

**NTP Technical Report  
on the Toxicity Studies of**

**3,3',4,4'-Tetrachloroazobenzene**

(CAS No. 14047-09-7)

**Administered by Gavage  
to F344/N Rats and B6C3F<sub>1</sub> Mice**

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Public Health Service  
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## FOREWORD

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## PEER REVIEW

The draft report on the toxicity studies of 3,3',4,4'-tetrachloroazobenzene 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.

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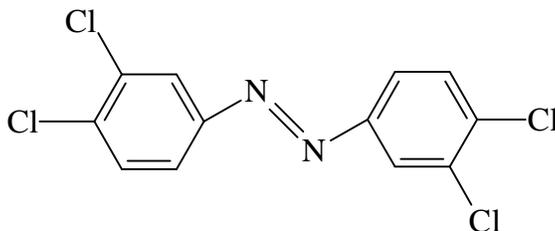
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# CONTENTS

<b>ABSTRACT</b> .....	5
<b>INTRODUCTION</b> .....	9
Chemical and Physical Properties .....	9
Production, Use, and Human Exposure .....	10
Absorption, Distribution, Metabolism, and Excretion .....	10
Toxicity .....	12
Reproductive and Developmental Toxicity .....	14
Carcinogenicity .....	14
Genetic Toxicity .....	15
Study Rationale and Design .....	15
<b>MATERIALS AND METHODS</b> .....	17
Procurement and Characterization of 3,3',4,4'-Tetrachloroazobenzene .....	17
Preparation and Analysis of Dose Formulations .....	17
16-Day Studies .....	18
13-Week Studies .....	18
Statistical Methods .....	24
Quality Assurance Methods .....	24
Genetic Toxicology .....	25
<b>RESULTS</b> .....	27
Rats .....	27
Mice .....	40
Genetic Toxicology .....	46
<b>DISCUSSION</b> .....	47
<b>REFERENCES</b> .....	61
<b>APPENDIXES</b>	
Appendix A Summary of Nonneoplastic Lesions .....	A-1
Appendix B Hematology and Clinical Chemistry Results .....	B-1
Appendix C Organ Weights and Organ-Weight-to-Body-Weight Ratios .....	C-1
Appendix D Reproductive Tissue Evaluations and Estrous Cycle Characterization .....	D-1
Appendix E Hepatic Cytochrome P <sub>450</sub> Results .....	E-1
Appendix F Genetic Toxicology .....	F-1

## ABSTRACT



### 3,3',4,4'-TETRACHLOROAZOBENZENE

CAS No. 14047-09-7

Chemical Formula:  $C_{12}H_6Cl_4N_2$       Molecular Weight: 320.0

**Synonyms:** Azobenzene, 3,3',4,4'-tetrachloro-(8Cl); diazene, bis(3,4-dichlorophenyl)-(9Cl); TCAB

3,3',4,4'-Tetrachloroazobenzene is not commercially manufactured but is formed as an unwanted byproduct in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives Propanil®, Linuron®, and Diuron®. In addition, environmental contamination by 3,3',4,4'-tetrachloroazobenzene occurs from the degradation of chloranilide herbicides and the photolysis and biolysis of 3,4-dichloroaniline. 3,3',4,4'-Tetrachloroazobenzene 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'-tetrachloroazobenzene. The toxicity of 3,3',4,4'-tetrachloroazobenzene was evaluated in 16-day and 13-week gavage studies in male and female F344/N rats and B6C3F<sub>1</sub> mice. In addition to histopathology, evaluations included hematology (rats only), clinical chemistry, thyroid hormone analyses (rats only), cytochrome P<sub>450</sub>1A immunohistochemical staining in the liver (rats only), and assessments of male reproductive endpoints and estrous cycle length. 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'-tetrachloroazobenzene in corn oil by gavage 5 days a week at doses of 0, 12.5, 32, 80, 200, or 500 mg per kg body weight. Groups of five male and five female mice received 3,3',4,4'-tetrachloroazobenzene in corn oil by gavage 5 days a week at doses of 0, 1, 3.2, 10, 32, or 100 mg/kg. Major effects included increases in liver, lung, and spleen weights of rats

and liver and heart weights of mice and decreases in thymus weights of rats and mice. No effects were found on survival or mean body weight gains of rats or mice. Incidences of hematopoietic cell proliferation in the spleen were increased in all groups of dosed male rats, in female rats that received 32 mg/kg or greater, and in 100 mg/kg male and female mice. Renal tubule hyaline droplet accumulation in the cytoplasm of renal cortical epithelial cells and chronic nephropathy were observed microscopically in male rats in the 80, 200, and 500 mg/kg groups. Female mice in the 100 mg/kg group had atrophy of the thymus.

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

In the 13-week rat study, the major effects included a decrease in the mean body weight gain of 30 mg/kg females and final mean body weights of 30 mg/kg males and females, decreased thymus weights of males and females in the 10 and 30 mg/kg groups accompanied by thymic atrophy observed microscopically, increased incidences of hematopoietic cell proliferation in the spleen in 10 and 30 mg/kg males and females, a responsive anemia in 10 and 30 mg/kg males and females at week 13, and decreased platelet counts in 10 and 30 mg/kg males and females on day 21 and at week 13. Spleen weights were increased in 10 and 30 mg/kg males and females. Liver weights were increased in males that received 1 mg/kg or greater and in 10 and 30 mg/kg females. Furthermore, hepatic cytochrome P<sub>450</sub>1A staining presence and intensity were increased in 30 mg/kg males and females. Sharp decreases in circulating thyroxine concentrations were observed in males and females at all doses. In spite of this sharp decrease, thyroid-stimulating hormone concentrations were marginally increased. Incidences of hyperplasia of the forestomach were increased in males administered 3 mg/kg or greater and females administered 30 mg/kg.

In the 13-week mouse study, the major effects included increases in liver and spleen weights of 10 and 30 mg/kg males and females and increased incidences of hyperplasia of the forestomach in males and females that received 1 mg/kg or greater. Furthermore, a decrease in thymus weight of 30 mg/kg males, an increase in centrilobular hypertrophy of hepatocytes in males that received 3 mg/kg or greater, and an increase in the incidences of hematopoietic cell proliferation in the spleen in males that received 3 mg/kg or greater were observed. A significant decrease in epididymal spermatozoal concentration was observed in 3 and 30 mg/kg males.

3,3',4,4'-Tetrachloroazobenzene was mutagenic in *S. typhimurium* strain TA97 in the presence of rat liver S9 activation enzymes; no mutagenic activity was detected in strain TA98, TA100, TA1535, or TA1537 with or without S9. *In vivo*, the frequency of micronucleated erythrocytes was significantly increased in peripheral blood samples from male and female mice given 3,3',4,4'-tetrachloroazobenzene by gavage for 13 weeks. However,

results of a 3-day exposure of up to 200 mg/kg by intraperitoneal injection did not demonstrate induction of micronuclei in bone marrow erythrocytes of male mice.

In summary, 3,3',4,4'-tetrachloroazobenzene caused typical dioxin-like effects, such as thymic atrophy, an increase in liver weights, induction of hepatic cytochrome P<sub>450</sub>1A, and decreased mean body weight gains. Furthermore, in the 13-week studies, a sharp decrease in circulating thyroxine concentrations was observed even at the lowest dose (0.1 mg/kg) tested in rats. Other effects included a decrease in epididymal spermatozoal concentration in mice, major effects on the hematopoietic system, and increased incidences of hyperplasia of the forestomach in 3 and 30 mg/kg males and 30 mg/kg females. A no-observable-adverse-effect-level (NOAEL) was not reached in rats. The NOAEL in mice was 0.1 mg/kg. Comparison of various dioxin-like effects in these studies with those reported in the literature indicate that 3,3',4,4'-tetrachloroazobenzene is six to two orders of magnitude less potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

