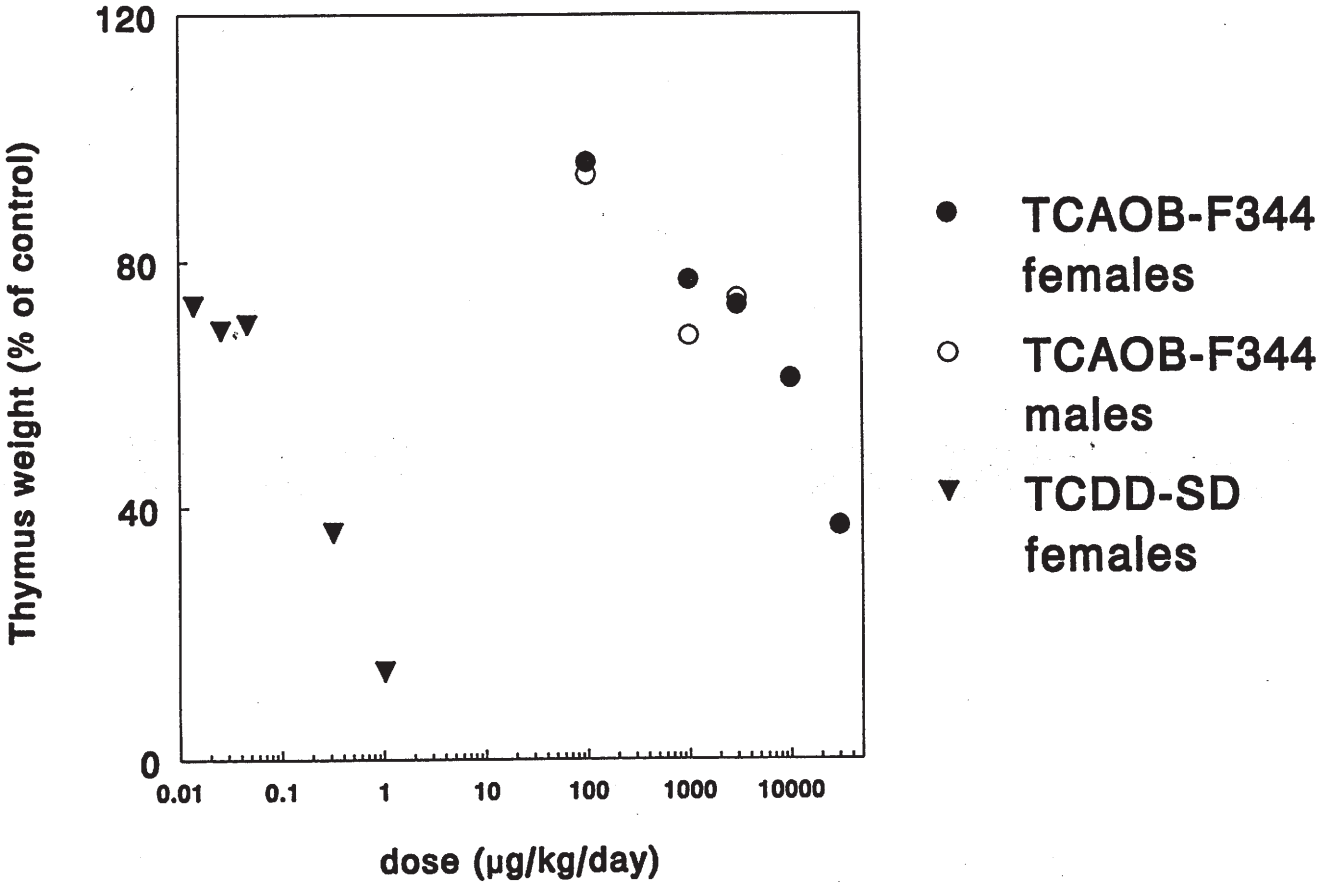


FIGURE 4
Decrease in Thymus Weights (% of Controls) in the 13-Week Studies of 3,3′ N,4,4′-Tetrachloroazoxybenzene (TCAOB) and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD).
The 3,3′,4,4′-tetrachloroazoxybenzene data for male and female F344/N rats were obtained from the current gavage study. The 13-week 2,3,7,8-tetrachlorodibenzo-*p*-dioxin feed study was performed in female Sprague-Dawley (SD) rats (Van Birgelen *et al*., 1995b).

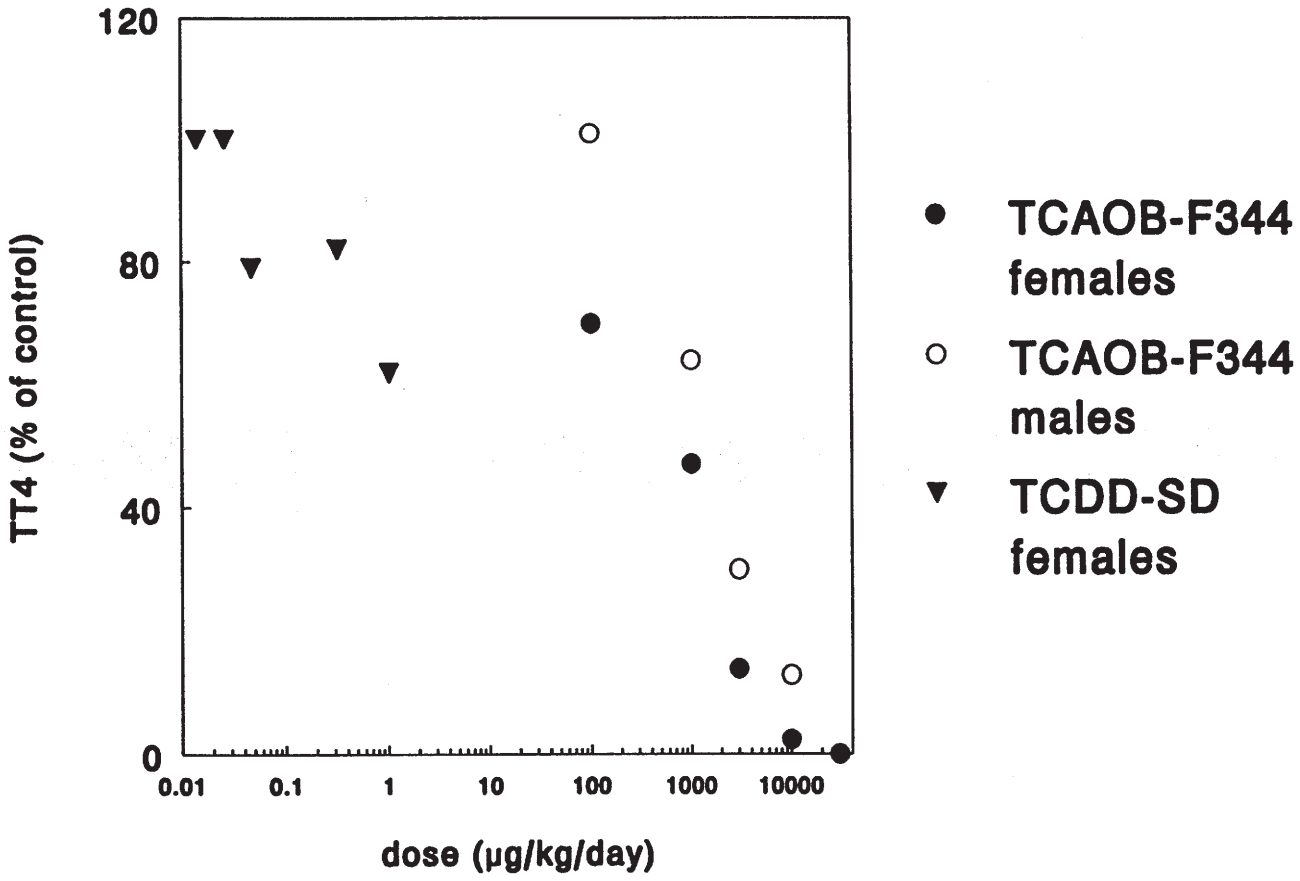


FIGURE 5
Decrease in Total Circulating Thyroxine (TT4) Concentrations (% of Controls) in the 13-Week Studies of 3,3′,4,4′-Tetrachloroazoxybenzene (TCAOB) and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD).
The 3,3′,4,4′-tetrachloroazoxybenzene data for male and female F344/N rats were obtained from the current gavage study. The 13-week 2,3,7,8-tetrachlorodibenzo-*p*-dioxin feed study was performed in female Sprague-Dawley (SD) rats (Van Birgelen *et al*., 1995b).

Based on the production levels of dichloroaniline and the concentration of 3,3′,4,4′-tetrachloroazoxybenzene in this compound, the production of 3,3′,4,4′-tetrachloroazoxybenzene might be as high as 3.6 kg per year in the United States (Sundström *et al.*, 1978; Hill *et al.*, 1981; USEPA, 1985). Assuming 3,3′,4,4′-tetrachloroazoxybenzene is indeed six to two orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, this could lead to an annual release of 3.6 mg to 0.036 kg of toxic equivalents into the environment due to 3,3′,4,4′-tetrachloroazoxybenzene alone. For comparison, the annual release in the environment of 3,3′,4,4′-tetrachloroazobenzene was estimated to be as much as 160 kg of toxic equivalents (NTP, 1998).

In general, hematologic, splenic, and thymic effects were observed at doses that induced no histopathologic alterations in the liver. This was also observed after administration of 3,3′,4,4′-tetrachloroazobenzene (NTP, 1998). This is in contrast to the pattern of effects observed in rodents exposed to other dioxin-like compounds in which the liver is one of the most sensitive organs (Kociba *et al.*, 1976; Chu *et al.*, 1994, 1995). This discrepancy might be explained by the fact that the liver-to-fat ratio of other dioxin-like compounds in rodents is at least one order of magnitude higher than that for 3,3′,4,4′-tetrachloroazoxybenzene (Table 15). A low liver-to-fat ratio means that relatively more of the test compound can be distributed to organs other than the liver before an effective concentration in the liver is reached. The liver is the most commonly affected organ in 2-year bioassays in rodents with dioxin-like compounds (IARC, 1997). Based on the difference in the liver-to-fat ratio between 3,3′,4,4′-tetrachloroazoxybenzene and other dioxin-like compounds, 3,3′,4,4′-tetrachloroazoxybenzene could induce neoplasms mainly in organs other than the liver.

TABLE 15
Liver-to-Fat Ratios of Dioxin-Like Compounds in Rats and Mice

Compound	Liver-to-Fat Ratio	Reference
Rats		
3,3′,4,4′-Tetrachloroazoxybenzene	0.06–0.1	Zeigler *et al.* (1996)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	2–6	Abraham *et al.* (1989); Van Birgelen *et al.* (1995b)
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	13	Abraham *et al.* (1989)
Hexachlorinated dibenzo-*p*-dioxins	34	Abraham *et al.* (1989)
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	66	Abraham *et al.* (1989)
2,3,7,8-Tetrachlorodibenzofuran	2	Abraham *et al.* (1989)
2,3,4,7,8-Pentachlorodibenzofuran	43	Abraham *et al.* (1989)
Mice		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	0.5–2.5	DeVito *et al.* (1995)
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	5-9	DeVito *et al.* (1995)
2,3,7,8-Tetrachlorodibenzofuran	1–5	DeVito *et al.* (1995)
2,3,4,7,8-Pentachlorodibenzofuran	7–47	DeVito *et al.* (1995)

In summary, 3,3′,4,4′-tetrachloroazoxybenzene caused typical dioxin-like effects, including thymic atrophy, increased liver weights, induction of hepatic cytochrome P_{450}1A, and decreased mean body weight gains. Furthermore, a marked decrease in circulating thyroxine concentrations was observed in male and female rats, even at the lowest dose (0.1 mg/kg) in female rats. A decrease in epididymal sperm motility was observed at all doses in rats. Effects on the hematopoietic system occurred at doses including and lower than those that caused histopathologic alterations in the liver. A no-observable-adverse-effect level (NOAEL) was not reached in rats. In male and female mice, the NOAEL was 1 and 0.1 mg/kg, respectively. Furthermore, treatment-related effects included increased incidences of hyperplasia of the forestomach epithelium in rats and mice, chronic active inflammation of the vasculature of the lung in rats, increased incidences and/or severities of cardiomyopathy and nephropathy in rats, and dilatation of the hair follicles in mice. Comparison of various dioxin-like effects in these studies with those reported in the literature indicate that 3,3′,4,4′-tetrachloroazoxybenzene is six to two orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						7
Natural deaths						3
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, rectum	(10)				(10)	(7)
Epithelium, hemorrhage					1 (10%)	
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation						6 (60%)
Hepatodiaphragmatic nodule		2 (20%)				
Bile duct, hyperplasia						3 (30%)
Hepatocyte, vacuolization cytoplasmic					1 (10%)	
Hepatocyte, periportal, hypertrophy					1 (10%)	
Hepatocyte, centrilobular, degeneration					5 (50%)	10 (100%)
Mesentery	(1)					
Fat, necrosis	1 (100%)					
Pancreas	(10)				(10)	(9)
Atrophy, focal					1 (10%)	
Acinus, atrophy, focal	2 (20%)				1 (10%)	
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, hyperplasia, focal			1 (10%)	6 (60%)	8 (80%)	9 (90%)
Stomach, glandular	(10)				(10)	(10)
Epithelium, inflammation, acute	1 (10%)					
Cardiovascular System						
Heart	(10)	(9)	(10)	(10)	(10)	(10)
Hemorrhage, focal	1 (10%)					
Atrium, epicardium, hemorrhage						1 (10%)
Myocardium, cardiomyopathy	10 (100%)	9 (100%)	9 (90%)	8 (80%)	6 (60%)	10 (100%)
Endocrine System						
Adrenal cortex	(10)				(10)	(10)
Vacuolization cytoplasmic, focal						1 (10%)
Pituitary gland	(10)				(10)	(10)
Pars distalis, cyst, focal					1 (10%)	
Thyroid gland	(10)				(10)	(10)
Ultimobranchial cyst, focal	3 (30%)				3 (30%)	4 (40%)
General Body System						
None						

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

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Genital System						
Preputial gland	(9)				(10)	(10)
Atrophy						10 (100%)
Inflammation, chronic	1 (11%)					
Inflammation, chronic active	3 (33%)				1 (10%)	
Seminal vesicle	(10)				(10)	(10)
Atrophy						10 (100%)
Testes	(10)				(10)	(10)
Inflammation, granulomatous					1 (10%)	
Bilateral, germinal epithelium, degeneration						1 (10%)
Germinal epithelium, atrophy					1 (10%)	
Hematopoietic System						
Lymph node, mandibular	(10)		(10)	(10)	(10)	(10)
Atrophy					2 (20%)	1 (10%)
Hemorrhage	1 (10%)		1 (10%)	2 (20%)		
Hyperplasia, lymphoid			2 (20%)			
Inflammation, chronic				1 (10%)		
Lymph node, mesenteric	(10)		(10)	(10)	(10)	(10)
Atrophy			1 (10%)	1 (10%)	2 (20%)	1 (10%)
Hyperplasia, histiocytic				1 (10%)		
Inflammation, chronic					1 (10%)	
Inflammation, focal, granulomatous					1 (10%)	
Spleen	(10)	(10)	(10)	(10)	(10)	(9)
Accessory spleen		1 (10%)				
Hematopoietic cell proliferation						9 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage	2 (20%)	1 (10%)				3 (30%)
Thymocyte, atrophy				9 (90%)	10 (100%)	10 (100%)
Integumentary System						
Mammary gland	(10)				(9)	(10)
Duct, inflammation, chronic					1 (11%)	
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage			1 (10%)			
Hemorrhage, focal	1 (10%)			2 (20%)	3 (30%)	
Artery, inflammation, chronic active					4 (40%)	10 (100%)
Bronchus, necrosis					1 (10%)	
Interstitium, inflammation, chronic	10 (100%)	10 (100%)	10 (100%)	10 (100%)	8 (80%)	9 (90%)
Trachea	(10)				(10)	(9)
Inflammation, acute					1 (10%)	
Epithelium, hyperplasia					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(9)
Hemorrhage, focal						1 (11%)
Nephropathy	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (100%)
Pelvis, dilatation						1 (11%)
Pelvis, transitional epithelium, hyperplasia					2 (20%)	

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						3
Natural deaths						4
Survivors						
Terminal sacrifice	10	10	10	10	10	3
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)				(10)	(10)
Muscularis, inflammation, chronic					1 (10%)	
Muscularis, inflammation, chronic, focal	1 (10%)					
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Eosinophilic focus						1 (10%)
Hematopoietic cell proliferation					1 (10%)	6 (60%)
Hepatodiaphragmatic nodule		2 (20%)			1 (10%)	1 (10%)
Necrosis, focal					1 (10%)	
Bile duct, hyperplasia					3 (30%)	3 (30%)
Hepatocyte, hypertrophy					1 (10%)	
Hepatocyte, centrilobular, degeneration					2 (20%)	9 (90%)
Mesentery				(1)		
Fat, necrosis				1 (100%)		
Pancreas	(10)				(10)	(10)
Acinus, atrophy, focal	1 (10%)					
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, hyperplasia, focal					10 (100%)	8 (80%)
Stomach, glandular	(10)				(10)	(10)
Erosion, focal					1 (10%)	
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage, focal					1 (10%)	
Endocardium, hemorrhage						1 (10%)
Myocardium, cardiomyopathy	5 (50%)	8 (80%)	7 (70%)	9 (90%)	3 (30%)	10 (100%)
Endocrine System						
Thyroid gland	(10)				(10)	(9)
Ultimobranchial cyst, focal	2 (20%)				2 (20%)	5 (56%)
General Body System						
None						

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Genital System						
Clitoral gland	(10)				(10)	(10)
Atrophy						8 (80%)
Inflammation, acute					1 (10%)	1 (10%)
Inflammation, chronic, focal	1 (10%)				1 (10%)	
Inflammation, chronic active	2 (20%)				2 (20%)	
Bilateral, inflammation	1 (10%)					
Ovary	(10)				(10)	(10)
Periovarian tissue, cyst						1 (10%)
Uterus	(10)	(1)		(1)	(10)	(10)
Hydrometra	4 (40%)	1 (100%)		1 (100%)		
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Atrophy					1 (10%)	1 (10%)
Lymph node, mandibular	(10)		(10)	(10)	(10)	(10)
Atrophy				3 (30%)		1 (10%)
Hemorrhage			1 (10%)			1 (10%)
Hyperplasia, histiocytic						1 (10%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy						4 (40%)
Hemorrhage						1 (10%)
Hyperplasia, histiocytic			3 (30%)	4 (40%)	3 (30%)	3 (30%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation					5 (50%)	7 (70%)
Pigmentation	2 (20%)					
Lymphoid follicle, atrophy					1 (10%)	
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Cyst, focal						1 (10%)
Thymocyte, atrophy				1 (10%)	10 (100%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
Bone	(10)	(1)			(10)	(10)
Cranium, atrophy		1 (100%)				
Nervous System						
Brain	(10)	(1)			(10)	(10)
Cerebellum, hemorrhage, focal						1 (10%)
Cerebrum, hemorrhage, focal						1 (10%)
Meninges, cerebrum, infiltration cellular, lymphocyte		1 (100%)				

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage, focal	1 (10%)					2 (20%)
Artery, inflammation, chronic active						8 (80%)
Interstitium, inflammation, chronic	5 (50%)	10 (100%)	10 (100%)	9 (90%)	10 (100%)	9 (90%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy		1 (10%)	2 (20%)	6 (60%)	7 (70%)	9 (90%)
Renal tubule, degeneration						1 (10%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatocyte, centrilobular, hypertrophy				3 (30%)	6 (60%)	10 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, hyperplasia			1 (10%)	1 (10%)	7 (70%)	10 (100%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, cyst, focal	1 (10%)					
Cardiovascular System						
None						
Endocrine System						
Thyroid gland	(10)					(10)
Ectopic thymus	1 (10%)					
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Cyst						1 (10%)
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Red pulp, hematopoietic cell proliferation, diffuse	2 (20%)	2 (20%)	2 (20%)	4 (40%)	6 (60%)	8 (80%)
Red pulp, pigmentation, diffuse		4 (40%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Thymus	(10)	(10)	(9)	(10)	(10)	(9)
Atrophy, diffuse				1 (10%)		2 (22%)
Thymocyte, necrosis	2 (20%)				8 (80%)	6 (67%)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Hair follicle, dilatation		1 (10%)	1 (10%)	3 (30%)	10 (100%)	10 (100%)

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Alveolar epithelium, hyperplasia, focal	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)					(10)
Hydronephrosis	1 (10%)					

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of 3,3′4,4′,-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Dosing accident					1	1
Survivors						
Terminal sacrifice	10	10	10	10	9	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus				1 (10%)		
Hepatocyte, vacuolization cytoplasmic				1 (10%)		
Hepatocyte, centrilobular, hypertrophy				1 (10%)	4 (40%)	6 (60%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(9)
Epithelium, hyperplasia					3 (30%)	7 (78%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(9)
Fibrosis				1 (10%)		
Cardiovascular System						
Heart	(10)					(10)
Pericardium, inflammation, chronic, diffuse						1 (10%)
Endocrine System						
Adrenal cortex	(10)					(10)
Hemorrhage, acute	1 (10%)					
Parathyroid gland	(4)					(3)
Infiltration cellular, lymphocyte	1 (25%)					
Inflammation, focal, granulomatous	1 (25%)					
General Body System						
Tissue NOS						(1)
Mediastinum, inflammation						1 (100%)
Genital System						
None						

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of 3,3′4,4′,-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Hematopoietic System						
Bone marrow	(10)				(1)	(10)
Myeloid cell, hyperplasia					1 (100%)	1 (10%)
Spleen	(10)	(10)	(10)	(10)	(10)	(9)
Red pulp, hematopoietic cell proliferation, diffuse	2 (20%)	6 (60%)	6 (60%)	4 (40%)	7 (70%)	9 (100%)
Red pulp, pigmentation, diffuse	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)	9 (100%)
Thymus	(10)	(10)	(9)	(10)	(8)	(8)
Atrophy, diffuse					1 (13%)	
Thymocyte, necrosis	1 (10%)	1 (10%)		2 (20%)	5 (63%)	8 (100%)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Hair follicle, dilatation					1 (10%)	8 (80%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(1)	(10)
Infiltration cellular, histiocyte						2 (20%)
Inflammation, focal					1 (100%)	1 (10%)
Perivascular, inflammation						1 (10%)
Special Senses System						
None						
Urinary System						
None						

APPENDIX B
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	8	0[b]
Hematology						
Automated hematocrit (%)						
Day 3	38.2 ± 0.4	38.6 ± 0.5	39.1 ± 0.5	38.8 ± 0.3	39.8 ± 0.6	37.8 ± 1.3
Day 21	41.1 ± 0.7	40.6 ± 0.5	39.9 ± 0.4	40.1 ± 0.5	41.1 ± 0.6	39.3 ± 0.3
Week 13	42.9 ± 0.4	42.0 ± 0.5	41.6 ± 0.6	39.0 ± 0.4**	37.2 ± 0.3**	—
Manual hematocrit (%)						
Day 3	44.1 ± 0.4	44.5 ± 0.6	45.4 ± 0.6	45.2 ± 0.4	46.3 ± 0.7*	45.2 ± 0.4
Day 21	46.6 ± 0.7	46.9 ± 0.7	45.4 ± 0.4	46.0 ± 0.6	46.6 ± 0.6	44.1 ± 0.5*
Week 13	46.6 ± 0.4	45.7 ± 0.5	44.7 ± 0.5**	42.9 ± 0.3**	40.4 ± 0.3**	—
Hemoglobin (g/dL)						
Day 3	14.2 ± 0.1	14.1 ± 0.1	14.2 ± 0.1	14.2 ± 0.1	14.6 ± 0.2	13.8 ± 0.4
Day 21	15.2 ± 0.2	14.8 ± 0.2	14.6 ± 0.1	14.8 ± 0.2	15.0 ± 0.1	14.2 ± 0.2**
Week 13	15.4 ± 0.1	15.1 ± 0.1	14.7 ± 0.2**	13.9 ± 0.1**	13.1 ± 0.2**	—
Erythrocytes (10^6/μL)						
Day 3	6.34 ± 0.07	6.37 ± 0.10	6.45 ± 0.08	6.48 ± 0.05	6.63 ± 0.09	6.26 ± 0.21
Day 21	6.83 ± 0.12	6.75 ± 0.08	6.73 ± 0.07	6.81 ± 0.07	7.11 ± 0.10*	6.85 ± 0.05
Week 13	8.59 ± 0.09	8.39 ± 0.10	8.35 ± 0.12	7.73 ± 0.09**	6.96 ± 0.11**	—
Reticulocytes (10^6/μL)						
Day 3	0.29 ± 0.04	0.21 ± 0.02	0.25 ± 0.04	0.25 ± 0.03	0.23 ± 0.02	0.22 ± 0.02
Day 21	0.12 ± 0.01	0.11 ± 0.01	0.17 ± 0.03	0.16 ± 0.02	0.13 ± 0.01	0.23 ± 0.03**
Week 13	0.07 ± 0.01	0.09 ± 0.02	0.11 ± 0.01**	0.09 ± 0.01*	0.14 ± 0.01**	—
Nucleated erythrocytes (10^3/μL)						
Day 3	0.13 ± 0.05	0.10 ± 0.03	0.25 ± 0.07	0.15 ± 0.04	0.15 ± 0.05	0.19 ± 0.04
Day 21	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.03
Week 13	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	—
Mean cell volume (fL)						
Day 3	60.2 ± 0.2	60.6 ± 0.3	60.7 ± 0.1	59.9 ± 0.4	60.0 ± 0.3	60.4 ± 0.3
Day 21	60.1 ± 0.3	60.2 ± 0.3	59.3 ± 0.2*	58.9 ± 0.3**	57.9 ± 0.3**	57.3 ± 0.3**
Week 13	49.9 ± 0.1	50.0 ± 0.2	49.8 ± 0.1	50.4 ± 0.3	53.6 ± 0.4**	—
Mean cell hemoglobin (pg)						
Day 3	22.3 ± 0.1	22.1 ± 0.2	22.0 ± 0.1	22.0 ± 0.1	22.0 ± 0.1	22.0 ± 0.1
Day 21	22.3 ± 0.1	22.0 ± 0.1	21.7 ± 0.1*	21.8 ± 0.1*	21.1 ± 0.2**	20.7 ± 0.2**
Week 13	17.9 ± 0.1	18.0 ± 0.1	17.6 ± 0.1	18.0 ± 0.1	18.8 ± 0.1**	—
Mean cell hemoglobin concentration (g/dL)						
Day 3	37.1 ± 0.1	36.5 ± 0.3	36.3 ± 0.2*	36.7 ± 0.3	36.7 ± 0.2	36.4 ± 0.2
Day 21	37.1 ± 0.2	36.5 ± 0.2	36.6 ± 0.3	37.0 ± 0.2	36.5 ± 0.3	36.0 ± 0.2*
Week 13	35.8 ± 0.2	36.0 ± 0.2	35.4 ± 0.2	35.7 ± 0.3	35.2 ± 0.2	—
Platelets (10^3/μL)						
Day 3	962.7 ± 9.4	996.4 ± 20.9	1,009.6 ± 13.1	959.7 ± 19.9	954.2 ± 17.8	914.3 ± 35.5
Day 21	841.3 ± 9.7	839.8 ± 13.4	860.6 ± 15.0	790.4 ± 16.3*	760.1 ± 15.4**	574.6 ± 14.5**
Week 13	716.8 ± 26.0	753.3 ± 21.7	681.8 ± 12.3	617.0 ± 17.6**	291.3 ± 22.5**	—

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	8	0
Hematology (continued)						
Leukocytes (10^3/μL)						
Day 3	8.40 ± 0.38	8.54 ± 0.41	8.41 ± 0.37	8.95 ± 0.45	9.20 ± 0.47	9.05 ± 0.57
Day 21	10.10 ± 0.34	9.63 ± 0.47	8.52 ± 0.42*	8.28 ± 0.20**	8.10 ± 0.32**	6.99 ± 0.27**
Week 13	10.92 ± 0.65	9.26 ± 0.75	9.85 ± 0.62	7.70 ± 0.52**	6.75 ± 0.27**	—
Segmented neutrophils (10^3/μL)						
Day 3	0.72 ± 0.16	1.26 ± 0.12	1.06 ± 0.19	1.00 ± 0.20	1.06 ± 0.17	1.04 ± 0.10
Day 21	1.37 ± 0.17	1.20 ± 0.13	1.10 ± 0.10	1.04 ± 0.07	0.91 ± 0.09	1.20 ± 0.08
Week 13	1.56 ± 0.26	1.31 ± 0.14	1.96 ± 0.41	1.43 ± 0.17	1.18 ± 0.15	—
Lymphocytes (10^3/μL)						
Day 3	7.61 ± 0.35	7.20 ± 0.26	7.30 ± 0.19	7.94 ± 0.41	8.06 ± 0.49	8.03 ± 0.49
Day 21	8.45 ± 0.25	8.29 ± 0.41	7.30 ± 0.38*	7.05 ± 0.22**	6.98 ± 0.26**	5.74 ± 0.24**
Week 13	9.01 ± 0.41	7.73 ± 0.62	7.66 ± 0.32*	6.16 ± 0.44**	5.42 ± 0.20**	—
Monocytes (10^3/μL)						
Day 3	0.12 ± 0.04	0.13 ± 0.04	0.14 ± 0.03	0.09 ± 0.03	0.19 ± 0.06	0.12 ± 0.04
Day 21	0.23 ± 0.06	0.12 ± 0.05	0.10 ± 0.03	0.19 ± 0.04	0.20 ± 0.06	0.08 ± 0.03
Week 13	0.26 ± 0.08	0.20 ± 0.06	0.20 ± 0.04	0.10 ± 0.05	0.14 ± 0.03	—
Eosinophils (10^3/μL)						
Day 3	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.01
Day 21	0.05 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01*	0.01 ± 0.01*	0.00 ± 0.00**
Week 13	0.09 ± 0.03	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	—
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	20.7 ± 0.4	19.6 ± 1.2	18.8 ± 0.4	18.4 ± 0.5**	18.9 ± 0.7	19.5 ± 0.5
Day 21	20.7 ± 0.4	20.1 ± 0.3	18.9 ± 0.3**	19.4 ± 0.5**	18.5 ± 0.3**	19.1 ± 0.5**
Week 13	19.6 ± 0.3	20.3 ± 0.6	18.9 ± 0.5	17.8 ± 0.5	19.6 ± 0.9	—
Creatinine (mg/dL)						
Day 3	0.60 ± 0.01	0.57 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.59 ± 0.02	0.57 ± 0.02
Day 21	0.60 ± 0.01	0.60 ± 0.01	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.01	0.58 ± 0.01
Week 13	0.74 ± 0.03	0.70 ± 0.00	0.73 ± 0.02	0.70 ± 0.01	0.71 ± 0.01	—
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.5 ± 0.1
Day 21	6.4 ± 0.1	6.4 ± 0.0	6.5 ± 0.1	6.8 ± 0.1**	6.8 ± 0.2**	6.6 ± 0.1*
Week 13	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.2	7.5 ± 0.1	7.6 ± 0.1**	—
Albumin (g/dL)						
Day 3	4.1 ± 0.0	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1
Day 21	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.1**	4.7 ± 0.1*	4.7 ± 0.1
Week 13	4.7 ± 0.0	4.8 ± 0.1	4.9 ± 0.1*	5.1 ± 0.1**	5.1 ± 0.1**	—
Albumin/globulin ratio						
Day 3	2.8 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
Day 21	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.3 ± 0.0*	2.5 ± 0.0
Week 13	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1*	2.1 ± 0.1	—

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	10	8	0
Clinical Chemistry (continued)						
Alanine aminotransferase (IU/L)						
Day 3	48 ± 2	45 ± 2	43 ± 1	45 ± 2	42 ± 1	48 ± 2
Day 21	41 ± 1	39 ± 1	34 ± 1**	36 ± 1**	32 ± 1**	30 ± 1**
Week 13	60 ± 2	57 ± 3	50 ± 3*	47 ± 1**	48 ± 2**	—
Alkaline phosphatase (IU/L)						
Day 3	787 ± 19	772 ± 11	807 ± 23	809 ± 23	810 ± 21	833 ± 23
Day 21	523 ± 17	486 ± 10	485 ± 16	479 ± 6**	481 ± 9*	464 ± 11**
Week 13	288 ± 8	301 ± 6	318 ± 15*	318 ± 11*	329 ± 11**	—
Sorbitol dehydrogenase (IU/L)						
Day 3	20 ± 1	18 ± 1	16 ± 1	18 ± 1	21 ± 2	19 ± 1
Day 21	18 ± 1	18 ± 1	20 ± 1	19 ± 1	20 ± 1	20 ± 1
Week 13	19 ± 1	16 ± 1	18 ± 1	16 ± 2	21 ± 2	—
Bile acids (μmol/L)						
Day 3	39.1 ± 6.6	37.3 ± 4.3	41.1 ± 3.9	42.0 ± 5.0	42.0 ± 3.6	43.8 ± 4.6
Day 21	28.8 ± 5.4	38.9 ± 5.0	33.2 ± 4.6	29.9 ± 4.2	29.3 ± 2.3	33.6 ± 3.6
Week 13	40.9 ± 3.8	34.3 ± 4.1	37.8 ± 4.6	45.8 ± 4.7	56.0 ± 6.7	—
Thyroid-stimulating hormone (ng/mL)						
Week 13	1.6 ± 0.2	2.6 ± 0.2*	2.9 ± 0.5*	3.1 ± 0.5*	3.5 ± 0.6**	—
Total Triiodothyronine (ng/dL)						
Week 13	98 ± 5	118 ± 7	102 ± 6	117 ± 8	99 ± 7	—
Total thyroxine (μg/dL)						
Week 13	2.9 ± 0.1	3.0 ± 0.1	1.9 ± 0.1**	0.9 ± 0.1**	0.4 ± 0.1**	—
Female						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Hematology						
Automated hematocrit (%)						
Day 3	41.8 ± 0.6	41.1 ± 0.7	41.5 ± 0.5	41.1 ± 0.5	42.0 ± 0.6	42.6 ± 0.5
Day 21	42.7 ± 0.5	42.0 ± 0.6	41.2 ± 0.5	42.9 ± 0.3	41.3 ± 0.5	40.9 ± 1.0
Week 13	41.0 ± 0.6	41.9 ± 0.7	40.6 ± 0.5	41.0 ± 0.3	38.8 ± 0.5*	18.5 ± 1.4**
Manual hematocrit (%)						
Day 3	45.4 ± 0.8	45.2 ± 0.7	45.0 ± 0.6	45.3 ± 0.7	45.7 ± 0.6	46.2 ± 0.8
Day 21	45.4 ± 0.4	44.9 ± 0.5	43.8 ± 0.6	45.9 ± 0.5	44.3 ± 0.6	43.9 ± 1.0
Week 13	44.7 ± 0.5	45.8 ± 0.7	44.1 ± 0.6	44.8 ± 0.3	42.8 ± 0.5*	20.3 ± 1.2**
Hemoglobin (g/dL)						
Day 3	14.8 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	15.0 ± 0.2	15.1 ± 0.2
Day 21	15.4 ± 0.1	15.2 ± 0.2	14.6 ± 0.1**	15.3 ± 0.2	14.6 ± 0.1**	14.4 ± 0.3**
Week 13	14.9 ± 0.1	15.1 ± 0.2	14.6 ± 0.2	14.5 ± 0.1	13.6 ± 0.1**	6.9 ± 0.5**

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Hematology (continued)						
Erythrocytes (10^6/μL)						
Day 3	6.90 ± 0.09	6.80 ± 0.12	6.82 ± 0.09	6.81 ± 0.08	6.91 ± 0.10	7.01 ± 0.11
Day 21	7.04 ± 0.09	6.94 ± 0.11	6.83 ± 0.09	7.21 ± 0.07	6.97 ± 0.09	7.11 ± 0.19
Week 13	7.45 ± 0.09	7.58 ± 0.14	7.42 ± 0.09	7.51 ± 0.04	7.06 ± 0.08*	2.92 ± 0.26**
Reticulocytes (10^6/μL)						
Day 3	0.17 ± 0.01	0.23 ± 0.01*	0.21 ± 0.02	0.21 ± 0.02	0.24 ± 0.03	0.20 ± 0.01
Day 21	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01*	0.15 ± 0.01*	0.17 ± 0.01**	0.19 ± 0.01**
Week 13	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.12 ± 0.01**	0.19 ± 0.02**
Nucleated erythrocytes (10^3/μL)						
Day 3	0.05 ± 0.02	0.13 ± 0.04	0.10 ± 0.03	0.12 ± 0.04	0.17 ± 0.03	0.16 ± 0.05
Day 21	0.08 ± 0.03	0.07 ± 0.03	0.09 ± 0.03	0.07 ± 0.02	0.07 ± 0.02	0.14 ± 0.04
Week 13	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Mean cell volume (fL)						
Day 3	60.5 ± 0.3	60.5 ± 0.3	60.9 ± 0.4	60.4 ± 0.2	60.8 ± 0.3	60.8 ± 0.3
Day 21	60.7 ± 0.2	60.6 ± 0.2	60.4 ± 0.2	59.6 ± 0.2**	59.3 ± 0.3**	57.6 ± 0.2**
Week 13	55.0 ± 0.3	55.3 ± 0.3	54.7 ± 0.2	54.5 ± 0.2	55.0 ± 0.2	63.5 ± 1.0
Mean cell hemoglobin (pg)						
Day 3	21.5 ± 0.1	21.7 ± 0.2	21.6 ± 0.1	21.6 ± 0.1	21.7 ± 0.1	21.6 ± 0.2
Day 21	21.8 ± 0.2	21.9 ± 0.1	21.4 ± 0.1	21.3 ± 0.1*	21.0 ± 0.2**	20.2 ± 0.1**
Week 13	20.0 ± 0.1	20.0 ± 0.2	19.7 ± 0.1	19.3 ± 0.1**	19.3 ± 0.1**	23.7 ± 0.8
Mean cell hemoglobin concentration (g/dL)						
Day 3	35.5 ± 0.3	35.8 ± 0.3	35.5 ± 0.2	35.8 ± 0.2	35.7 ± 0.2	35.5 ± 0.2
Day 21	36.0 ± 0.2	36.2 ± 0.2	35.4 ± 0.2*	35.7 ± 0.2	35.5 ± 0.3	35.1 ± 0.2**
Week 13	36.4 ± 0.2	36.1 ± 0.2	36.1 ± 0.3	35.4 ± 0.2	35.2 ± 0.2*	37.4 ± 0.6
Platelets (10^3/μL)						
Day 3	953.6 ± 20.3	910.8 ± 16.1	936.8 ± 14.6	877.7 ± 13.2*	925.9 ± 19.4	947.8 ± 21.8
Day 21	819.1 ± 20.3	814.5 ± 16.2	817.1 ± 12.9	713.0 ± 9.8**	727.2 ± 11.3**	613.0 ± 6.8**
Week 13	700.8 ± 21.8	637.0 ± 14.9*	591.0 ± 12.6**	525.2 ± 7.0**	475.9 ± 20.1**	65.7 ± 13.7**
Leukocytes (10^3/μL)						
Day 3	9.96 ± 0.38	10.09 ± 0.20	8.93 ± 0.43	9.80 ± 0.36	9.20 ± 0.30	9.88 ± 0.38
Day 21	10.05 ± 0.41	10.96 ± 0.36	9.57 ± 0.43	9.57 ± 0.67	9.32 ± 0.30	8.82 ± 0.27*
Week 13	5.96 ± 0.55	7.08 ± 0.33	6.90 ± 0.70	7.30 ± 0.56	6.19 ± 0.23	2.70 ± 0.46
Segmented neutrophils (10^3/μL)						
Day 3	0.87 ± 0.13	0.97 ± 0.12	0.94 ± 0.16	1.17 ± 0.15	0.88 ± 0.15	1.39 ± 0.11*
Day 21	1.13 ± 0.14	1.47 ± 0.17	1.20 ± 0.11	1.20 ± 0.12	0.98 ± 0.08	0.92 ± 0.11
Week 13	0.82 ± 0.06	1.05 ± 0.10	0.83 ± 0.10	1.08 ± 0.11	1.07 ± 0.11	0.32 ± 0.06
Lymphocytes (10^3/μL)						
Day 3	8.92 ± 0.33	9.01 ± 0.17	7.95 ± 0.34	8.53 ± 0.35	8.23 ± 0.28	8.39 ± 0.39
Day 21	8.70 ± 0.42	9.36 ± 0.26	8.09 ± 0.38	8.15 ± 0.59	8.11 ± 0.23	7.88 ± 0.30
Week 13	5.00 ± 0.52	5.93 ± 0.27	5.94 ± 0.69	6.08 ± 0.48	5.04 ± 0.23	2.37 ± 0.41

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Hematology (continued)						
Monocytes (10^3/μL)						
Day 3	0.10 ± 0.03	0.11 ± 0.04	0.08 ± 0.03	0.12 ± 0.05	0.14 ± 0.03	0.20 ± 0.05
Day 21	0.18 ± 0.03	0.14 ± 0.05	0.27 ± 0.05	0.19 ± 0.05	0.21 ± 0.04	0.08 ± 0.03
Week 13	0.06 ± 0.02	0.05 ± 0.02	0.11 ± 0.02	0.10 ± 0.02	0.07 ± 0.03	0.01 ± 0.01
Eosinophils (10^3/μL)						
Day 3	0.06 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01
Day 21	0.04 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
Week 13	0.09 ± 0.03	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.01 ± 0.01*	0.01 ± 0.01
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	19.8 ± 0.8	19.5 ± 0.5	19.0 ± 0.7	18.4 ± 0.5	18.5 ± 0.7	18.4 ± 0.9
Day 21	21.5 ± 0.6	20.6 ± 0.7	20.4 ± 0.6	20.7 ± 0.6	18.9 ± 0.4*	20.6 ± 0.6
Week 13	19.3 ± 1.1	20.6 ± 0.7	19.1 ± 0.9	17.5 ± 0.7	19.1 ± 0.3	24.3 ± 0.9
Creatinine (mg/dL)						
Day 3	0.59 ± 0.01	0.55 ± 0.02	0.56 ± 0.02	0.60 ± 0.00	0.57 ± 0.02	0.57 ± 0.02
Day 21	0.61 ± 0.01	0.64 ± 0.02	0.63 ± 0.02	0.62 ± 0.02	0.60 ± 0.01	0.63 ± 0.02
Week 13	0.79 ± 0.01	0.74 ± 0.03	0.75 ± 0.02	0.72 ± 0.02*	0.71 ± 0.01**	0.67 ± 0.03**
Total protein (g/dL)						
Day 3	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.6 ± 0.1*
Day 21	6.2 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.1**	6.7 ± 0.1**
Week 13	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.0	7.0 ± 0.1	6.6 ± 0.2
Albumin (g/dL)						
Day 3	4.0 ± 0.0	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.0*
Day 21	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.6 ± 0.1	4.8 ± 0.1**	4.8 ± 0.1**
Week 13	5.1 ± 0.1	5.1 ± 0.1	5.2 ± 0.0	5.3 ± 0.0	5.1 ± 0.1	5.1 ± 0.2
Albumin/globulin ratio						
Day 3	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
Day 21	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
Week 13	2.7 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.6 ± 0.1	3.5 ± 0.0*
Alanine aminotransferase (IU/L)						
Day 3	39 ± 2	38 ± 1	35 ± 2	37 ± 1	36 ± 2	37 ± 2
Day 21	35 ± 2	36 ± 1	30 ± 1	35 ± 1	34 ± 1	33 ± 2
Week 13	54 ± 5	48 ± 3	43 ± 3**	46 ± 3*	36 ± 2**	42 ± 5*
Alkaline phosphatase (IU/L)						
Day 3	596 ± 15	634 ± 13	656 ± 18*	659 ± 9**	691 ± 14**	712 ± 19**
Day 21	396 ± 6	406 ± 8	395 ± 6	384 ± 7	373 ± 10	347 ± 9**
Week 13	250 ± 8	279 ± 12	298 ± 10*	309 ± 11**	288 ± 6	241 ± 8
Sorbitol dehydrogenase (IU/L)						
Day 3	17 ± 1	17 ± 1	20 ± 1	17 ± 1	18 ± 2	17 ± 1
Day 21	15 ± 1	21 ± 1**	20 ± 1**	26 ± 2**	32 ± 2**	49 ± 4**
Week 13	16 ± 2	18 ± 1	16 ± 2	21 ± 1	15 ± 2	38 ± 20

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 13	10	10	10	10	10	3
Clinical Chemistry (continued)						
Bile acids (µmol/L)						
Day 3	32.0 ± 3.4	42.7 ± 4.4	34.0 ± 3.4	39.1 ± 3.0	43.1 ± 3.4	42.0 ± 4.0
Day 21	36.4 ± 4.1	28.8 ± 2.5	37.4 ± 4.7	43.8 ± 4.0	58.1 ± 4.1**	49.8 ± 8.0*
Week 13	28.9 ± 3.9	31.6 ± 2.9	33.2 ± 2.6	36.9 ± 3.2*	52.4 ± 4.4**	117.2 ± 19.6**
Thyroid-stimulating hormone (ng/mL)						
Week 13	1.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.2
Total triiodothyronine (ng/dL)						
Week 13	97 ± 6	90 ± 7	89 ± 5	83 ± 3	64 ± 4**	83 ± 6*
Total thyroxine (µg/dL)						
Week 13	2.5 ± 0.2	1.7 ± 0.2*	1.2 ± 0.1**	0.3 ± 0.1**	0.1 ± 0.0**	0.0 ± 0.0**

* Significantly different (P 0.05) from the vehicle control group by Dunn's or Shirley's test
** P 0.01
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] No data available due to 100% mortality.

TABLE B2
Clinical Chemistry Data for Mice in the 13-Week Gavage Study of 3,3′4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	30.1 ± 1.6	31.8 ± 0.6	30.3 ± 1.2	28.0 ± 1.5	29.1 ± 1.7	29.3 ± 1.1
Creatinine (mg/dL)	0.47 ± 0.02	0.47 ± 0.02	0.47 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.43 ± 0.02
Total protein (g/dL)	6.1 ± 0.1	6.2 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.1**	5.6 ± 0.1**
Albumin (g/dL)	4.1 ± 0.0	4.2 ± 0.1	4.1 ± 0.0	4.1 ± 0.1	4.1 ± 0.1	4.0 ± 0.0
Albumin/globulin ratio	2.0 ± 0.0	2.1 ± 0.0	2.3 ± 0.1**	2.2 ± 0.1**	2.5 ± 0.1**	2.6 ± 0.1**
Alanine aminotransferase (IU/L)	28 ± 4	32 ± 4	27 ± 3	27 ± 3	31 ± 2[b]	36 ± 2*
Alkaline phosphatase (IU/L)	60 ± 1	59 ± 1	56 ± 2	57 ± 2	56 ± 2	51 ± 1**
Sorbitol dehydrogenase (IU/L)	48 ± 2	47 ± 1	43 ± 2	46 ± 2	42 ± 2	50 ± 2
Bile acids (µmol/L)	15.6 ± 0.8	16.8 ± 0.9	16.1 ± 0.4	17.2 ± 1.0	17.1 ± 0.5	16.5 ± 0.8
Female						
n	10	10	10	10	9	9
Urea nitrogen (mg/dL)	18.4 ± 1.0	21.0 ± 1.5	21.8 ± 1.0	22.8 ± 1.0*	21.3 ± 1.2	17.8 ± 1.0
Creatinine (mg/dL)	0.47 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	0.49 ± 0.02	0.42 ± 0.01	0.41 ± 0.02
Total protein (g/dL)	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Albumin (g/dL)	4.4 ± 0.0	4.5 ± 0.1	4.4 ± 0.0	4.5 ± 0.1	4.6 ± 0.0*	4.6 ± 0.1**
Albumin/globulin ratio	3.5 ± 0.2	3.6 ± 0.2	3.7 ± 0.2	4.1 ± 0.1*	4.5 ± 0.2**	4.4 ± 0.2**
Alanine aminotransferase (IU/L)	32 ± 5	29 ± 4	27 ± 3	24 ± 1	27 ± 3	28 ± 2
Alkaline phosphatase (IU/L)	134 ± 5	131 ± 6	134 ± 6	133 ± 4	128 ± 5	134 ± 5
Sorbitol dehydrogenase (IU/L)	44 ± 3	48 ± 4	51 ± 2	46 ± 1	48 ± 3	49 ± 2
Bile acids (µmol/L)	16.4 ± 0.2	17.3 ± 0.6	16.9 ± 0.8	16.5 ± 0.6	17.0 ± 0.5	17.8 ± 0.6

* Significantly different (P 0.05) from the vehicle control group by Dunn's or Shirley's test
** Significantly different (P 0.01) from the vehicle control group by Shirley's test
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n = 9

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

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	12.5 mg/kg	32 mg/kg	80 mg/kg	200 mg/kg	500 mg/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	217 ± 8	205 ± 3	201 ± 5	189 ± 7**	176 ± 5**	163 ± 10**
Heart						
Absolute	0.783 ± 0.019	0.823 ± 0.009	0.791 ± 0.014	0.804 ± 0.033	0.698 ± 0.025*	0.637 ± 0.040**
Relative	3.62 ± 0.07	4.01 ± 0.06*	3.95 ± 0.07	4.26 ± 0.17**	3.97 ± 0.07	3.92 ± 0.08
R. Kidney						
Absolute	0.940 ± 0.036	0.966 ± 0.017	0.962 ± 0.032	0.977 ± 0.035	0.942 ± 0.035	0.871 ± 0.037
Relative	4.34 ± 0.10	4.70 ± 0.06*	4.79 ± 0.07**	5.16 ± 0.10**	5.36 ± 0.14**	5.39 ± 0.14**
Liver						
Absolute	9.976 ± 0.449	12.389 ± 0.327*	12.353 ± 0.352*	13.630 ± 0.455**	13.318 ± 0.505**	11.710 ± 0.801
Relative	45.94 ± 0.71	60.34 ± 1.40**	61.53 ± 0.77**	72.03 ± 0.86**	75.71 ± 1.54**	71.87 ± 0.84**
Lung						
Absolute	1.119 ± 0.056	1.232 ± 0.089	1.456 ± 0.017**	1.420 ± 0.074*	1.250 ± 0.041	1.103 ± 0.082
Relative	5.15 ± 0.12	5.99 ± 0.39	7.27 ± 0.25**	7.53 ± 0.44**	7.11 ± 0.08**	6.77 ± 0.19**
Spleen						
Absolute	0.598 ± 0.013	0.627 ± 0.019	0.662 ± 0.020	0.650 ± 0.029	0.554 ± 0.023	0.527 ± 0.032
Relative	2.77 ± 0.07	3.05 ± 0.07*	3.30 ± 0.07**	3.43 ± 0.04**	3.15 ± 0.10**	3.25 ± 0.09**
R. Testis						
Absolute	1.256 ± 0.041	1.207 ± 0.033	1.195 ± 0.044	1.195 ± 0.028	1.146 ± 0.017*	1.102 ± 0.022**
Relative	5.80 ± 0.07	5.88 ± 0.16	5.94 ± 0.09	6.32 ± 0.13	6.53 ± 0.11**	6.86 ± 0.36**
Thymus						
Absolute	0.480 ± 0.029	0.249 ± 0.014**	0.194 ± 0.011**	0.158 ± 0.011**	0.130 ± 0.008**	0.126 ± 0.015**
Relative	2.21 ± 0.10	1.21 ± 0.07**	0.97 ± 0.05**	0.83 ± 0.03**	0.74 ± 0.04**	0.77 ± 0.06**

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	12.5 mg/kg	32 mg/kg	80 mg/kg	200 mg/kg	500 mg/kg
Female						
n	5	5	5	5	5	5
Necropsy body wt	144 ± 5	140 ± 2	141 ± 4	132 ± 3*	127 ± 4**	121 ± 2**
Heart						
Absolute	0.570 ± 0.011	0.589 ± 0.022	0.626 ± 0.023	0.548 ± 0.013	0.521 ± 0.023	0.498 ± 0.006**
Relative	3.96 ± 0.06	4.22 ± 0.10	4.44 ± 0.09**	4.15 ± 0.10	4.09 ± 0.10	4.14 ± 0.05
R. Kidney						
Absolute	0.645 ± 0.017	0.659 ± 0.014	0.676 ± 0.031	0.638 ± 0.016	0.658 ± 0.033	0.624 ± 0.019
Relative	4.48 ± 0.07	4.72 ± 0.04	4.79 ± 0.15*	4.83 ± 0.09*	5.16 ± 0.11**	5.17 ± 0.10**
Liver						
Absolute	6.438 ± 0.325	7.535 ± 0.237*	9.100 ± 0.363**	9.232 ± 0.227**	9.430 ± 0.371**	8.536 ± 0.249**
Relative	44.57 ± 1.31	53.94 ± 0.99**	64.49 ± 1.95**	69.86 ± 0.93**	73.92 ± 0.77**	70.79 ± 1.76**
Lung						
Absolute	0.850 ± 0.030	1.016 ± 0.041*	1.012 ± 0.045*	0.992 ± 0.038*	1.010 ± 0.032*	0.914 ± 0.027
Relative	5.89 ± 0.08	7.27 ± 0.21**	7.17 ± 0.26**	7.53 ± 0.41**	7.93 ± 0.17**	7.58 ± 0.14**
Spleen						
Absolute	0.433 ± 0.017	0.421 ± 0.012	0.459 ± 0.024	0.425 ± 0.014	0.446 ± 0.008	0.387 ± 0.009
Relative	3.00 ± 0.07	3.02 ± 0.08	3.24 ± 0.11	3.22 ± 0.08	3.51 ± 0.04**	3.21 ± 0.04
Thymus						
Absolute	0.381 ± 0.016	0.210 ± 0.012**	0.207 ± 0.017**	0.107 ± 0.019**	0.116 ± 0.011**	0.103 ± 0.007**
Relative	2.64 ± 0.09	1.50 ± 0.08**	1.46 ± 0.09**	0.81 ± 0.14**	0.91 ± 0.07**	0.85 ± 0.06**
Uterus						
Absolute	0.376 ± 0.025	0.313 ± 0.048	0.361 ± 0.035	0.271 ± 0.027	0.291 ± 0.046	0.194 ± 0.019**
Relative	2.61 ± 0.15	2.24 ± 0.33	2.55 ± 0.21	2.05 ± 0.18	2.31 ± 0.40	1.61 ± 0.16*

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

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n	10	10	10	10	10	0[b]
Necropsy body wt	366 ± 5	357 ± 8	351 ± 6	332 ± 5**	301 ± 13**	
Heart						
Absolute	1.057 ± 0.018	1.039 ± 0.019	1.028 ± 0.023	1.058 ± 0.014	1.131 ± 0.051	
Relative	2.89 ± 0.03	2.91 ± 0.03	2.93 ± 0.04	3.19 ± 0.05	3.88 ± 0.36**	
R. Kidney						
Absolute	1.210 ± 0.022	1.250 ± 0.031	1.272 ± 0.035	1.238 ± 0.024	1.200 ± 0.044	
Relative	3.31 ± 0.04	3.50 ± 0.03**	3.62 ± 0.05**	3.73 ± 0.04**	4.00 ± 0.06**	
Liver						
Absolute	12.854 ± 0.218	13.433 ± 0.285	14.563 ± 0.419*	14.068 ± 0.251*	14.039 ± 0.625*	
Relative	35.14 ± 0.35	37.67 ± 0.45**	41.46 ± 0.63**	42.37 ± 0.29**	46.59 ± 0.28**	
Lung						
Absolute	1.975 ± 0.115	1.715 ± 0.088	1.655 ± 0.042*	1.800 ± 0.097	1.884 ± 0.080	
Relative	5.41 ± 0.32	4.81 ± 0.24	4.72 ± 0.13	5.42 ± 0.28	6.31 ± 0.27*	
Spleen						
Absolute	0.751 ± 0.013	0.807 ± 0.043	0.789 ± 0.026	0.769 ± 0.023	0.738 ± 0.023	
Relative	2.06 ± 0.04	2.25 ± 0.07*	2.25 ± 0.05*	2.32 ± 0.06**	2.48 ± 0.10**	
R. Testis						
Absolute	1.503 ± 0.024	1.469 ± 0.032	1.477 ± 0.034	1.452 ± 0.038	1.373 ± 0.055	
Relative	4.11 ± 0.03	4.12 ± 0.07	4.21 ± 0.05	4.37 ± 0.08*	4.59 ± 0.13**	
Thymus						
Absolute	0.409 ± 0.016	0.384 ± 0.017	0.279 ± 0.006**	0.301 ± 0.011**	0.249 ± 0.030**	
Relative	1.12 ± 0.04	1.08 ± 0.04	0.80 ± 0.02**	0.91 ± 0.03**	0.81 ± 0.08**	

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female						
n	10	10	10	10	10	3
Necropsy body wt	197 ± 3	192 ± 3	191 ± 2	191 ± 3	185 ± 4*	159 ± 5**
Heart						
Absolute	0.689 ± 0.018	0.668 ± 0.015	0.647 ± 0.005	0.673 ± 0.011	0.735 ± 0.026	0.892 ± 0.074**
Relative	3.51 ± 0.06	3.48 ± 0.06	3.39 ± 0.04	3.53 ± 0.05	3.97 ± 0.08**	5.10 ± 0.35**
R. Kidney						
Absolute	0.681 ± 0.013	0.671 ± 0.016	0.674 ± 0.010	0.690 ± 0.011	0.699 ± 0.019	0.797 ± 0.028**
Relative	3.47 ± 0.05	3.50 ± 0.07	3.52 ± 0.05	3.62 ± 0.05	3.78 ± 0.05**	4.56 ± 0.10**
Liver						
Absolute	6.266 ± 0.190	6.489 ± 0.196	6.713 ± 0.159	6.980 ± 0.193*	8.160 ± 0.314**	9.923 ± 0.110**
Relative	31.91 ± 0.90	33.78 ± 0.83	35.08 ± 0.70*	36.57 ± 0.57**	44.09 ± 1.16**	56.85 ± 1.34**
Lung						
Absolute	1.154 ± 0.058	1.210 ± 0.054	1.155 ± 0.042	1.211 ± 0.025	1.659 ± 0.179**	1.390 ± 0.023*
Relative	5.88 ± 0.29	6.31 ± 0.29	6.03 ± 0.19	6.36 ± 0.17	8.99 ± 0.98**	7.96 ± 0.22**
Spleen						
Absolute	0.447 ± 0.017	0.449 ± 0.019	0.457 ± 0.017	0.468 ± 0.017	0.545 ± 0.037**	0.565 ± 0.033*
Relative	2.28 ± 0.09	2.34 ± 0.08	2.38 ± 0.07	2.45 ± 0.06	2.95 ± 0.19**	3.23 ± 0.18**
Thymus						
Absolute	0.274 ± 0.010	0.264 ± 0.014	0.212 ± 0.007**	0.201 ± 0.009**	0.166 ± 0.011**	0.101 ± 0.006**
Relative	1.40 ± 0.05	1.37 ± 0.06	1.11 ± 0.03**	1.05 ± 0.04**	0.90 ± 0.05**	0.58 ± 0.03**
Uterus						
Absolute	0.709 ± 0.089	0.573 ± 0.055	0.644 ± 0.033	0.609 ± 0.063	0.529 ± 0.030	0.460 ± 0.046
Relative	3.63 ± 0.47	2.99 ± 0.30	3.36 ± 0.17	3.19 ± 0.32	2.87 ± 0.16	2.64 ± 0.29

* Significantly different (P 0.05) from the vehicle control group by Williams' or Dunnett's test
** P 0.01
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No data available due to 100% mortality.

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	1 mg/kg	3.2 mg/kg	10 mg/kg	32 mg/kg	100 mg/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	25.4 ± 1.0	26.2 ± 0.7	25.7 ± 0.4	25.9 ± 0.4	26.1 ± 0.4	26.6 ± 0.3
Heart						
Absolute	0.130 ± 0.005	0.142 ± 0.006	0.137 ± 0.004	0.143 ± 0.003	0.138 ± 0.003	0.142 ± 0.002
Relative	5.14 ± 0.18	5.39 ± 0.13	5.32 ± 0.18	5.53 ± 0.09	5.30 ± 0.08	5.33 ± 0.10
R. Kidney						
Absolute	0.238 ± 0.008	0.259 ± 0.011	0.267 ± 0.015	0.266 ± 0.011	0.268 ± 0.006	0.260 ± 0.011
Relative	9.41 ± 0.25	9.89 ± 0.24	10.39 ± 0.54	10.23 ± 0.30	10.31 ± 0.33	9.77 ± 0.38
Liver						
Absolute	1.307 ± 0.042	1.435 ± 0.075	1.582 ± 0.052**	1.635 ± 0.029**	1.778 ± 0.035**	1.912 ± 0.025**
Relative	51.56 ± 0.42	54.66 ± 2.23	61.47 ± 1.57**	63.11 ± 0.83**	68.33 ± 2.06**	72.00 ± 0.98**
Lung						
Absolute	0.202 ± 0.012	0.197 ± 0.011	0.194 ± 0.006	0.191 ± 0.007	0.198 ± 0.007	0.190 ± 0.006
Relative	7.97 ± 0.28	7.56 ± 0.52	7.57 ± 0.25	7.37 ± 0.24	7.61 ± 0.24	7.13 ± 0.18
Spleen						
Absolute	0.069 ± 0.002	0.065 ± 0.002	0.064 ± 0.002	0.065 ± 0.002	0.065 ± 0.003	0.070 ± 0.005
Relative	2.72 ± 0.09	2.46 ± 0.04	2.48 ± 0.07	2.52 ± 0.06	2.49 ± 0.13	2.62 ± 0.17
R. Testis						
Absolute	0.110 ± 0.004	0.108 ± 0.005	0.107 ± 0.004	0.104 ± 0.002	0.106 ± 0.002	0.104 ± 0.002
Relative	4.36 ± 0.16	4.10 ± 0.08	4.16 ± 0.10	4.03 ± 0.08*	4.06 ± 0.04*	3.92 ± 0.05**
Thymus						
Absolute	0.050 ± 0.003	0.044 ± 0.003	0.040 ± 0.002**	0.030 ± 0.001**	0.031 ± 0.002**	0.021 ± 0.002**
Relative	1.97 ± 0.05	1.67 ± 0.08**	1.54 ± 0.08**	1.17 ± 0.04**	1.17 ± 0.05**	0.80 ± 0.07**

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	1 mg/kg	3.2 mg/kg	10 mg/kg	32 mg/kg	100 mg/kg
Female						
n	5	5	5	5	5	5
Necropsy body wt	20.3 ± 0.5	20.6 ± 0.4	20.4 ± 0.3	20.5 ± 0.4	20.4 ± 0.7	20.2 ± 0.6
Heart						
Absolute	0.104 ± 0.002	0.111 ± 0.003	0.108 ± 0.002	0.113 ± 0.003	0.112 ± 0.004	0.114 ± 0.002
Relative	5.10 ± 0.06	5.38 ± 0.10	5.26 ± 0.06	5.51 ± 0.16*	5.51 ± 0.08*	5.65 ± 0.16**
R. Kidney						
Absolute	0.160 ± 0.009	0.164 ± 0.006	0.166 ± 0.004	0.168 ± 0.004	0.174 ± 0.005	0.171 ± 0.006
Relative	7.87 ± 0.32	7.96 ± 0.15	8.12 ± 0.10	8.21 ± 0.14	8.57 ± 0.10*	8.46 ± 0.19*
Liver						
Absolute	1.065 ± 0.058	1.193 ± 0.022*	1.219 ± 0.026*	1.293 ± 0.037**	1.345 ± 0.042**	1.498 ± 0.047**
Relative	52.36 ± 2.27	58.00 ± 0.78**	59.64 ± 1.20**	63.12 ± 0.69**	66.01 ± 0.37**	74.14 ± 1.06**
Lung						
Absolute	0.155 ± 0.006	0.166 ± 0.005	0.159 ± 0.006	0.172 ± 0.005	0.171 ± 0.009	0.165 ± 0.006
Relative	7.60 ± 0.15	8.08 ± 0.24	7.79 ± 0.27	8.41 ± 0.27	8.36 ± 0.28	8.19 ± 0.27
Spleen						
Absolute	0.073 ± 0.003	0.070 ± 0.002	0.073 ± 0.003	0.068 ± 0.004	0.067 ± 0.003	0.077 ± 0.004
Relative	3.58 ± 0.08	3.42 ± 0.08	3.56 ± 0.16	3.30 ± 0.13	3.29 ± 0.06	3.81 ± 0.12
Thymus						
Absolute	0.069 ± 0.003	0.066 ± 0.004	0.057 ± 0.001*	0.049 ± 0.003**	0.039 ± 0.003**	0.032 ± 0.004**
Relative	3.40 ± 0.16	3.19 ± 0.17	2.78 ± 0.07*	2.40 ± 0.12**	1.91 ± 0.18**	1.60 ± 0.20**
Uterus						
Absolute	0.108 ± 0.014	0.126 ± 0.012	0.118 ± 0.018	0.103 ± 0.013	0.084 ± 0.010	0.127 ± 0.014
Relative	5.31 ± 0.68	6.09 ± 0.54	5.81 ± 0.92	4.99 ± 0.53	4.13 ± 0.44	6.26 ± 0.59

* Significantly different (P 0.05) from the vehicle control group by Williams' test
** P 0.01
Trend is significantly increased (P 0.01) by Jonckheere's test.
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	36.7 ± 0.9	37.1 ± 1.0	36.7 ± 1.3	37.5 ± 1.1	35.5 ± 0.8	38.1 ± 0.9
Heart						
Absolute	0.154 ± 0.003	0.156 ± 0.006	0.164 ± 0.005	0.165 ± 0.004	0.163 ± 0.003	0.169 ± 0.004*
Relative	4.22 ± 0.17	4.24 ± 0.19	4.49 ± 0.11	4.44 ± 0.15	4.61 ± 0.07	4.44 ± 0.09
R. Kidney						
Absolute	0.293 ± 0.006	0.296 ± 0.009	0.309 ± 0.010	0.315 ± 0.012	0.313 ± 0.007	0.321 ± 0.007*
Relative	8.02 ± 0.22	8.05 ± 0.34	8.45 ± 0.19	8.41 ± 0.18	8.84 ± 0.21	8.46 ± 0.24
Liver						
Absolute	1.742 ± 0.041	1.827 ± 0.062	1.899 ± 0.067	2.046 ± 0.086**	2.011 ± 0.071**	2.307 ± 0.071**
Relative	47.68 ± 1.42	49.30 ± 1.35	51.96 ± 1.59	54.68 ± 1.93**	56.60 ± 1.40**	60.55 ± 1.24**
Lung						
Absolute	0.249 ± 0.017	0.229 ± 0.012	0.230 ± 0.012	0.223 ± 0.011	0.204 ± 0.005*	0.224 ± 0.009
Relative	6.81 ± 0.50	6.22 ± 0.37	6.33 ± 0.39	5.93 ± 0.19	5.77 ± 0.18	5.89 ± 0.23
Spleen						
Absolute	0.073 ± 0.002	0.074 ± 0.003	0.075 ± 0.003	0.073 ± 0.002	0.075 ± 0.002	0.075 ± 0.002
Relative	1.99 ± 0.05	2.01 ± 0.07	2.05 ± 0.07	1.96 ± 0.08	2.11 ± 0.07	1.98 ± 0.04
R. Testis						
Absolute	0.125 ± 0.002	0.119 ± 0.004	0.125 ± 0.003	0.124 ± 0.001	0.120 ± 0.001	0.121 ± 0.002
Relative	3.42 ± 0.07	3.22 ± 0.15	3.42 ± 0.07	3.32 ± 0.09	3.38 ± 0.07	3.20 ± 0.09
Thymus						
Absolute	0.051 ± 0.003	0.046 ± 0.004	0.043 ± 0.004	0.037 ± 0.003**	0.032 ± 0.004**	0.029 ± 0.002**
Relative	1.41 ± 0.10	1.25 ± 0.11	1.16 ± 0.09	1.01 ± 0.07**	0.90 ± 0.09**	0.78 ± 0.06**

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Female						
n	10	10	10	10	9	9
Necropsy body wt	27.7 ± 0.8	27.9 ± 0.5	27.0 ± 0.8	26.7 ± 0.7	25.9 ± 0.6	26.1 ± 0.6
Heart						
Absolute	0.127 ± 0.001	0.135 ± 0.004	0.133 ± 0.003	0.132 ± 0.002	0.129 ± 0.002	0.133 ± 0.003
Relative	4.61 ± 0.10	4.84 ± 0.15	4.94 ± 0.14	4.96 ± 0.12*	5.01 ± 0.10*	5.08 ± 0.05**
R. Kidney						
Absolute	0.172 ± 0.003	0.181 ± 0.005	0.181 ± 0.002	0.182 ± 0.003	0.188 ± 0.004**	0.188 ± 0.005**
Relative	6.23 ± 0.12	6.52 ± 0.16	6.76 ± 0.19*	6.84 ± 0.14**	7.27 ± 0.16**	7.20 ± 0.08**
Liver						
Absolute	1.157 ± 0.021	1.264 ± 0.050	1.355 ± 0.057**	1.367 ± 0.035**	1.456 ± 0.048**	1.600 ± 0.045**
Relative	41.93 ± 0.86	45.29 ± 1.25*	50.08 ± 1.31**	51.28 ± 0.93**	56.16 ± 0.91**	61.21 ± 0.96**
Lung						
Absolute	0.197 ± 0.005	0.199 ± 0.006	0.203 ± 0.008	0.202 ± 0.012	0.190 ± 0.003	0.188 ± 0.005
Relative	7.17 ± 0.25	7.17 ± 0.23	7.59 ± 0.41	7.58 ± 0.45	7.35 ± 0.15	7.21 ± 0.15
Spleen						
Absolute	0.084 ± 0.003	0.089 ± 0.004	0.095 ± 0.005	0.085 ± 0.002	0.085 ± 0.002	0.086 ± 0.002
Relative	3.05 ± 0.11	3.19 ± 0.15	3.53 ± 0.16*	3.19 ± 0.10	3.30 ± 0.09	3.31 ± 0.07
Thymus						
Absolute	0.047 ± 0.004	0.043 ± 0.002[b]	0.041 ± 0.001	0.042 ± 0.002	0.034 ± 0.001**	0.032 ± 0.002**
Relative	1.66 ± 0.09	1.55 ± 0.08[b]	1.53 ± 0.05	1.58 ± 0.07	1.31 ± 0.06**	1.22 ± 0.09**
Uterus						
Absolute	0.126 ± 0.011	0.144 ± 0.011	0.125 ± 0.014	0.129 ± 0.016	0.115 ± 0.006	0.143 ± 0.014
Relative	4.53 ± 0.38	5.21 ± 0.43	4.68 ± 0.56	4.94 ± 0.67	4.45 ± 0.20	5.52 ± 0.60

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

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg
n	10	9	10	8
Weights (g)				
Necropsy body wt	366 ± 5	351 ± 6[b]	332 ± 5**	318 ± 7**
L. cauda epididymis	0.1663 ± 0.0051	0.1733 ± 0.0037[b]	0.1662 ± 0.0065	0.1681 ± 0.0039
L. epididymis	0.4954 ± 0.0102	0.4923 ± 0.0133[b]	0.4712 ± 0.0121	0.4914 ± 0.0084
L. testis	1.5627 ± 0.0237	1.5335 ± 0.0211[b]	1.4890 ± 0.0510	1.5186 ± 0.0335
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.81 ± 0.21	9.21 ± 0.20	8.82 ± 0.22	9.70 ± 0.50
Spermatid heads (10^7/testis)	13.74 ± 0.29	14.09 ± 0.30	13.12 ± 0.48	14.66 ± 0.59
Spermatid count (mean/10^{-4} mL suspension)	68.70 ± 1.46	70.47 ± 1.49	65.60 ± 2.40	73.28 ± 2.94
Epididymal spermatozoal measurements				
Motility (%)	80.58 ± 0.94	73.90 ± 2.34*	70.63 ± 3.61**	68.06 ± 6.33*
Concentration (10^6/g cauda epididymal tissue)	443 ± 40	355 ± 42	426 ± 32	457 ± 32

* Significantly different (P 0.05) from the vehicle control group by Shirley's test
** Significantly different (P 0.01) from the vehicle control group by Williams' test (body weights) or by Shirley's test (motility)
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Data are presented as mean ± standard error. Differences from the vehicle control group for spermatid parameters and epididymal spermatozoal concentration are not significant by Dunn's test; differences from the vehicle control group for tissue weights are not significant by Dunnett's test.
[b] n = 10

TABLE D2
Estrous Cycle Characterization for Female Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	1 mg/kg	3 mg/kg	10 mg/kg
n	10	10	10	10
Necropsy body wt (g)	197 ± 3	191 ± 2	191 ± 3	185 ± 4**
Estrous cycle length (days)	4.90 ± 0.07	5.05 ± 0.05	4.95 ± 0.09	5.25 ± 0.11*
Estrous stages (% of cycle)				
Diestrus	41.7	40.8	38.3	38.3
Proestrus	15.8	19.2	19.2	20.0
Estrus	23.3	20.0	23.3	23.3
Metestrus	19.2	20.0	19.2	18.3

* Significantly different (P 0.05) from the vehicle control group by Dunn's test
** Significantly different (P 0.01) from the vehicle control group by Williams' test
Trend is significantly decreased (P 0.01) by Jonckheere's test.
[a] Weights and estrous cycle lengths are presented as mean ± standard error. By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	3 mg/kg	10 mg/kg	30 mg/kg
n	9	6	7	9
Weights (g)				
Necropsy body wt	37.2 ± 0.7	37.7 ± 1.2	35.3 ± 0.9	38.1 ± 1.0
L. cauda epididymis	0.0227 ± 0.0013	0.0215 ± 0.0013	0.0271 ± 0.0011*	0.0264 ± 0.0012*
L. epididymis	0.0561 ± 0.0023	0.0560 ± 0.0027	0.0590 ± 0.0017	0.0598 ± 0.0020
L. testis	0.1223 ± 0.0019	0.1217 ± 0.0024	0.1171 ± 0.0015	0.1174 ± 0.0019
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.10 ± 0.46	17.88 ± 0.44	18.60 ± 0.42	17.56 ± 0.64
Spermatid heads (10^7/testis)	2.21 ± 0.06	2.17 ± 0.03	2.18 ± 0.05	2.06 ± 0.06
Spermatid count (mean/10^{-4} mL suspension)	69.14 ± 1.89	67.83 ± 0.97	68.07 ± 1.62	64.25 ± 1.78
Epididymal spermatozoal measurements				
Motility (%)	81.00 ± 0.98	77.53 ± 3.93	81.30 ± 1.79[b]	82.46 ± 1.08[c]
Concentration (10^6/g cauda epididymal tissue)	548 ± 77	492 ± 78	410 ± 66[b]	316 ± 65[c]

* Significantly different (P 0.05) from the vehicle control group by Williams' test
Trend is significantly increased (P 0.01) by Jonckheere's test.

[a] Data are presented as mean ± standard error. Differences from the vehicle control group for are not significant by Dunnett's test (left epididymal and testis weights) or by Dunn's test (spermatid and epididymal spermatozoal measurements).

[b] n = 6

[c] n = 7

TABLE D4
Estrous Cycle Characterization for Female Mice in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	3 mg/kg	10 mg/kg	30 mg/kg
n	10	10	9	9
Necropsy body wt (g)	27.7 ± 0.8	26.7 ± 0.7	25.9 ± 0.6	26.1 ± 0.6
Estrous cycle length (days)	4.15 ± 0.11	4.20 ± 0.15	4.22 ± 0.17	4.39 ± 0.18
Estrous stages (% of cycle)				
Diestrus	34.2	22.5	26.9	29.6
Proestrus	16.7	15.8	15.7	11.1
Estrus	27.5	38.3	34.3	38.0
Metestrus	21.7	23.3	23.1	21.3

[a] Weights and estrous cycle lengths are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.

APPENDIX E
HEPATIC CELL PROLIFERATION RESULTS

TABLE E1
Hepatic Cell Proliferation Data for Male and Female Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene[a]

	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
n	5	5	5	5	5	5
Day 31						
Range	0.0 – 1.1	0.3 – 0.6	0.4 – 0.8	0.3 – 0.3[b]	0.0 – 0.7[c]	0.1 – 1.6
Mean ± standard deviation	0.4 ± 0.4	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.0[b]	0.2 ± 0.3[c]	0.9 ± 0.6
Fold increase over vehicle control		—[d]	1.5	—	—	2.3
n	5	5	5	5	5	0[e]
Day 87						
Range	0.3 – 0.7	0.0 – 0.3	0.0 – 0.8	0.1 – 0.6	0.1 – 0.7	
Mean ± standard deviation	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	
Fold increase over vehicle control		—	—	—	—	
Female						
n	5	5	5	5	5	5
Day 31						
Range	0.1 – 1.7	0.2 – 1.6	0.2 – 1.9	0.7 – 1.8	0.5 – 1.0	0.2 – 0.5
Mean ± standard deviation	0.9 ± 0.6	0.8 ± 0.6	0.9 ± 0.7	1.2 ± 0.4	0.7 ± 0.2	0.3 ± 0.1
Fold increase over vehicle control		—	—	1.3	—	—
n	5	5	5	5	5	0
Day 87						
Range	1.2 – 6.3	1.6 – 4.2	0.2 – 1.2[c]	0.3 – 1.7	0.8 – 3.8	
Mean ± standard deviation	2.8 ± 2.0	2.5 ± 1.1	0.6 ± 0.5[c]	0.8 ± 0.5	1.7 ± 1.2	
Fold increase over vehicle control		—	—	—	—	

[a] Data presented are the labeling index generated from BrdU labeling.
[b] n = 3
[c] n = 4
[d] No increase in cell proliferation observed.
[e] No data available due to 100% mortality.

APPENDIX F
HEPATIC CYTOCHROME P_{450} RESULTS

TABLE F1
Summary of Hepatic P_{450}1A Staining Presence and Intensity for Rats in the 13-Week Gavage Study of 3,3′,4,4′-Tetrachloroazoxybenzene

Parameter	Vehicle Control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male						
Number examined	10	10	10	10	10	0
Number with staining	0	0	5* (0.6)[a]	8** (1.0)	1 (0.1)	0
Female						
Number examined	10	10	10	10	10	3
Number with staining	3 (0.3)	4 (0.4)	6 (0.6)	9** (1.1)	10** (1.2)	3 (2.3)

* Significantly different (P 0.05) from the vehicle controls by the Fisher exact test
** P 0.01
[a] Intensity scale: 1 = minimal, 2 = mild, 3 = moderate

APPENDIX G
GENETIC TOXICOLOGY

TABLE G1
Mutagenicity of 3,3′,4,4′-Tetrachloroazoxybenzene in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]		
		-S9	+30% hamster S9	+30% rat S9
TA100	0	101 ± 5.3	93 ± 4.2	114 ± 11.7
	100	75 ± 6.1	97 ± 14.9[c]	123 ± 9.4[c]
	333	78 ± 3.3	83 ± 3.2[c]	106 ± 6.1[c]
	1,000	81 ± 2.9	92 ± 4.7[c]	112 ± 13.1[c]
	3,333	80 ± 3.0[c]	90 ± 2.5[c]	114 ± 5.7[c]
	10,000	80 ± 1.7[c]	85 ± 5.1[c]	107 ± 14.0[c]
Trial summary		Negative	Negative	Negative
Positive control[d]		456 ± 26.4	779 ± 4.5	520 ± 4.4
TA1535	0	14 ± 2.8	20 ± 6.5	18 ± 2.3
	100	12 ± 2.9	13 ± 2.7	22 ± 2.8
	333	11 ± 0.9	17 ± 4.2	19 ± 0.6
	1,000	10 ± 2.7	20 ± 4.5	18 ± 1.7
	3,333	9 ± 3.1[c]	19 ± 4.2[c]	16 ± 3.4[c]
	10,000	11 ± 3.8[c]	19 ± 1.2[c]	23 ± 3.3[c]
Trial summary		Negative	Negative	Negative
Positive control		538 ± 6.1	396 ± 45.1	51 ± 0.6
TA97	0	89 ± 4.7	150 ± 11.7	200 ± 3.4
	100	92 ± 8.5	159 ± 2.8	219 ± 14.5
	333	88 ± 3.2	160 ± 13.1	211 ± 23.6
	1,000	75 ± 2.6	148 ± 18.3	232 ± 9.1
	3,333	85 ± 5.0[c]	169 ± 13.7[c]	210 ± 4.2[c]
	10,000	73 ± 3.2[c]	162 ± 11.9[c]	213 ± 2.3[c]
Trial summary		Negative	Negative	Negative
Positive control		253 ± 11.3	815 ± 48.5	520 ± 31.0
TA98	0	31 ± 3.1	41 ± 4.7	31 ± 2.6
	100	34 ± 3.5	39 ± 2.5[c]	33 ± 3.2[c]
	333	24 ± 1.5	38 ± 2.8[c]	34 ± 4.5[c]
	1,000	30 ± 1.3	32 ± 6.7[c]	32 ± 1.5[c]
	3,333	35 ± 3.8	31 ± 3.0[c]	37 ± 2.3[c]
	10,000	34 ± 2.7	31 ± 2.3[c]	35 ± 0.7[c]
Trial summary		Negative	Negative	Negative
Positive control		153 ± 9.7	887 ± 3.9	224 ± 5.0

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented in Zeiger *et al*. (1987). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Precipitate on plate
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-animoanthracene.

TABLE G2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with 3,3′,4,4′-Tetrachloroazoxybenzene by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]
Corn oil[d]		5	0.3 ± 0.2	
Cyclophosphamide[e]	25	5	2.3 ± 0.7	
3,3′,4,4′-Tetrachloroazoxybenzene				
	50	5	0.7 ± 0.3	0.1635
	100	5	0.3 ± 0.1	0.500
	150	5	0.7 ± 0.4	0.1635
	200	5	1.1 ± 0.5	0.0488
			P = 0.058[f]	

[a] Study was performed at Integrated Laboratory Systems. The detailed protocol is presented in Shelby *et al*. (1993). PCE = polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison of treated group with solvent control micronuclei frequency; significant at P 0.006
[d] Solvent control
[e] Positive control
[f] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P 0.025 (ILS, 1990)

TABLE G3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with 3,3′,4,4′-Tetrachloroazoxybenzene by Gavage for 13 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]
Male				
Corn oil[d]		5	3.1 ± 0.3	
3,3′,4,4′-Tetrachloroazoxybenzene				
	0.1	5	4.2 ± 0.5	0.099
	1	5	4.2 ± 0.6	0.099
	3	5	4.7 ± 0.6	0.035
	10	5	6.4 ± 0.5	0.000
	30	5	5.0 ± 0.3	0.017
			P = 0.046[e]	
Female				
Corn oil		5	1.8 ± 0.3	
3,3′,4,4′-Tetrachloroazoxybenzene				
	0.1	5	2.2 ± 0.3	0.263
	1	5	2.4 ± 0.2	0.177
	3	5	3.2 ± 0.3	0.024
	10	5	3.9 ± 0.3	0.003
	30	5	4.0 ± 0.4	0.002
			P = 0.001	

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented in MacGregor *et al*. (1990). NCE = normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison of treated group with solvent control micronuclei frequency; significant at P 0.005.
[d] Solvent control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P 0.025 (ILS, 1990). Exclusion of the 30 mg/kg male mouse data results in a trend P value of 0.001.