Final

Report on Carcinogens Background Document for

4,4'-Thiodianiline

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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of 4,4' thiodianiline. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Executive Summary

Introduction
4,4'-Thiodianiline (TDA) is used as an intermediate in the preparation of several diazo dyes. TDA was nominated for listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences because a two-year study conducted by the National Cancer Institute showed TDA to be carcinogenic in F344 male and female rats and $B6C3F_1$ male and female mice. The International Agency for Research on Cancer (IARC) has classified TDA as possibly carcinogenic to humans (Group 2B) (IARC 1987).

Human Exposure
Use. TDA has been used almost exclusively as a chemical intermediate in the production of three dyes: C.I. mordant yellow 16, milling red G, and milling red FR; only C.I. mordant yellow is believed to have any current commercial significance in the United States. C.I. mordant yellow 16 has been used to dye wool; for printing on wool, silk, and cotton; and as an indicator in the United States government's nerve gas detector program. However, because the government has been phasing out the use of C.I. mordant yellow in the nerve gas detector program, TDA probably is no longer used in the United States to produce C.I. mordant yellow. TDA was used in veterinary medicine as a fasciolicide (i.e., a treatment for liver flukes) but is no longer used for that purpose.

Production. TDA probably is prepared by reaction of aniline with sulfur. United States production of TDA was first reported for 1941 to 1943; however, TDA is no longer produced in the United States. Small amounts may still be produced in India. The United States Dye Manufacturers speculate that only a few hundred pounds of TDA are imported into the United States each year. Currently, there are three domestic suppliers of TDA. The United States International Trade Commission indicated that C.I. mordant yellow 16 was produced in the United States in 1980, 1990, and 1991. Separate statistics for this dye were not available; however, total mordant dye production was 410,000 lb (186,000 kg) in 1980 and 19,841 lb (9,000 kg) in 1990.

Occupational exposure. Dye workers may be exposed to TDA through dermal, eye, oral, or inhalation exposure. However, no information was found regarding quantitation or documentation of such exposures.

Human Cancer Studies
No studies have been reported on the relationship between human cancer and exposure to TDA.

Studies in Experimental Animals
IARC (1982) concluded that there was sufficient evidence for carcinogenicity of TDA in experimental animals. Dietary administration of TDA increased the incidences of thyroid follicular-cell and hepatocellular tumors in both male and female $B6C3F_1$ mice. Many of the tumors were malignant, and some had metastasized to one or more distal locations. In validation studies for a rapid carcinogenicity testing system, dietary administration of

TDA for 24 weeks induced thyroid follicular hyperplasia and adenoma in both transgenic and nontransgenic mice within 26 weeks. In transgenic mice, the incidence of lung adenoma was significantly increased, and the incidences of several malignant tumors (e.g., lung adenocarcinoma, spleen hemangiosarcoma, and hepatocellular carcinoma) were increased, though not significantly. Dietary administration of TDA to F344 rats for up to 72 weeks significantly increased the incidences of thyroid, liver, and ear-canal (Zymbal gland) tumors in males and the incidences of thyroid and uterine tumors in females. In addition, colon tumors in male rats and ear-canal tumors in female rats were attributed to TDA exposure; however, incidences were not significantly increased. There was some evidence that gavage administration of TDA to young female Sprague-Dawley rats induced mammary tumors. Other studies provided evidence of synergistic effects when TDA was coadministered with other carcinogens; however, the relative role of TDA could not be determined.

Genotoxicity

TDA induced reverse mutation in *Salmonella typhimurium* strains TA98 and TA100 with or without metabolic activation but was not mutagenic in strains TA1535 or TA1537. TDA was mutagenic in strain TA97, but only with metabolic activation. Orally administered TDA caused DNA damage in the brain, liver, urinary bladder, and lungs of mice.

Other Relevant Data

Little is known about the absorption, distribution, metabolism, or excretion of TDA. Data on the hemoglobin binding index of TDA correlate with carcinogenic potency and demonstrate that TDA undergoes acetylation. Two groups have used structure-activity analysis to suggest that the aryl-amino group of TDA is most likely involved in carcinogenicity, although the C"-S-C= fragment also has been proposed. TDA significantly increased the incidences of tumors in a variety of tissues in rats and mice, including liver, thyroid, ear canal (Zymbal gland), and uterus. Aniline and dapsone, on the other hand, caused tumors of the spleen. Some similarity in the organ-specific DNA damage induced by TDA and aniline in the comet assay was reported; however, no genotoxic effects of dapsone have been reported. Three other dianilines (4,4'-oxydianiline, 4,4'-methylenedianiline, and 4,4'-methylenebis[2-chloroaniline]) currently are listed in the Report on Carcinogens. These dianilines have been reported to induce tumors in organs and tissues in which TDA induces tumors, and at lower doses than TDA.

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1 Introduction

4,4'-Thiodianiline (TDA) has been produced commercially since the early 1940s as an intermediate in the preparation of several diazo dyes. Human exposure may occur by inhalation and by skin absorption during production of these dyes. TDA was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences because a two-year study conducted by the National Cancer Institute (NCI) showed TDA to be carcinogenic in F344 male rats (tumors of the liver, thyroid, colon, and Zymbal gland of the ear canal), female rats (tumors of the thyroid, uterus, and Zymbal gland of the ear canal), and B6C3F$_1$ male and female mice (liver and thyroid tumors) (NCI 1978). In addition, TDA is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B) (IARC 1987).

1.1 Chemical identification

TDA ($C_{12}H_{12}N_2S$, mol wt 216.30, CASRN 139-65-1) occurs as brown to brown-violet powder or needles. It also is known as *p*,*p*′-thiodianiline, 4,4'-thiobisbenzenamine, *p*,*p*′-diaminodiphenyl sulfide, 4,4'-diaminodiphenyl sulfide, bis(*p*-aminophenyl)sulfide, bis(4-aminophenyl)sulfide thioaniline, and thiodi-*p*-phenylenediamine. Its RTECS number is BY9625000 (ChemFinder 2001). The structure of TDA is illustrated in Figure 1-1.

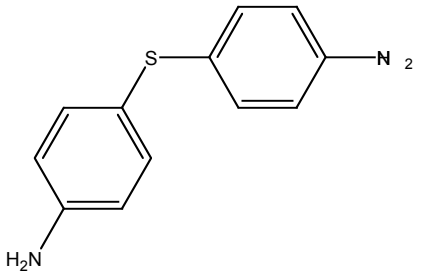

Figure 1-1. Structure of TDA

1.2 Physical-chemical properties

TDA is stable under normal laboratory conditions. Solutions in 95% ethanol are stable for 24 hours (NTP 2001). TDA should be stored in a tightly closed container in a cool, dry, well-ventilated area away from oxidizing agents. It is slightly soluble in water. TDA is noncombustible but, when heated, may decompose to form irritating and toxic fumes. Hazardous decomposition products include nitrogen oxides, carbon monoxide, carbon dioxide, nitrogen, and sulfur oxides (Fisher Scientific 2000). The physical and chemical properties of TDA are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of TDA

Property	Information	Reference
Molecular weight	216.30	ChemFinder 2001, Fisher Scientific 2000
Color	brown or brown-violet	ChemFinder 2001, Fisher Scientific 2000
Physical state	powder or needles	Fisher Scientific 2000
Melting point (^{o}C)	108–111	ChemFinder 2001, Fisher Scientific 2000
Solubility:		
water	slightly soluble	Lide 1999
ethanol	very soluble	Lide 1999
ether	very soluble	Lide 1999
benzene	very soluble	Lide 1999

1.3 Identification of metabolites and derivatives

No information was found regarding the metabolism of TDA.

A commercial derivative of TDA is the dye C.I. mordant yellow 16, the chemical structure of which is shown in Figure 1-2. It is soluble in water and almost insoluble in ethanol (SDC 1982). No information on other physical-chemical properties of this dye was found.

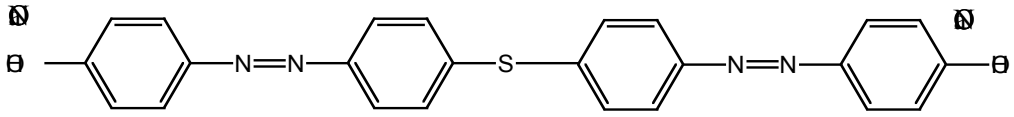

Figure 1-2. Structure of C.I. mordant yellow 16

1.4 Identification of analogues

TDA may be considered a derivative of aniline (CASRN 62-53-3; structure shown in Figure 1-3); however, no metabolic pathway linking TDA to release of aniline was found in a search of the published literature. An analogue of TDA is 4,4'-sulfonyldianiline (dapsone, CASRN 80-08-0; structure shown in Figure 1-4), a drug used for the treatment of leprosy. As in the case of aniline, no evidence for metabolic conversion of TDA to dapsone or vice versa could be found. The Report on Carcinogens lists three additional dianilines: 4,4'-oxydianiline, 4,4'-methylene dianiline and its dihydrochloride, and methylenebis(2-chloroaniline) (structures shown in Figures 1-5 through 1-7). The tumorigenicity and genotoxicity of TDA, aniline, dapsone, 4,4'-oxydianiline, 4,4'-methylene dianiline, and 4,4'-methylenebis(2-chloroaniline) are discussed in Section 6.4 and summarized in Table 6-1.

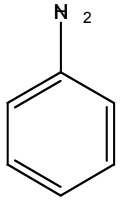

Figure 1-3. Structure of aniline

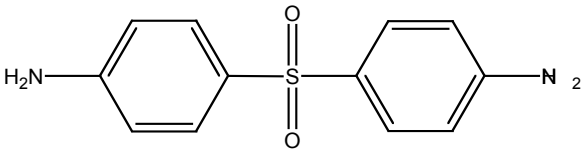

Figure 1-4. Structure of dapsone

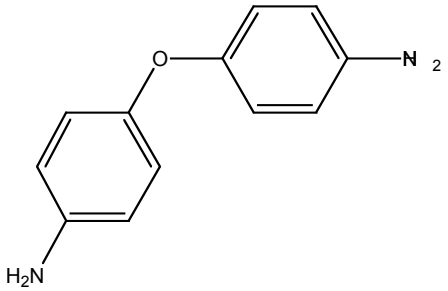

Figure 1-5. Structure of 4,4'-oxydianiline

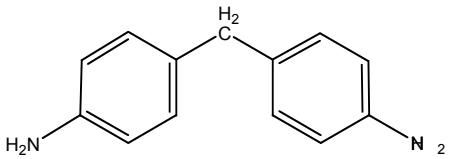

Figure 1-6a. Structure of 4,4'-methylenedianiline

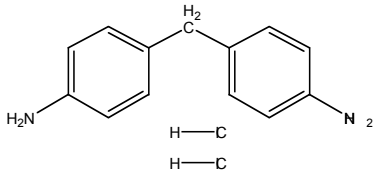

Figure 1-6b. Structure of 4,4'-methylenedianiline dihydrochloride

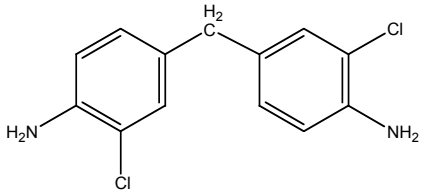

Figure 1-7. Structure of 4,4'-methylenebis(2-chloroaniline)

2 Human Exposure

2.1 Use

TDA has been used almost exclusively as a chemical intermediate in the production of three dyes: C.I. mordant yellow 16, milling red G, and milling red FR. Only C.I. mordant yellow 16 is believed to have any current commercial significance in the United States. C.I. mordant yellow 16 (see structure in Figure 1-2) is produced by reaction of TDA with salicylic acid. It is used to dye wool and for printing on wool, silk, and cotton (SDC 1971). C.I. mordant yellow also has been used as an indicator in the U.S. government's nerve gas detector program. However, because the government has been phasing out this use of C.I. mordant yellow, TDA is probably no longer used in the United States to produce C.I. mordant yellow (personal communication, U.S. Dye Manufacturers Operating Committee of the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers, 2002). TDA was at one time used in veterinary medicine as a fasciolicide (i.e., a treatment for liver flukes) (HSDB 2000), but no current use for this purpose was identified.

2.2 Production

TDA was first prepared by Merz and Weith in 1871 by boiling of sulfur with aniline for several days (Prager *et al.* 1930, as cited in IARC 1982). No information is available on commercial methods of production, though it probably involves reaction of aniline with sulfur (IARC 1982, HSDB 2000).

U.S. production of TDA was first reported for 1941 to 1943 (IARC 1982). TDA is no longer produced in the United States. Small amounts may still be produced in India. It appears that TDA is not produced in large enough quantities to be listed in some sources. For example, SRI's Directory of Chemical Producers lists only chemicals that are produced in commercial quantities of at least 5,000 lb or $10,000 in value annually. TDA was listed in the Directory of Chemical Producers in 1983 and 1984, indicating that it was produced in those years, but has not been listed in this directory since 1985 (SRI 1983, 1984, 2001).

The Colour Index indicates that no dye except C.I. mordant yellow 16 is produced from TDA. The U.S. Dye Manufacturers speculate that only a few hundred pounds of TDA are imported into the United States each year (personal communication, U.S. Dye Manufacturers Operating Committee of the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers, 2002). Currently, there are three domestic suppliers of TDA (ChemFinder 2001).

The U.S. International Trade Commission (USITC) indicated that C.I. mordant yellow 16 was produced in the United States in 1980, 1990, and 1991. Separate statistics for this dye were not available; however, total mordant dye production was 410,000 lb (186,000 kg) in 1980 and 19,841 lb (9,000 kg) in 1990. No separate mordant dye production values were available for 1991 (USITC 1990, 1991, 1993). Production of C.I. mordant yellow 16 was not reported for the other years in which the USITC reported synthetic organic chemical production (USITC 1987, 1988, 1990, 1994a, 1994b, 1995).

2.3 Analysis

Thin-layer chromatography (TLC) with four different solvents has been used to separate and identify the components of a group of diamines, including TDA. Paper chromatography and gas chromatography also have been used to separate TDA from other aromatic diamines (IARC 1982).

TDA sulfide, a metabolite of TDA sulfoxide, was identified in human urine by use of TLC (HSDB 2000). TDA bound to hemoglobin was detected by high-performance liquid chromatography with electrochemical detection (Sabbioni and Schütze 1998).

2.4 Environmental occurrence

TDA does not occur naturally in the environment (IARC 1982). No data on its environmental occurrence were found.

2.5 Environmental fate

No information was found regarding TDA's environmental fate or potential persistence in the environment after release.

2.6 Environmental exposure

No information was found regarding environmental exposure to TDA.

2.7 Occupational exposure

Dye workers may be exposed to TDA through dermal, eye, oral, or inhalation exposure (HSDB 2000). However, no information was found regarding quantitation or documentation of such exposures.

2.8 Biological indices of exposure

TDA, as either the diamine or the monoacetyl-diamine (*N*-acetylamine), was found to bind to hemoglobin in Wistar rats. Cleavage products released from the TDA-hemoglobin adducts (i.e., the diamine and *N*-acetylamine) were measured to determine the amount of TDA bound to hemoglobin. The hemoglobin binding index (HBI) reflects the relative binding affinity of chemicals for hemoglobin and is determined by the following formula: compound bound (millimoles per gram of hemoglobin)/dose (millimoles per kilogram of body weight) (Sabbioni and Schütze 1998). The HBI for TDA is 8.2 ± 1.3 (mean ± standard deviation) for the diamine and 7.4 ± 0.6 for the *N*-acetylamine, for a total of 15.6 ± 1.9 [± 1.4 as calculated by the RoC Review Committee]. The data presented were insufficient to provide a basis for assessing exposure to TDA. No additional information was found regarding TDA binding with human hemoglobin.

2.9 Regulations

TDA is regulated by the U.S. Environmental Protection Agency (EPA) under the Emergency Planning and Community Right-to-Know Act (also referred to as the Toxics Release Inventory). Table 2-1 lists the EPA regulation.

Table 2-1. EPA regulations

Regulatory action	Effect of regulation or other comments
40 CFR 372 – PART 372 – TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Codes: 42 U.S.C. 11013, 11028. The *de minimis* concentration for TDA is 0.1%.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of the Superfund Amendments Reauthorization Act (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and to aid in the development of regulations, guidelines, and standards.

Source: The regulations in this table have been updated through the 2001 Code of Federal Regulations 40 CFR, 1 July 2001.

3 Human Cancer Studies

No studies have been reported on the relationship between human cancer and exposure to TDA.

4 Studies of Cancer in Experimental Animals

TDA was tested for carcinogenicity in mice and rats in a few studies published between 1968 and 1991. IARC (1982) reviewed three studies (Griswold *et al.* 1968, NCI 1978, Cueto and Chu 1979) that investigated the carcinogenicity of TDA in experimental animals. Based on these studies, IARC (1982) concluded that there was sufficient evidence for carcinogenicity of TDA in experimental animals. The NCI (1978) report includes a list of other chemicals that were tested concurrently with TDA; animals exposed to these other chemicals were housed in the same room as the animals used in the TDA studies. A complete list of these chemicals is included in Appendix B, pages B-11 through B-13. This section summarizes the results from the carcinogenicity studies included in the IARC monograph and several more recent studies. The IARC (1982) and NCI (1978) reports are provided as Appendices A and B, respectively.

4.1 Studies with mice

TDA was tested in transgenic mice (Yamamoto *et al.* 1998a, b), male Swiss mice (NCI 1978), and male and female B6C3F_1 mice (NCI 1978) in studies lasting from 90 days to about 18 months.

4.1.1 Subchronic exposure studies

4.1.1.1 Transgenic mouse model

TDA, along with 34 other chemicals, was studied in a transgenic mouse model, and the experimental results were compared and contrasted with results obtained in standard long-term carcinogenicity studies. Although details of the specific methods and results for TDA were not published separately, the results for TDA were summarized in two review papers (Yamamoto *et al.* 1998a, b).

Transgenic male C57BL/6J mice were crossed with normal female BALB/cByJ mice, and the F_1 offspring were screened for the presence of the human prototype c-Ha-*ras* gene. The transgenic mice (also known as the *ras*H2 mouse) and their nontransgenic littermates were fed a diet containing TDA at a concentration of 2,000 or 4,000 ppm for 24 weeks, and the study was terminated by sacrifice after week 26 (Yamamoto *et al.* 1998a, b). At both dose levels, incidences of thyroid follicular-cell hyperplasia and adenoma were significantly greater in both transgenic and nontransgenic mice than in control mice fed a TDA-free diet. The incidence of lung adenoma also was significantly increased in female transgenic mice fed TDA at 2,000 ppm but was lower in the 4,000-ppm group, indicating that TDA was toxic at the higher dose level (i.e., the high-dose mice died before tumors formed). In transgenic mice, the incidences of lung adenocarcinoma, spleen hemangiosarcoma, forestomach papilloma, altered liver foci, and hepatocellular carcinoma also were greater in mice fed TDA than in the control group, but the differences were not statistically significant (Fisher's exact test). Few or none of the malignant tumors occurred in nontransgenic mice.

4.1.1.2 NCI subchronic toxicity study

The dose levels used in the NCI carcinogenicity study with $B6C3F_1$ mice (described in Section 4.1.2) were based on the results of a subchronic toxicity study with male Swiss mice (NCI 1978). Five animals per group were fed diets containing TDA at a concentration of 2,000, 5,000, 10,000, 25,000, or 50,000 ppm for 45 days and observed for an additional 45 days. The control group contained 20 animals. Two animals in the 10,000-ppm group and all animals in the 25,000- and 50,000-ppm groups died before or during the third week of the study. Body weight gain in all TDA-exposed groups was less than that of controls at 45 days; however, at 90 days, body weight gain in the 2,000- and 10,000-ppm groups was comparable to that of controls. Body weight gain in the 5,000-ppm group was 46% and 65% of the control value after 45 and 90 days, respectively. No gross abnormalities were found at necropsy. Detailed pathology data are not provided in the NCI report.

4.1.2 NCI 18-month carcinogenicity study

Groups of $B6C3F_1$ mice (35 of each sex) were fed a diet containing either 2,500 or 5,000 ppm TDA (99% pure) for 77 to 79 weeks (NCI 1978, Cueto and Chu 1979). The study duration differed slightly between the exposure groups because of differences in survival. Animals received the test diet five days/week and a TDA-free diet two days/week. The control group (14 mice of each sex) received a TDA-free diet. Animals were observed twice daily for signs of toxicity and were weighed every two weeks. Moribund animals were sacrificed and necropsied, and surviving animals (all of which were in the control group) were sacrificed at 91 weeks. All major organs and tissues and gross lesions (approximately 30 tissues and organs) were examined microscopically. A few tissues from some animals were not examined, because these animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Details of the pathology findings are provided in Appendix B.

All high-dose animals died by week 77, and all low-dose animals died by week 79 (see Appendix B, p. B-40, Figure 4 in NCI 1978). The Tarone test for positive dose-related trend in mortality was significant ($P < 0.001$). Body weight was significantly reduced in both sexes; however, the high and low doses caused about the same reduction in body weight (see Appendix B, p. B-39, Figure 3 in NCI 1978).

This study demonstrated the carcinogenic potential of TDA in male and female mice (Table 4-1). The liver and thyroid gland were the primary target tissues in both sexes. The time to the first observed liver tumor in mice exposed to TDA at 5,000 ppm was 40 weeks in female mice and 50 to 54 weeks in male mice. At the low dose (2,500 ppm), liver tumors appeared at 54 weeks in both sexes. Spontaneous liver tumors occurred at 88 weeks in male controls. The first thyroid tumors appeared at 40 weeks in high-dose females, 54 weeks in high-dose males, 59 weeks in low-dose females, and 63 weeks in low-dose males.

The incidence of hepatocellular carcinoma was significantly increased in both sexes at both dose levels. These tumors were metastatic to the lungs and kidney. The incidence of

thyroid follicular-cell carcinoma was significantly increased at both dose levels in males, but only at the high dose in females. These tumors were metastatic to the lungs. In addition, two unspecified thyroid adenomas occurred in the high-dose females. The incidences of total tumors of the thyroid gland (follicular-cell adenoma or carcinoma combined) and liver (hepatocellular adenoma or carcinoma combined) were significantly increased in both sexes at both dose levels. The NCI (1978) concluded that TDA was carcinogenic in $B6C3F_1$ mice.

Table 4-1. Tumor incidence in $B6C3F_1$ mice following dietary exposure to TDA for up to 79 weeks

Sex	Exposure group (ppm)	Tumor incidence (no. with tumors/no. examined)					
		Thyroid[a]			Liver[b]		
		FCA	FCC	FCA/C	HA	HC	HA/C
Male	0	0/14	0/14	0/14	3/13	1/13	4/13
	2,500	8/33*[d]	15/33***	22/33***	1/34	32/34***	33/34***
	5,000	0/23	20/23***	20/23***	1/24	22/24***	23/24***
	Trend[c]	NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$
Female	0	0/11	0/11	0/11	0/12	0/12	0/12
	2,500	9/33[e]	3/33	11/33*	0/34	32/34***	32/34***
	5,000	5/30	15/30**	18/30***	2/31	30/31***	30/31***
	Trend[c]	NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$

Sources: NCI 1978, Cueto and Chu 1979.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ (Fisher's exact test); NS = not significant.

[a]FCA, follicular-cell adenoma; FCC, follicular-cell carcinoma; FCA/C, follicular-cell adenoma or carcinoma combined.

[b]HA, hepatocellular adenoma; HC hepatocellular carcinoma; HA/C, hepatocellular adenoma or carcinoma combined.

[c]The Cochran-Armitage test for linear trend.

[d][$P = 0.044$, calculated by the RoC Review Committee using Fisher's exact test.]

[e][$P = 0.054$, calculated by the RoC Review Committee using Fisher's exact test.]

In addition to the neoplastic lesions, several degenerative, proliferative, and inflammatory changes occurred in mice of the TDA-exposed and control groups. Although most of these lesions are typical for aged mice, follicular-cell hyperplasia of the thyroid was observed only in the TDA-exposed mice and was believed to be exposure-related. This lesion occurred in 29 of 33 males and 31 of 33 females in the low-dose groups and in 4 of 23 males and 26 of 30 females in the high-dose groups.

4.2 Studies with rats

TDA was tested in male and female Sprague-Dawley rats (Griswold *et al.* 1968, NCI 1978) and in male and female F344 rats (NCI 1978, Cueto and Chu 1979) in studies lasting from 90 days to about two years.

4.2.1 NCI subchronic toxicity study

The dose levels used in the NCI (1978) carcinogenicity study with F344 rats (described in Section 4.2.2) were based on the results of a subchronic toxicity study with male Sprague-Dawley rats (NCI 1978). Five rats per group were fed diets containing TDA at a concentration of 1,200, 3,000, 6,000, 15,000, or 30,000 ppm for 45 days and observed for an additional 45 days. The control group contained 20 animals. All rats in the 30,000-ppm group died during week 3 of the study, and one animal in the 3,000-ppm group died during week 2. After 45 days, mean body weight gains in rats fed TDA at 1,200, 3,000, or 6,000 ppm were only 56%, 27%, and 10%, respectively, of the control value. Body weight remained depressed in all TDA-fed groups at 90 days; however, no gross abnormalities were found at necropsy. Detailed pathology data are not provided in the NCI (1978) report.

4.2.2 NCI two-year carcinogenicity study

Groups of F344 rats (35 of each sex) were fed a diet containing 1,500 or 3,000 ppm TDA (99% pure) for 68 to 72 weeks, depending on survival time (NCI 1978, Cueto and Chu 1979). Animals received the test diet five days/week and a TDA-free diet two days/week. The control groups (15 rats of each sex) received a TDA-free diet and were observed for 104 weeks. Animals were weighed every two weeks for the entire study and were observed twice daily for signs of toxicity. Moribund animals were sacrificed and necropsied. All major organs and tissues and all gross lesions (approximately 30 tissues and organs) were examined microscopically (see Appendix B for details of the pathology findings).

A significant ($P \leq 0.001$, Tarone test) dose-related trend in mortality was observed. All animals in the control groups survived as long as week 52, and 6 males (40%) and 5 females (33%) survived until the end of the study (104 weeks). Among TDA-exposed males, 23 (66%) in the low-dose group and 18 (51%) in the high-dose group survived to week 52. Survival of females was a little higher, with 32 (91%) in the low-dose group and 21 (60%) in the high-dose group surviving to week 52. However, all high-dose rats died by week 69, and all low-dose rats died by week 72 (see Appendix B, p. B-26, Figure 2 in NCI 1978). Throughout the study, body weight was significantly lower in all exposed groups than in the control group (see Appendix B, p. B-25, Figure 1 in NCI 1978).

TDA was carcinogenic in both male and female F344 rats. All TDA-exposed rats except one had tumors at one or more sites, including the ear canal, lung, liver, and thyroid gland. Additionally, skin and colon tumors occurred in males, and uterine tumors in females. Table 4-2 shows tumor incidences that were signficantly increased in at least one sex, and Table 4-3 shows the incidences of other tumors observed. The times to the first observed tumors in TDA-exposed rats were 25 weeks for ear-canal tumors in males, 32 weeks for thyroid tumors in males, 44 weeks for liver and colon tumors in males and thyroid and uterine tumors in females, 46 weeks for ear-canal tumors in females, and 61 weeks for liver tumors in females. Liver and colon tumors appeared earlier in the low-dose groups than in the high-dose groups, as did ear-canal tumors in females. Skin tumors

occurred only in low-dose males and were observed at 48 weeks. Lung tumors were observed at 50 weeks in low-dose males and 63 weeks in low-dose females.

The incidences of liver, thyroid, and ear-canal tumors were significantly increased in male rats, and the incidences of thyroid and uterine tumors were significantly increased in female rats. Although the increased incidences of colon tumors in males and ear-canal tumors in females were not statistically significant, they were considered to be related to TDA administration because they were not observed in the concurrent controls or in 235 historical control animals. For similar reasons, the increased incidence of skin tumors in male rats may have been associated with TDA exposure (tumors were not observed in concurrent controls and were observed in only 1 of 235 historical controls). [The RoC Review Committee noted that tumor comparisons were not adjusted for survival and that the number of control animals was small.]

All tumors were epithelial in origin, and most of them were malignant. These included squamous-cell papilloma and carcinoma of the skin; squamous-cell papilloma and carcinoma of the external ear canal and adjacent subcutaneous tissues; squamous-cell carcinoma, alveolar-cell carcinoma, and bronchiolar adenoma of the lungs; hepatocellular adenoma and carcinoma; adenocarcinoma of the colon; follicular-cell adenoma and carcinoma of the thyroid; and adenocarcinoma of the uterus. Many of these tumors had invaded surrounding tissue or metastasized to the lungs, lymph nodes, liver, or spleen (NCI 1978). The ear-canal tumors currently are classified as Zymbal gland tumors (Copeland-Haines and Eustis 1990).

In addition to the neoplastic lesions, a number of chemically induced degenerative, proliferative, and inflammatory lesions were observed. These included lesions in the lung (epidermal inclusion cyst formation, alveolar-cell hyperplasia, and alveolar and bronchiolar squamous metaplasia), liver (hepatocellular nodular hyperplasia and bile duct hyperplasia), and thyroid gland (follicular-cell hyperplasia). These lesions were not observed in any of the control animals. Epidermal inclusion cysts, alveolar-cell hyperplasia, and alveolar and bronchiolar squamous metaplasia were observed, respectively, in 5, 15, and 12 of 33 low-dose males and 2, 13, and 4 of 32 low-dose females. Thyroid follicular-cell hyperplasia occurred in 1 of 33 low-dose males and 7 of 33 low-dose females. Thyroid and lung lesions were not observed in the high-dose groups. Liver nodular hyperplasia occurred in 4 of 33 low-dose and 10 of 33 high-dose males and 1 of 32 low-dose and 9 of 33 high-dose females. Bile-duct hyperplasia occurred in 8 of 33 low-dose and 25 of 33 high-dose males and in 6 of 33 low-dose and 12 of 33 high-dose females.

Table 4-2. Significantly increased tumor incidences in F344 rats (at least one sex) following dietary exposure to TDA for up to 72 weeks

Sex	Exposure group (ppm)	Tumor incidence (no. with tumors/no. examined)									
		Thyroid[a]			Liver[b]			Ear canal[c]			Uterus[d]
		FCA	FCC	FCA/C	HA	HC	HA/C	SCP	SCC	SCP/C	AC
Male	0	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	NAP
	1,500	2/33	28/33***	30/33***	2/33	21/33***	23/33***	10/33*[f]	5/33	15/33***	
	3,000	0/33	32/33***	32/33***	2/33	10/33*	12/33**	2/33	6/33	8/33*	
	Trend[e]	NS	$P < 0.001$	$P < 0.001$	NS	NS	NS	NS	NS	NS	
Female	0	0/14	0/14	0/14	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	2,500	0/33	24/33***	24/33***	1/32	5/32	6/32	5/33	1/33	6/33	31/33***
	5,000	0/32	32/32***	32/32***	2/33	1/33	3/33	3/33	0/33	3/33	23/32***
	Trend[e]	NS	$P < 0.001$	$P < 0.001$	NS	NS	NS	NS	NS	NS	$P < 0.001$

Source: NCI 1978, Cueto and Chu 1979.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ (Fisher's exact test); NS = not significant.

[a]FCA, follicular-cell adenoma; FCC, follicular-cell carcinoma; FCA/C, follicular-cell adenoma or carcinoma.

[b]HA, hepatocellular adenoma; HC, hepatocellular carcinoma; HA/C, hepatocellular adenoma or carcinoma.

[c]SCP, squamous-cell papilloma; SCC, squamous-cell carcinoma; SCP/C, squamous-cell papilloma or carcinoma.

[d]AC, adenocarcinoma; NAP, not applicable.

[e]The Cochran-Armitage test for linear trend.

[f][$P \leq 0.05$, calculated by the RoC Review Committee using Fisher's exact test.]

Table 4-3. Other tumors observed in F344 rats following dietary exposure to TDA for up to 72 weeks

Sex	Exposure conc. (ppm)	Tumor incidence (no. with tumors/no. examined)						
		Skin[a]		Lung[b]				Colon[c]
		SCP	SCC	SCC	ABA	ABC	ASC	AC
Male	0	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	1,500	4/33	1/33	4/33	1/33	3/33	0/33	6/32
	3,000	0/33	0/33	0/33	0/33	0/33	0/33	1/33
Female	0	0/15	0/15	0/15	0/15	0/15	0/15	0/14
	1,500	0/33	0/33	0/32	0/32	2/32	1/32	0/33
	3,000	0/33	0/33	0/32	0/32	0/32	0/32	0/32

Source: NCI 1978, Cueto and Chu 1979.

[a] SCP, squamous-cell papilloma; SCC, squamous-cell carcinoma.

[b]SCC, squamous-cell carcinoma; ABA, alveolar/bronchiolar adenoma; ABC, alveolar/bronchiolar carcinoma; ASC, adenosquamous carcinoma.

[c]AC, adenocarcinoma, not otherwise specified.

Griswold *et al.* (1968) tested 35 aromatic and heterocyclic nitro or amino derivatives, including TDA, for carcinogenic effects on the mammary gland in female Sprague-Dawley rats. TDA dissolved in sesame oil (usually 1 mL/dose) was administered by gavage every three days for 30 days. Twenty 40-day-old rats were given a total of 400 mg of TDA per animal in 10 equal doses. Because of excessive early mortality in this group (40% died in the first 45 days), a second group of 10 animals was added and given a total of 300 mg of TDA. Eight rats in this group survived until the end or nearly the end of the study. The vehicle control group (140 animals) and positive control group (40 animals) were administered sesame oil and 7,12-dimethylbenz[*a*]anthracene, respectively.

Animals were weighed and examined weekly throughout the nine-month observation period. The surviving animals, 12 in the 400-ppm group and 8 in the 300-ppm group, were necropsied. Mammary glands, intestinal tract, pituitary, liver, ovaries, adrenals, and all grossly observed lesions were fixed and prepared for histologic examination. Mammary-gland carcinoma was observed in 3 of 12 rats (25%) in the 400-ppm group and in 1 of 8 (13%) in the 300-ppm group. Not all animals in the control groups were necropsied, presumably because of early deaths. In the 132 vehicle-control rats that were necropsied, 3 mammary-gland carcinomas (all in one rat) and 1 mammary fibroadenoma were observed. Mammary-gland lesions, including hyperplasia, fibroadenoma, and carcinoma, were observed in all of the 29 positive-control rats that were necropsied. The authors concluded that TDA was a relatively weak carcinogen in this test system.

4.3 Administration with other carcinogens

Takayama *et al.* (1989) fed 30 male F344/DuCrj rats a diet containing a mixture of 40 experimental carcinogenic chemicals, including TDA, for 102 weeks. The concentration of each chemical in the diet was 1/50 the dose associated with a 50% tumor incidence (TD_{50}) in earlier carcinogenicity studies. Neoplastic nodules of the liver occurred in 17 of 29 rats in the exposed group (59%, $P < 0.01$), compared with 1 of 30 in the control group, and thyroid gland follicular-cell tumors occurred in 5 of 29 rats in the exposed group (17%, $P < 0.05$), compared with 0 of 29 rats in the control group. The role of TDA in these carcinogenic responses could not be determined.

Hasegawa *et al.* (1991) investigated the possibility of synergistic effects of three thyroid carcinogens in male F344 rats. TDA, 2,4-diaminoanisole sulfate (DAAS), and *N,N'*-diethylthiourea (DETU) were mixed in the diet at one-third of their reported TD_{50} levels (46 ppm for TDA, 200 ppm for DAAS, and 610 ppm for DETU). Groups of 20 or 21 rats were fed a diet containing all three chemicals (group 1), each chemical separately (groups 2, 3, and 4), or the control diet (group 5) for up to 52 weeks. Four rats in group 1 and one rat in group 3 died before the end of the experiment.

Final mean body weight was significantly lower in rats fed all three chemicals (371 ± 24 g) or TDA alone (390 ± 22 g) than in the control group (462 ± 21 g). Tumor incidences are summarized in Table 4-4. Thyroid follicular-cell carcinoma occurred in 18 (100%) of the rats fed all three chemicals but in only 2 (10%) of the animals fed TDA alone and in no control animals. Most of the thyroid tumors in group 1 were large and had invaded surrounding tissue. Lung metastasis was observed in 3 rats in group 1, but not in any of the other groups. Incidences of liver and lung tumors also were significantly increased in group 1. Of rats fed TDA alone, 3 (15%) had hepatocellular carcinoma, but this increased incidence was not statistically significant. All animals fed all three chemicals or TDA alone had lung hyperplasia. Neither lung or liver tumors nor pulmonary hyperplasia were observed in the other groups. The authors concluded that the increased incidences of malignant tumors of the thyroid gland and liver resulted from apparent synergistic interactions among TDA, DAAS, and DETU. However, the relative roles of the three components of the chemical mixture could not be determined.

Table 4-4. Tumor incidence in F344 rats following dietary exposure to TDA alone or combined with other carcinogens (DAAS and DETU) for up to 52 weeks

Exposure group	No. of rats	Tumor incidence (%)			
		Thyroid	Liver	Lung	
		Follicular-cell carcinoma	Hepatocellular carcinoma	Hyperplasia	Adenoma
1. Combined	18[a]	18 (100)***	9 (50)***	18 (100)***	6 (33)**
2. DAAS	21	0	0	0	0
3. DETU	21	1 (5)	0	0	0
4. TDA	20	2 (10)	3 (15)	20 (100)***	4 (20)
5. Control	20	0	0	0	0

Source: Hasegawa *et al*. 1991.

**$P \leq 0.01$; ***$P \leq 0.001$ (Fisher's exact test).

[a]Includes one rat that died at week 49.

4.4 Summary

IARC (1982) concluded that there was sufficient evidence for carcinogenicity of TDA in experimental animals. That conclusion is supported by the results of more recent studies in transgenic animals.

4.4.1 Mice

Dietary administration of TDA increased the incidences of thyroid follicular-cell and hepatocellular tumors in both male and female B6C3F_1 mice. Many of the tumors were malignant, and some had metastasized to one or more distal locations.

In validation studies for a rapid carcinogenicity testing system, dietary administration of TDA for 24 weeks induced thyroid follicular hyperplasia and adenoma in both transgenic and nontransgenic mice within 26 weeks. In transgenic mice, the incidence of lung adenoma was significantly increased, and the incidences of several malignant tumors (e.g., lung adenocarcinoma, spleen hemangiosarcoma, and hepatocellular carcinoma) were increased, though not significantly.

4.4.2 Rats

Dietary administration of TDA to F344 rats for up to 72 weeks significantly increased the incidences of thyroid, liver, and ear-canal (Zymbal gland) tumors in males and the incidences of thyroid and uterine tumors in females. In addition, colon tumors in male rats and ear-canal tumors in female rats were attributed to TDA exposure; however, incidences were not significantly increased. There was some evidence that gavage administration of TDA to young female Sprague-Dawley rats induced mammary tumors. Other studies provided evidence of synergistic effects when TDA was coadministered with other carcinogens; however, the relative role of TDA could not be determined.

5 Genotoxicity

IARC (1982) reviewed the genotoxicity of TDA. The only study reviewed showed that TDA induced reverse mutation in *Salmonella typhimurium* strains TA98 and TA100 when tested with induced rat liver S9 metabolic activation (Lavoie *et al.* 1979).

Since the IARC review, additional studies have reported testing of TDA for genotoxicity, usually as part of a study to evaluate the genotoxicity of a number of chemicals. TDA has been assessed for mutagenicity in the Ames assay and for the ability to induce DNA damage, as measured by alkaline single-cell gel electrophoresis (the comet assay).

5.1 Prokaryotic systems: Induction of mutation in *S. typhimurium*

Zeiger *et al*. (1988) reported that TDA, with or without induced rat or hamster liver S9, did not induce reverse mutation in strains TA1535 or TA1537. TDA induced reverse mutation in strains TA100 and TA98 with or without S9 and in strain TA97 only with S9.

5.2 Mammalian systems: DNA damage in tissues of exposed mice

Male ddY mice were administered TDA by gavage at a dose of 500 mg/kg body weight (b.w.) and sacrificed at 0, 3, 8, or 24 h after administration (Sasaki *et al.* 1999a, b). DNA damage was assessed with the comet assay, in nuclei isolated from stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow. The amount of DNA damage in the brain had increased significantly at 3 h (Dunnett's test, $0.01 < P < 0.05$) and remained elevated at 24 h. At 24 h after TDA administration, the liver, urinary bladder, and lungs showed significantly increased DNA damage (Dunnett's test, $P < 0.001$, $P < 0.001$, and $0.01 < P < 0.05$, respectively). No DNA damage was detected in the stomach, colon, kidneys, or bone marrow of exposed mice. The authors noted that the induction of DNA damage in the liver correlated with the increased incidence of liver tumors in TDA-exposed mice (see Section 4).

5.3 Summary

TDA induced reverse mutation in *S. typhimurium* strains TA98 and TA100 with or without metabolic activation but was not mutagenic in strains TA1535 or TA1537. TDA was mutagenic in strain TA97, but only with metabolic activation. Orally administered TDA caused DNA damage in the brain, liver, urinary bladder, and lungs of mice.

6 Other Relevant Data

For the IARC (1982) review of TDA, no data were available on TDA's toxicity to humans or its absorption, distribution, metabolism, or excretion in humans. This section summarizes information on TDA's toxicity, absorption, metabolism, and excretion in animals; prediction of TDA's carcinogenic potential; and comparative tumorigenicity and genotoxicity of TDA, aniline, and other dianilines.

6.1 Toxicity

IARC (1982) reviewed the toxicity of TDA in experimental animals. The oral dose of TDA causing 50% mortality (LD_{50}) in rats (strain not specified) was reported to be 1,100 mg/kg b.w. TDA also was a reproductive toxin in mice (strain not specified); orally administered TDA (50 mg/kg b.w. on days 1 to 5 of pregnancy) slightly reduced implantation, and doses ≥ 100 mg/kg b.w. prevented implantation (IARC 1982). When TDA was administered in the diet for 90 days to male Sprague-Dawley rats (1,200 to 30,000 ppm) and male Swiss mice (2,000 to 50,000 ppm), all rats fed diets containing TDA at 30,000 ppm and all mice fed diets containing TDA at 25,000 ppm or more died during the study (NCI 1978). TDA also was toxic at dietary concentrations of 1,500 and 3,000 ppm in male and female Fischer 344 rats and 2,500 and 5,000 ppm in male and female $B6C3F_1$ mice in chronic exposure studies (NCI 1978). Body weight gain was depressed at both dose levels, and all animals exposed to TDA died by 72 weeks (rats) or 91 weeks (mice).

6.2 Mammalian absorption, metabolism, and excretion

The only study on mammalian absorption, metabolism, or excretion of TDA published since the IARC (1982) review is an investigation of hemoglobin adduct formation in female Wistar rats (Sabbioni and Schütze 1998).

6.2.1 Human studies

No data on human absorption, metabolism, or excretion of TDA were found in the literature published since the IARC (1982) review.

6.2.2 Animal studies

No specific data on absorption and excretion of TDA in experimental animals were found in the literature published since the IARC (1982) review. However, Sabbioni and Schütze (1998) investigated the biological availability of several known carcinogenic diamines and *N*-hydroxylamines of *ortho*-substituted diamines in female Wistar rats by measuring hemoglobin adducts. TDA was administered by gavage, and hemoglobin was isolated and hydrolyzed in 0.1 M sodium hydroxide. TDA had bound to hemoglobin as both the diamine and *N*-acetylamine; however, no data were presented on the mechanism for acetylation of TDA or the potential role of acetyl-TDA in carcinogenicity. The extent of adduct formation was positively correlated with carcinogenic potency as demonstrated in rodent bioassays.

6.3 Prediction of carcinogenic potential

Because of the time and expense involved in the standard two-year bioassays for carcinogenicity, many researchers have attempted to identify chemical characteristics that may allow screening of large numbers of chemicals to predict carcinogenic potential.

6.3.1 Electron attachment rate constant (k_e) test

Bakale and McCreary (1992) proposed that a sufficiently electrophilic chemical might be a potential carcinogen. They used the k_e test, a physicochemical screening test for carcinogens based on the affinity of molecules for free electrons, to test 105 chemicals that had been studied in long-term rodent bioassays. The k_e for TDA was below the empirical cutoff value considered predictive of carcinogenicity, so TDA was not identified as a potential carcinogen in this test.

6.3.2 Structure-activity relationships

Various structure-activity relationships have been used in attempts to identify potentially carcinogenic chemicals. Ashby and Tennant (1988) included TDA among 222 chemicals surveyed for concordance of structural alerts for potentially electrophilic sites, mutagenicity in *S. typhimurium*, and carcinogenicity in mice and rats. The aromatic amino group of TDA was identified as an alerting substructure, and its presence correlated with mutagenicity in *S. typhimurium* (see Section 5) and increased incidence of tumors in male and female rats and mice (see Section 4).

Rosenkranz and Klopman (1993) analyzed TDA and 48 other chemicals for structural feature determinants that might identify "genotoxic" and "non-genotoxic" carcinogens. They concluded that a C"-S-C= fragment in TDA, consisting of the carbon-sulfur-carbon moiety linking the two rings (see TDA's structure in Figure 1-1), was most likely associated with carcinogenicity. However, in re-examining the potential carcinogenicity of azathioprine, Gombar *et al*. (1993) used a toxicity-prediction program to analyze TDA and concluded that the aryl-NH_2 group was the structural feature associated with the highest probability of carcinogenicity; they assigned a much lower probability to the C"-S-C= fragment. The ultimate utility of these structure-activity relationships in predicting carcinogenicity remains to be determined.

6.4 Comparative tumorigenicity and genotoxicity of TDA, aniline, and some other dianilines

TDA is a derivative of aniline (see Figure 1-3), a compound that also has been examined for tumorigenicity and genotoxicity; however, no metabolic pathway by which TDA may be converted to aniline was found in a search of the published literature. TDA was tested by the NCI at the same time as its analog, 4,4'-sulfonyldianiline, which is the antileprosy drug dapsone. 4,4'-Sulfonyldianiline differs from TDA by the oxidation of the sulfide linkage to the sulfone (see Figure 1-4). The Report on Carcinogens also lists three other dianilines, 4,4'-oxydianiline (see Figure 1-5), 4,4'-methylene dianiline and its dihydrochloride (see Figure 1-6), and 4,4'-methylenebis(2-chloroaniline) (see Figure 1-7). The tumorigenicity and genotoxicity of TDA, aniline, dapsone, 4,4'-oxydianiline, 4,4'-methylene dianiline, and 4,4'-methylenebis(2-chloroaniline) are discussed below and summarized in Table 6-1.

6.4.1 Tumorigenicity

6.4.1.1 TDA

Data on the tumorigenicity of TDA are summarized in Section 4. Tumors occurring at significantly increased incidences in Fischer 344 rats exposed to TDA included hepatocellular adenoma and carcinoma (males only), follicular-cell adenoma and carcinoma of the thyroid gland (males and females), squamous-cell papilloma and carcinoma of the ear canal (males only), and adenocarcinoma of the uterus or cervix (females). Nonsignificant increases in the incidences of tumors of skin (males), lung (males and females), and colon (males) also were reported. Significant increases in thyroid carcinoma and adenoma or carcinoma combined and liver carcinoma and adenoma or carcinoma combined in male and female $B6C3F_1$ mice have been reported. Tumors of the lung (adenoma) were significantly increased in *ras*H2 transgenic mice, and tumors of the thyroid (follicular-cell adenoma) were significantly increased in both transgenic and nontransgenic mice exposed to TDA in the diet. The incidences of lung adenocarcinoma, spleen hemangiosarcoma, and hepatocellular carcinoma also were increased, though not significantly.

6.4.1.2 Aniline

The NCI (1978) tested aniline hydrochloride in a two-year bioassay (TR-130) in Fischer 344 rats and $B6C3F_1$ mice. Aniline hydrochloride was administered in the diet to groups of Fischer 344 rats (50 of each sex) at a concentration of 0.6% (6,000 ppm) or 0.3% (3,000 ppm) and $B6C3F_1$ mice (50 of each sex, except 49 females in the high-dose group) at a concentration of 1.2% (12,000 ppm) or 0.6% (6,000 ppm). The NCI concluded that administration of aniline hydrochloride was associated with increased incidences of hemangiosarcoma of the spleen and fibrosarcoma or sarcoma of the spleen and of multiple organs of the body cavity in male rats. A possible association also was reported between administration of aniline hydrochloride and the increased combined incidence of fibrosarcoma or sarcoma of the spleen or of multiple organs of the body cavity in female rats. The NCI also reported that there was no statistical evidence indicating that aniline hydrochloride was carcinogenic in male or female mice.

IARC (1987) reported that aniline hydrochloride did not increase tumor incidence in mice. In rats, aniline hydrochloride caused fibrosarcoma, sarcoma, and hemangiosarcoma of the spleen and peritoneal cavity. IARC concluded that aniline was not classifiable as to its carcinogenicity to humans (Group 3).

6.4.1.3 Dapsone (4,4'-sulfonyldianiline)

In an NCI bioassay (1977, TR-20) of dapsone, groups of Fischer 344 rats and $B6C3F_1$ mice (35 of each sex) were administered dapsone in the diet at a concentration of 600 or 1,200 ppm for rats and 500 or 1,000 ppm for mice for 78 weeks. The rats were observed for 26 to 28 weeks, and the mice for 28 to 30 weeks. Survival was unaffected by dapsone exposure. Dapsone caused tumors of the spleen and peritoneum in male rats but was not tumorigenic in female rats or in mice.

IARC (1987) reported that dapsone administered orally to rats and mice induced mesenchymal tumors of the spleen (three studies) and peritoneum (two studies) in male

rats. The incidence of thyroid tumors was increased in rats of both sexes in one study and in males in a second study. IARC concluded that dapsone was not classifiable as to its carcinogenicity to humans (Group 3).

6.4.1.4 4,4'-Oxydianiline

4,4'-Oxydianiline is listed in the Ninth Annual Report on Carcinogens (NTP 2001b) as *reasonably anticipated to be a human carcinogen,* based on sufficient evidence of carcinogenicity in experimental animals (IARC 1978, NCI 1978, IARC 1982). Diets containing 4,4'-oxydianiline at 200, 400, or 500 ppm were fed to groups of Fischer 344 rats (50 of each sex), and diets containing 4,4'-oxydianiline at 150, 300, or 800 ppm were fed to groups of B6C3F_1 mice (50 of each sex). Survival was significantly shortened in the high-dose female rats and in the low- and mid-dose mice of both sexes. When administered in the diet, 4,4'-oxydianiline increased the incidences of adenoma of the Harderian gland and hepatocellular adenoma or carcinoma (combined) in mice of both sexes, follicular-cell adenoma in female mice, and hepatocellular carcinoma or neoplastic nodules (combined) and follicular-cell adenoma or carcinoma (combined) in rats of both sexes. When administered by subcutaneous injection, the compound induced malignant liver-cell tumors in rats.

IARC (1982) reported that in two studies, 4,4'-oxydianiline (4,4'-diaminodiphenyl ether) administered orally or by subcutaneous injection to rats induced benign and malignant liver-cell tumors. Administered orally to rats in one study, it induced benign and malignant follicular-cell tumors of the thyroid. In one study in mice, oral administration of 4,4'-oxydianiline induced benign and malignant liver-cell tumors in high-dose females and low-dose males; Harderian gland tumors (adenoma) were observed in mice of both sexes. IARC concluded that 4,4'-oxydianiline was possibly carcinogenic to humans (Group 2B).

6.4.1.5 4,4'-Methylenedianiline and its dihydrochloride

4,4'-Methylenedianiline and its dihydrochloride are listed in the Ninth Annual Report on Carcinogens (NTP 2001b) as *reasonably anticipated to be a human carcinogen* (NTP 1983, IARC 1986, 1987). Groups of Fischer 344 rats and B6C3F_1 mice (50 of each sex) received drinking water containing 4,4'-methylenedianiline dihydrochloride at 150 or 300 ppm (dosage estimated as the free base) for 103 weeks. Survival was comparable among all groups except high-dose male mice, whose survival was lower ($P = 0.006$) that that of controls. 4,4'-Methylenedianiline dihydrochloride administered in drinking water increased the incidences of thyroid follicular-cell carcinoma and neoplastic nodules of the liver in male rats, follicular-cell and C-cell adenoma of the thyroid gland in female rats, thyroid follicular-cell adenoma and heptocellular carcinoma in mice of both sexes, adrenal pheochromocytoma in male mice, and hepatocellular adenoma and malignant lymphoma in female mice (NTP 1983). When 4,4'-methylenedianiline was administered to rats orally in combination with a known carcinogen, the incidence of thyroid tumors was greater than that produced by the known carcinogen alone (IARC 1986).

IARC (1986) reported that oral administration of 4,4'-methylenedianiline and its dihydrochloride resulted in exposure-related increases in the incidences of thyroid

follicular-cell carcinoma and hepatic nodules in male rats and thyroid follicular-cell adenoma in female rats. Increased incidences of thyroid follicular-cell adenoma and hepatocellular neoplasms also were observed in male and female mice. IARC concluded that 4,4'-methylenedianiline and its dihydrochloride were possibly carcinogenic to humans (Group 2B).

6.4.1.6 4,4'-Methylenebis(2-chloroaniline)

4,4'-Methylenebis(2-chloroaniline) (MBOCA) is listed in the Ninth Annual Report on Carcinogens (NTP 2001b) as *reasonably anticipated to be a human carcinogen,* based on sufficient evidence of carcinogenicity in experimental animals (IARC 1974, 1987). When administered in the diet, MBOCA increased the incidences of hemangiosarcoma in mice of both sexes and hepatoma in female mice. When administered in the diet, MBOCA induced lung adenoma and adenocarcinoma and some mesothelioma in rats of both sexes. In another study, when administered in the diet, MBOCA induced pulmonary adenoma, mammary adenocarcinoma, Zymbal gland carcinoma, and hepatocellular carcinoma in male rats. When administered by gavage, MBOCA induced transitional-cell carcinoma of the urinary bladder in dogs. When administered by subcutaneous injection, MBOCA induced liver-cell carcinoma and lung carcinoma in rats of both sexes.

IARC (1993) reported that MBOCA administered to groups of CD rats (25 of each sex) in the diet at a concentration of 500 or 1,000 ppm induced liver-cell tumors and malignant lung tumors in both sexes. Survival in this two-year study was similar for control and exposed rats (approximately 55% were alive at 20 to 22 months). A few liver-cell tumors were induced in male rats in a second study; lung adenocarcinoma and hepatocellular tumors in male and female rats in a third study; and malignant lung tumors, mammary gland adenocarcinoma, Zymbal gland carcinoma, and hepatocellular carcinoma in a fourth study. Hepatocellular carcinoma and malignant lung tumors also were observed after subcutaneous administration of MBOCA to rats. In a study of HaM/ICR mice (25 of each sex per group), dietary administration of MBOCA at a concentration of of 1,000 or 2,000 ppm increased the incidence of liver tumors in female mice. Survival was similar for control and exposed mice (approximately 55% were alive at 20 to 22 months). IARC concluded that 4,4'-methylenebis(2-chloroaniline) was probably carcinogenic to humans (Group 2A).

6.4.2 Genotoxicity

6.4.2.1 TDA

Data on the genotoxicity of TDA are summarized in Section 5. TDA induced reverse mutation in *S. typhimurium* strains TA98 and TA100 with or without metabolic activation but was not mutagenic in strains TA1535 or TA1537. TDA was mutagenic in strain TA97, but only with metabolic activation. Orally administered TDA caused DNA damage (assessed with the comet assay) in the brain, liver, urinary bladder, and lungs of mice (Sasaki *et al.* 1999a, b).

6.4.2.2 Aniline

IARC (1987) reported that aniline was not mutagenic in bacteria but did cause a number of genotoxic effects *in vitro* and *in vivo*. Aniline induced sister chromatid exchange

(SCE) in bone-marrow cells of mice exposed *in vivo* and in mammalian cells *in vitro,* and induced transformation of BALB/c 3T3 cells. Sasaki *et al.* (1999a, b) reported that aniline caused DNA damage (assessed with the comet assay) in mouse liver, kidney, urinary bladder, lung, brain, and bone marrow, but not in stomach or colon.

6.4.2.3 Dapsone

IARC (1987) reported that no data were available on the genetic effects of dapsone in humans and that it was not mutagenic in bacteria.

6.4.2.4 4,4'-Oxydianiline

In a summary of the testing status of 4,4'-oxydianiline, the National Toxicology Program (NTP 2002a) reported the following findings from genetic toxicology tests: positive for chromosomal aberrations, positive for SCE in some studies *(in vitro)* but negative in others, negative for sex-linked recessive lethal mutation or reciprocal translocation in *Drosophila*, positive for gene mutation in mouse lymphoma L5178Y cells, positive for induction of micronuclei in mouse peripheral-blood erythrocytes, and positive for reverse mutation in *S. typhimurium*.

6.4.2.5 4,4'-Methylenedianiline and its dihydrochloride

In a summary of the testing status of 4,4'-methylenedianiline, the NTP (2002b) reported the following findings from genetic toxicology tests: positive for chromosomal aberrations in some studies but negative in others, positive for SCE, positive for induction of micronuclei in mouse peripheral-blood erythrocytes, and positive for reverse mutation in *S. typhimurium*.

6.4.2.6 4,4'-Methylenebis(2-chloroaniline)

In a summary of the testing status of 4,4'-methylenebis(2-chloroaniline), the NTP (2002c) reported the following findings from genetic toxicology tests: negative for chromosomal aberrations; negative for SCE; positive for gene mutation in mouse lymphoma L5178Y cells; and positive, weakly positive, or inconclusive for reverse mutation in *S. typhimurium*.

6.5 Summary

Hardly any reports have been published on the absorption, distribution, metabolism, or excretion of TDA. Data on the hemoglobin binding index of TDA correlate with carcinogenic potency and demonstrate that TDA undergoes acetylation. Two groups have used structure-activity analysis to suggest that the aryl-amino group of TDA is most likely involved in carcinogenicity, although the C"-S-C= fragment also has been proposed. TDA significantly increased the incidences of tumors in a variety of tissues in rats and mice, including liver, thyroid, ear canal (Zymbal gland), and uterus. Aniline and dapsone, on the other hand, caused tumors of the spleen. Some similarity in the organ-specific DNA damage induced by TDA and aniline in the comet assay was reported; however, no genotoxic effects of dapsone have been reported. Three other dianilines (4,4'-oxydianiline, 4,4'-methylenedianiline, and 4,4'-methylenebis[2-chloroaniline]) currently are listed in the Report on Carcinogens. These dianilines have been reported to

induce tumors in organs and tissues in which TDA induces tumors, and at lower doses than TDA.

Table 6-1. Comparative carcinogenicity and mutagenicity of TDA, aniline, and some other dianilines[a]

	TDA	Aniline	Dapsone	Oxydianiline	Methylene-dianiline and its dihydrochloride	Methylenebis-(2-chloroaniline)
Carcinogenicity						
Liver	+ rats (M) + mice (M/F)			+ rats (M/F) + mice (M/F)	+ rats (M) + mice (M/F)	+ rats (M/F) + mice (F)
Thyroid	+ rats (M/F) + mice (M/F)		+ rats (M/F)	+ rats (M/F) + mice (F)	+ rats (M/F) + mice (M/F)	
Ear canal (Zymbal gland)	+ rats (M)					+ rats (M)
Uterus or cervix	+ rats (F)					
Spleen		+ rats (M)	+ rats (M)			
Peritoneal cavity		+ rats (M)	+ rats (M)			
Eye (Harderian gland)				+ mice (M/F)		
Adrenal gland					+ mice (M)	
Mammary gland						+ rats (M)
Lung						+ rats (M/F)
Vascular						+ mice (M/F)

	TDA	Aniline	Dapsone	Oxydianiline	Methylene-dianiline and its dihydrochloride	Methylenebis-(2-chloroaniline)
Mutagenicity						
S. typhimurium (reverse mutation)	+ TA98 (± S9) + TA100 (± S9) + TA97 (+ S9)	– strain not specified	– strain not specified	+ strain not specified	+ strain not specified	+ strain not specified
Comet assay (DNA damage in mice)	+ brain, liver, urinary bladder, lung	+ brain, liver, kidney, urinary bladder, lung, bone marrow	NR	NR	NR	NR
SCE	NR	+ mouse bone marrow	NR	+ *in vitro*	+	–
Transformation	NR	+ BALB/c 3T3 cells	NR	NR	NR	NR
Chromosomal aberration	NR	NR	NR	+	+	–
Micronuclei	NR	NR	NR	+	+	NR

[a]NR = not reported.

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4,4′-THIODIANILINE

This substance was considered by a previous Working Group, in June 1977 (IARC, 1978). Since that time new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

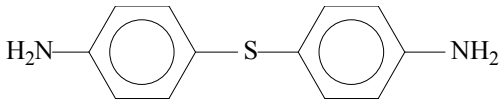
Chem. Abstr. Services Reg. No.: 139-65-1

Chem. Abstr. Name: Benzenamine, 4,4'-thiobis-

IUPAC Systematic Name: 4,4'-Thiodianiline

Synonyms: Bis(4-aminophenyl)sulphide; 4,4'-diaminodiphenyl sulphide; *para,para'*-diaminodiphenyl sulphide; 4,4'-diaminophenyl sulphide; di(*para*-aminophenyl)-sulphide; thioaniline; 4,4'-thiobis(aniline); *para,para'*-thiodianiline; thiodi-*para*-phenylenediamine

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2S$ Mol. wt: 216.3

1.3 Chemical and physical properties of the pure substance

From Weast (1979)

(a) Description: Needles

(b) Melting-point: 108-109º

(c) Spectroscopic data: Infra-red, nuclear magnetic resonance, ultra-violet (Sadtler Research Laboratories, Inc., undated) and mass spectral data (Mass Spectrometry Data Centre, 1974) have been published.

(d) Solubility: Slightly soluble in hot water; very soluble in ethanol, diethyl ether and hot benzene

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) Production

4,4'-Thiodianiline was prepared by Merz & Weith in 1871 by boiling sulphur with aniline for several days (Prager *et al.,* 1930). No information was available on the methods used for its commercial production.

Production of 4,4'-thiodianiline in the US was first reported in 1941-1943 (US Tariff Commission, 1945); currently, one company is believed to produce it. 4,4'-Thiodianiline is also produced in the USSR. No evidence was found that it is produced in commercial quantities in western Europe or Japan.

(b) Use

4,4'-Thiodianiline appears to be used almost exclusively as a dye intermediate. The Society of Dyers & Colourists (1971) reported that three dyes can be prepared from it. Only one of these, Mordant Yellow 16, has been produced commercially in the US; one company reported production of an undisclosed amount of this dye in 1979 (US International Trade Commission, 1980).

The USSR has established a ceiling value of 1 mg/m^3 of air for occupational exposure to 4,4'-thiodianiline (International Labour Office, 1977).

2.2 Occurrence

4,4'-Thiodianiline is not known to occur as a natural product. No data on its occurrence in the environment were available to the Working Group.

2.3 Analysis

An IARC manual (Egan *et al.,* 1981) gives selected methods for the analysis of aromatic amines.

Thin-layer chromatography, with four different solvent systems, has been used to separate and identify a group of aromatic diamines, including 4,4'-thiodianiline, and *N*-benzamides (Krasnov *et al*., 1970); and paper chromatography has been used to separate and identify a group of aromatic *para,para′*-diamines, including 4,4'-thiodianiline (Gasparic & Snobl, 1971). A group of aromatic amines, including this compound, has been separated and identified by gas chromatography (Kazinik *et al.*, 1971a,b).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Oral administration

Mouse: Groups of 35 male and 35 female B6C3F_1 mice, 46-47 days of age, were fed diets containing 2500 or 5000 mg/kg 4,4'-thiodianiline (purity probably greater than 97%, with at least one unspecified impurity detected by gas chromatography) on five days per week for 77-79 weeks. The doses were selected on the basis of a range-finding study in male Swiss mice [see section 3.2(*a*)]. A group of 14 mice of each sex served as matched controls. All animals under study received food and water *ad libitum.* Mean body weight gain of treated male and female mice was markedly reduced, and a positive dose-related trend in mortality was observed: by 52 weeks, 97% of the low-dose animals and 83% of the high-dose animals of both sexes were still alive, as compared with 86% of female and 100% of male controls. All low- and high-dose animals had died by the end of 77-79 weeks of treatment, while the controls were kept for observation for 91 weeks. Statistically significant increases in tumour incidences were observed for several neoplasms: (a) There was a dose-related increase ($P < 0.001$) in hepatocellular carcinomas in females: 0/12 controls, 32/34 low-dose ($P < 0.001$) and 30/31 high-dose animals ($P < 0.001$); in males, hepatocellular carcinomas were seen in 1/13 controls, 32/34 low-dose ($P < 0.001$) and 22/24 high-dose animals ($P < 0.001$). Such tumours metastasized to the lungs in 9-14% of females and to the kidneys in 3% of low-dose males and high-dose females. (b) There was a dose-related increase ($P < 0.001$) in follicular-cell carcinomas of the thyroid gland in females: 0/11 controls, 3/33 low-dose and 15/30 high-dose animals ($P = 0.002$); and in males, these tumours were seen in 0/14 controls, 15/33 low-dose ($P < 0.001$) and 20/23 high-dose animals ($P < 0.001$). Pulmonary metastases were found in some males. When the incidences of follicular-cell adenoma and carcinoma were grouped for analysis, a significant increase was observed for animals of each sex and at each dose level. In addition, the high incidence of thyroid follicular-cell hyperplasia was considered to be related to the treatment (National Cancer Institute, 1978; Cueto & Chu, 1979).

Rat: In an experiment designed to screen carcinogens that act on the mammary gland of young female Sprague-Dawley rats, 20 animals, 40 days of age, were given 10 doses of 40 mg/animal 4,4'-thiodianiline in 1 mL sesame oil every three days by gastric intubation (total dose, 400 mg); a further group of 10 females received a total dose of 300 mg/rat; 140

control rats received the vehicle alone. All surviving animals were killed after nine months. Of 12 surviving rats given 400 mg and which were autopsied, three developed mammary carcinomas; of those that received 300 mg/animal, 1/8 developed a mammary carcinoma. Among 132 controls autopsied, of which 127 survived nine months, three had mammary carcinomas and one a mammary fibroadenoma (Griswold *et al*., 1968). [The Working Group noted the inadequate duration of the experiment.]

Groups of 35 male and 35 female Fischer 344 rats, 47-48 days of age, were fed diets containing 1500 or 3000 mg/kg 4,4'-thiodianiline (purity probably greater than 97%, with at least one unspecified impurity detected by gas chromatography) on five days per week for 68-72 weeks. The doses were selected on the basis of a range-finding study in male Sprague-Dawley rats [see section 3.2(*a*)]. A group of 15 rats of each sex served as matched controls. All animals under study received food and water *ad libitum.* Mean body weight gain of treated male and female rats was markedly reduced, and a positive, dose-related trend in mortality was observed: by 52 weeks, 51-91% of the treated rats and 100% of controls were still alive. The low-dose animals had all died by 68-72 weeks of treatment and the high-dose animals by 68-69 weeks; the controls were observed up to 104 weeks. Statistically significant increases in tumour incidences were observed for several neoplasms, all found in treated animals only: (a) An increase occurred in follicular-cell carcinomas of the thyroid gland in females: 24/33 low-dose ($P < 0.001$), 32/32 high-dose ($P < 0.001$); and in males: 28/33 low-dose ($P < 0.001$), 32/33 high-dose ($P < 0.001$). In most cases, these tumours were bilateral, and infiltration of neoplastic cells was frequent. Pulmonary metastases were found in 34-52% of animals in the different groups; metastases to other sites were rare. (b) There was an increased incidence of adenocarcinomas of the uterus: 31/33 low-dose ($P < 0.001$), 23/32 high-dose ($P < 0.001$). Pulmonary metastases were observed in 44% of the low-dose group; metastases to other sites were rare. (c) An increase was observed in the incidence of squamous-cell papillomas or carcinomas of the ear canal in males: 15/33 low-dose ($P = 0.001$), 8/33 high-dose ($P = 0.037$). These tumours metastasized to the lungs in 2/66 animals. (d) An increase occurred in the incidence of hepatocellular carcinomas in males: 21/33 low-dose ($P < 0.001$), 10/33 high-dose ($P = 0.014$). The carcinomas metastasized to the lungs in 21% of animals in the low-dose group and in 3% of those in the high-dose group (National Cancer Institute, 1978; Cueto & Chu, 1979).

3.2 Other relevant biological data

(a) Experimental systems

Toxic Effects

The oral LD_{50} of 4,4'-thiodianiline in rats has been reported as 1100 mg/kg bw (Lewis & Tatken, 1979).

In 45-day subchronic feeding studies with male Sprague-Dawley rats and male Swiss mice, all animals that received diets containing 3% (rats) or 2.5% (mice) or more 4,4'-thiodianiline died. In chronic studies with Fischer 344 rats and $B6C3F_1$ mice, squamous metaplasia of alveolar and bronchiolar epithelium, nodular hyperplasia of the liver and hyperplasia of the bile ducts were observed (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

Oral administration of 50 mg/kg bw 4,4'-thiodianiline to mice on days 1-5 of pregnancy slightly reduced fetal implantation; doses ≥100 mg/kg bw prevented implantation (Kamboj & Kar, 1966).

Absorption, distribution, excretion and metabolism

No data were available to the Working Group.

Mutagenicity and other short-term tests

4,4'-Thiodianiline induced reverse mutations in *Salmonella typhimurium* strains TA98 and TA100 when tested in the presence of a liver activation system prepared from Aroclor 1254-induced rats (Lavoie *et al.,* 1979).

(b) Humans

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

4,4'-Thiodianiline was tested adequately for carcinogenicity in one experiment in mice and in one experiment in rats by dietary administration. It was carcinogenic for animals of both sexes of both species. In mice, it produced hepatocellular carcinomas and carcinomas

or adenomas of the thyroid gland in animals of both sexes. In rats, it produced metastatic thyroid gland carcinomas in animals of both sexes, hepatocellular carcinomas and ear-canal papillomas or carcinomas in males and adenocarcinomas of the uterus in females.

4,4'-Thiodianiline was mutagenic to *Salmonella typhimurium* with metabolic activation.

4.2 Human data

4,4'-Thiodianiline was first produced commercially in the early 1940s. Its use as a dye intermediate could lead to occupational exposure.

No case report or epidemiological study was available to the Working Group.

4.3 Evaluation

There is *sufficient evidence* for the carcinogenicity of 4,4'-thiodianiline in experimental animals.

In the absence of data on humans, 4,4'-thiodianiline should be regarded, for practical purposes, as if it presented a carcinogenic risk to humans.

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Appendix B: NCI TR 47 (1978). Bioassay of 4,4'-Thiodianiline for Possible Carcinogenicity. DHEW Publication No. 78-847. pp vii - 106.

BIOASSAY OF

4,4′-THIODIANILINE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-847

SUMMARY

A bioassay of 4,4′-thiodianiline for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were administered 4,4′-thiodianiline 5 days per week at one of the following doses, either 1,500 or 3,000 ppm for the rats and either 2,500 or 5,000 ppm for the mice. The period of administration of the chemical was 68–72 weeks for the rats and 77 or 79 weeks for the mice, depending on the length of survival time of the animals. Matched controls consisted of groups of 15 untreated rats and 14 untreated mice of each sex. All surviving matched-control rats were killed at 104 weeks; all surviving matched-control mice were killed at 91 weeks.

The administration of 4,4′-thiodianiline resulted in marked reduction in mean body weights of the rats and mice of each sex, and all dosed animals died prior to the scheduled end of the study.

Tumors of epithelial origin were found in many organs, and all dosed rats except one were affected at one or more sites (males: skin, ear canal, lungs, liver, colon, and thyroid; females: ear canal, lung, liver, thyroid, and uterus). These tumors were not found among any of the matched-control animals.

In male rats, several of these neoplastic lesions occurred with statistically significant incidences in one or both of the dosed groups. The incidences of hepatocellular carcinoma (controls 0/15, low-dose 21/33, high-dose 10/33) and of follicular-cell carcinoma of the thyroid (controls 0/15, low-dose 28/33, high-dose 32/33) were significant in each of the groups at $P \leq 0.014$. The combined incidences of squamous-cell carcinoma and squamous-cell papilloma of the ear canal in the low- and high-dose groups of males were both significantly higher (low-dose $P = 0.001$, high-dose $P = 0.037$) than that in the control group (controls 0/15, low-dose 15/33, high-dose 8/33). The first such tumor in the high-dose group was observed at 25 weeks.

Also in low-dose male rats, squamous-cell papilloma of the skin occurred in 4/33 animals, and squamous-cell carcinoma of the skin in 1/33, but no tumors of either type occurred in the controls. The incidences of these lesions were too low to have statistical significance. The majority of the squamous-cell tumors of the skin were located in one area near the commissure of the mouth. Only one such tumor occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the skin may be associated with administration of the chemical. Adenocarcinoma of the colon occurred in six low-dose male rats and in one high-dose male rat, but not in any of the controls. This incidence is not statistically significant; however, no such tumors occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the colon are considered to be related to administration of 4,4′-thiodianiline.

In female rats, the incidences of hepatocellular adenoma or carcinoma in the dosed groups were greater than those in the controls, but not statistically significant (controls 0/15, low-dose 6/32, high-dose 3/33). Follicular-cell carcinoma of the thyroid and adenocarcinoma of the uterus occurred in the females administered the test chemical at statistically significant incidences ($P < 0.001$) in both dosed groups (follicular-cell carcinoma: controls 0/14, low-dose 24/33, high-dose 32/32; adenocarcinoma: controls 0/15, low-dose 31/33, high-dose 23/32). Squamous-cell papilloma or carcinoma of the ear canal occurred at increased, but not statistically significant, incidences in female rats (controls 0/15, low-dose 6/33, high-dose 3/33). However, no such tumors occurred among the 235 historical-control female rats at this laboratory; thus, the tumors of the ear canal are considered to be related to administration of the chemical.

In mice of each sex, the incidence of hepatocellular carcinoma was statistically significant ($P < 0.001$) in each of the dosed groups (males: controls 1/13, low-dose 32/34, high-dose 22/24, females: controls 0/12, low-dose 32/34, high-dose 30/31). In the males, follicular-cell carcinoma of the thyroid occurred at statistically significant incidences ($P \leq 0.001$) in both the low- and high-dose groups (controls 0/14, low-dose 15/33, high-dose 20/23). In the females, the incidence was significant ($P = 0.002$) only at the high dose (controls 0/11, high-dose 15/30); however, when follicular-cell adenoma and carcinoma were combined, the incidences in both the low- and high-dose groups of

females were significantly higher (low-dose P = 0.025, high-dose P < 0.001) than that in the control group (controls 0/11, low-dose 11/33, high-dose 18/30).

It is concluded that under the conditions of this bioassay, 4,4′-thiodianiline was carcinogenic for Fischer 344 rats, inducing tumors in the liver, thyroid, colon, and ear canal of male rats, and the thyroid, uterus, and ear canal of female rats. 4,4′-Thiodianiline was carcinogenic for B6C3F1 mice, inducing tumors in the liver and thyroid of both males and females.

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FIGURES

I. INTRODUCTION

4,4′-Thiodianiline (CAS 139-65-1; NCI C01707) is an intermediate in the manufacture of several diazo dyes (Am. Assoc. Text. Chem. Color., 1958). It is an analog of 4,4′-sulfonyldianiline, which is the antileprosy drug dapsone; 4,4′-sulfonyldianiline differs from 4,4′-thiodianiline by the oxidation of the sulfide linkage to the sulfone. 4,4′-Thiodianiline has been considered to be weakly active in inducing mammary tumors in female Sprague-Dawley rats (Griswold et al., 1968).

4,4′-Thiodianiline was selected for the Carcinogenesis Testing Program because of this activity and for comparison with the analog 4,4′-sulfonyldianiline, which was tested at the same time.

II. MATERIALS AND METHODS

A. Chemical

The 4,4′-thiodianiline used in the chronic study was manufactured by Carroll Products, Wood River Junction, Rhode Island. Its stated purity was 97% (nitrite absorption). Purity as determined by nonaqueous titration of the amine groups was 99.11 ± 0.01%. Thin-layer chromatography (tlc) revealed one impurity at the origin and three trace impurities. The trace impurities were shown by two-dimensional chromatography to result from decomposition of 4,4′-thiodianiline on the tlc plate. One impurity, estimated as 0.05%, was detected by vapor-phase chromatography. The melting point was 107-109°C (literature: 108-109°C). Elemental analyses (C, H, N, S) were correct for $C_{12}H_{12}N_2S$, the molecular formula of the chemical. Infrared and nuclear magnetic resonance spectra were consistent with spectra for 4,4′-thiodianiline in the literature.

The single batch of the chemical used in the chronic studies was stored at 5°C in amber bottles.

B. Dietary Preparation

Test diets were prepared every 2 weeks by mixing a known amount of sifted 4,4′-thiodianiline with a small amount of Wayne® Lab

Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer, then adding this mixture to the required amount of animal meal and mixing in a twin-shell blender for 10 minutes. No analyses of concentration or determinations of stability of the chemical in feed were performed. The prepared diets were stored at room temperature in sealed plastic containers.

C. Animals

For the subchronic studies, male Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic studies, male and female Fischer 344 rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories. On arrival at the laboratory, the animals, which were 30 or 31 days of age, were quarantined (rats for 17 days, mice for 16 days). Animals with no clinical signs of disease were then assigned to control or dosed groups and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. Room air was changed 15 times

per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Food and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and the cage tops were covered with disposable filter bonnets; the mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective dosed animals. Animals administered 4,4′-thiodianiline were maintained in the same rooms as animals of the same species administered the following chemicals:

RATS

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)

5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
(pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine
hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
ethionamide (CAS 536-33-4)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide
(acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide
(chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
(pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine
hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
(phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)

acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of 4,4′-thiodianiline, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. Male Sprague-Dawley rats were administered the chemical in feed at concentrations of 1,200, 3,000, 6,000, 15,000, or 30,000 ppm. Male Swiss mice were administered the chemical at concentrations of 2,000, 5,000 10,000, 25,000, or 50,000 ppm. The animals were fed the test diet 7 days per week for 45 days and were then observed for an additional 45 days. Five male animals of each species were dosed at each concentra-

tion, and 20 male animals of each species were maintained as untreated controls.

At 45 days, the mean body weight gain in rats administered 1,200 ppm was 56% of that of the controls; at 3,000 ppm, 27%; and at 6,000 ppm, 10%. The mean body weights of animals administered 15,000 ppm were below the initial mean. At 90 days, mean body weights of the dosed groups were still less than those of the controls. All rats fed at 30,000 ppm died during week 3 on study, and one animal fed at 3,000 ppm died during week 2. No gross abnormalities were found at necropsy. For rats, the low and high doses for the chronic studies were set at 1,500 and 3,000 ppm.

At 45 days, the mean body weight gain in mice administered 2,000 ppm was 77% of that of the controls; at 5,000 ppm, 46%; and at 10,000 ppm, 46%. At 90 days, mice administered 2,000 or 10,000 ppm had mean body weight gains which were comparable to those of controls. In the group administered 5,000 ppm, however, mean body weight gain was only 65% of that of the controls. All mice fed diets containing 25,000 or 50,000 ppm had died by week 3, and two animals fed a diet containing 10,000 ppm died during week 3. No gross abnormalities were found at necropsy. For mice, the low and high doses for the chronic studies were set at 2,500 and 5,000 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2. The time on study varied slightly for the dosed groups, because of differences in the times of death.

G. Clinical and Pathologic Examinations

All animals were observed twice per day for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed once every 2 weeks for the entire study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Whenever possible, peripheral blood smears were prepared from each animal. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in

Table 1. Design of 4,4′-Thiodianiline Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	4,4′-Thiodianiline in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	15	0		104
Low-Dose	35	1,500	68[c]	
High-Dose	35	3,000	68[c]	
Female				
Matched-Control	15	0		104
Low-Dose	35	1,500	72[c]	
High-Dose	35	3,000	69[c]	

[a]All rats were 47 or 48 days of age when placed on study.

[b]Dosed animals were fed test diets 5 days per week and control diets 2 days per week.

[c]Administration of the chemical to rats terminated at the times indicated due to death of all animals.

Table 2. Design of 4,4′-Thiodianiline Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	4,4′-Thiodianiline in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	14	0		91
Low-Dose	35	2,500	79[c]	
High-Dose	35	5,000	77[c]	
Female				
Matched-Control	14	0		91
Low-Dose	35	2,500	79[c]	
High-Dose	35	5,000	77[c]	

[a]All mice were 46 or 47 days of age when placed on study.

[b]Dosed animals were fed test diets 5 days per week and control diets 2 days per week.

[c]Administration of the chemical to mice terminated at the times indicated due to death of all animals.

paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental

results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could

have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relation-

ship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise

noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically signifi-

cant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

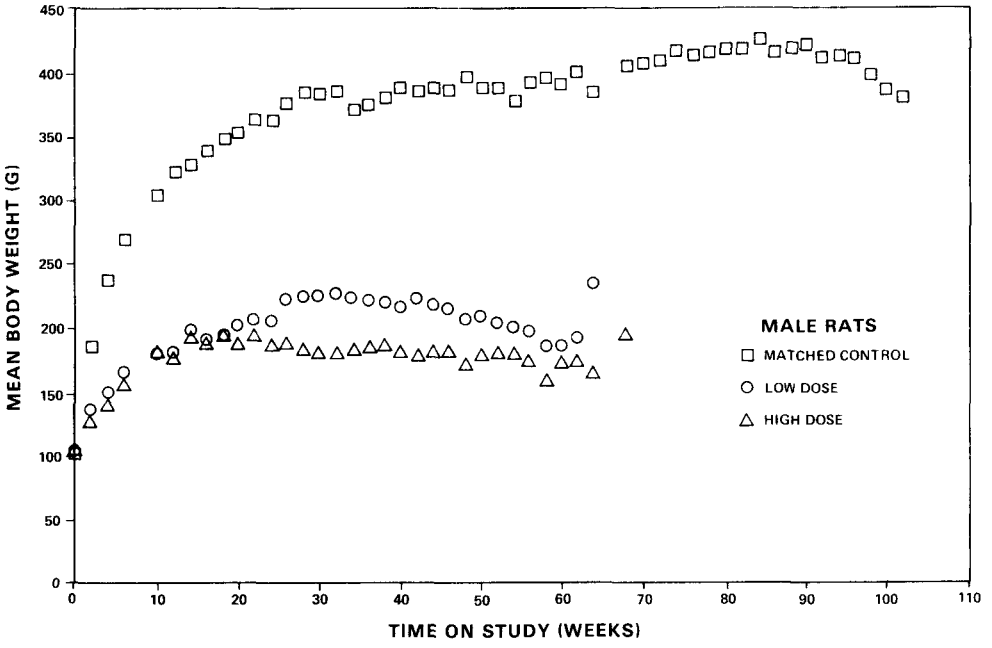
A. Body Weights and Clinical Signs (Rats)

Mean body weights of low- and high-dose groups of rats of each sex were markedly depressed in comparison with those of the controls during the entire period of administration of the chemical (figure 1). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. Several dosed rats had palpable masses in the external ear canal. No other clinical signs associated with administration of 4,4′-thiodianiline were recorded.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed 4,4′-thiodianiline in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2.

In each sex, the result of the Tarone test for positive dose-related trend in mortality is significant ($P < 0.001$), and a significant departure from linear trend ($P < 0.001$) is observed in male rats. In male rats, 18/35 (51%) of the high-dose group, 23/35 (66%) of the low-dose group, and all of the 15 matched



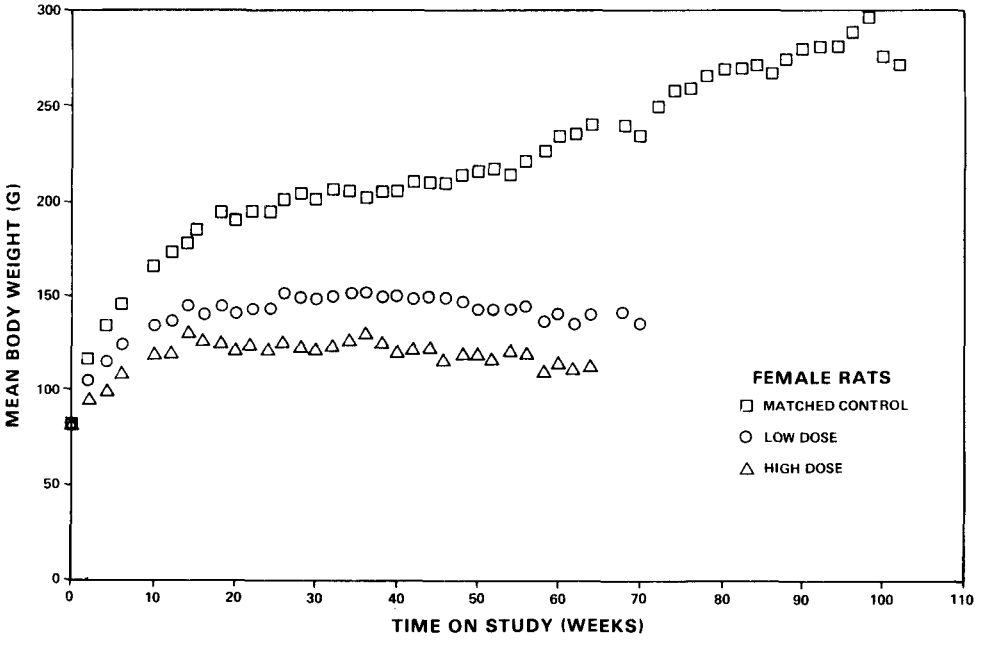


Figure 1. Growth Curves for Rats Fed 4, 4'-Thiodianiline in the Diet

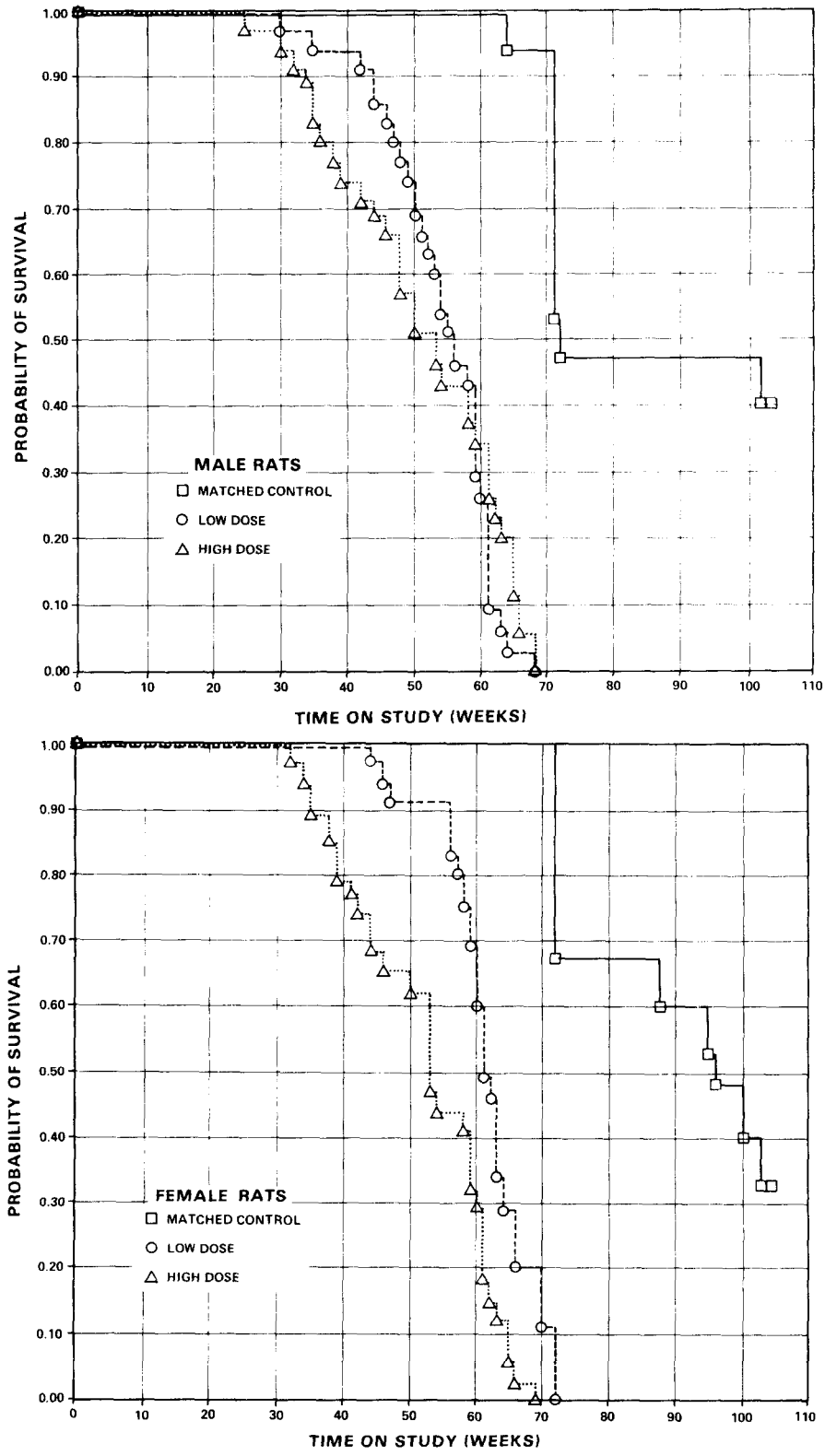


Figure 2. Survival Curves for Rats Fed 4, 4'-Thiodianiline in the Diet

control animals lived at least as long as week 52 on study. In females, 21/35 (60%) of the high-dose group, 32/35 (91%) of the low-dose group, and all of the 15 matched control animals lived beyond 1 year on study.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

A variety of neoplasms occurred in both the control and dosed groups. Some types of neoplasms occurred only, or with a greater frequency, in rats of dosed groups when compared with controls. These lesions are not uncommon in this strain of rat independent of the administration of any chemical. However, many of the tumors observed in the rats appeared to be chemically induced. These tumors occurred at a high incidence in the dosed groups when compared with the controls; most were malignant, and many had metastasized to one or more locations. The incidences of these tumors were as follows:

	RATS					
	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Skin*	(15)	(33)	(33)	(15)	(33)	(33)
Squamous-cell papilloma	0	4	0	0	0	0
Squamous-cell carcinoma	0	1	0	0	0	0
Ear Canal*	(15)	(33)	(33)	(15)	(33)	(33)
Squamous-cell papilloma	0	10	2	0	5	3
Squamous-cell carcinoma	0	5	6	0	1	0
Lungs**	(15)	(33)	(33)	(15)	(32)	(32)
Squamous-cell carcinoma	0	4	0	0	0	0
Alveolar/bronchiolar adenoma	0	1	0	0	0	0
Alveolar/bronchiolar carcinoma	0	3	0	0	2	0
Adenosquamous carcinoma	0	0	0	0	1	0
Liver**	(15)	(33)	(33)	(15)	(32)	(33)
Hepatocellular adenoma	0	2	2	0	1	2
Hepatocellular carcinoma	0	21	10	0	5	1
Colon**	(15)	(32)	(33)	(14)	(33)	(32)
Adenocarcinoma, NOS (not otherwise specified)	0	6	1	0	0	0
Thyroid**	(15)	(33)	(33)	(14)	(33)	(32)
Follicular-cell adenoma	0	2	0	0	0	0
Follicular-cell carcinoma	0	28	32	0	24	32
Uterus**				(15)	(33)	(32)
Adenocarcinoma				0	31	23

*Number of rats necropsied
**Number of rats with tissue examined microscopically

The majority of squamous-cell tumors arising from the skin were located near the commissure of the mouth. The squamous-cell carcinomas were characterized by large, polyhedral cells that

extended deep into the underlying dermis and subcutis. These well-differentiated neoplastic cells had a large, vesicular nucleus and prominent nucleolus, and many exhibited intercellular bridges. Numerous keratin pearls and some individual cell keratinization were also observed within the neoplasm.

Squamous-cell tumors involving the external ear canal and the subcutaneous tissues adjacent to the ear canal were observed as early as 25 weeks in male rats. They probably originated from the sebaceous glands of the ear canals (Zymbal's glands). In 1/33 (3%) high-dose males and 1/33 (3%) low-dose males, the ear canal papillomas were observed bilaterally. Squamous-cell carcinomas were metastatic to the lungs in 1/33 (3%) high-dose and 1/33 (3%) low-dose males. The morphology of the squamous-cell carcinomas and papillomas involving the ear canal is well documented (Pliss, 1973).

Malignant tumors arising from the lungs consisted of squamous-cell carcinomas, alveolar-cell adenocarcinomas, and an adenosquamous carcinoma. The morphology of the pulmonary squamous-cell carcinomas resembled that described for the skin, with marked keratinization and pearl formation being present. Neoplastic squamous cells had invaded through the pleura and transplanted to the parietal pleura in one case. The alveolar-cell carcinomas were comprised of cuboidal to columnar cells

aligned along the alveolar septa. Often the cells projected into the alveolar spaces, resulting in the formation of numerous papillary structures. The adenosquamous carcinoma present in one low-dose female was comprised of poorly differentiated cells arranged in a lobular pattern. The lobules were separated by a fine fibrovascular stroma. The neoplastic cells were characterized by a large vesicular nucleus and an eosinophilic cytoplasm. Few nucleoli or mitotic figures were observed. A bronchiolar adenoma was seen in one low-dose male.

In the hepatocellular carcinomas, there was considerable hepatocytomegaly, with many large hepatocytes having a large vesicular nucleus and a finely vacuolated cytoplasm. The disorganized hepatic cords were often surrounded by distended sinusoidal spaces, resulting in a trabecular pattern. In 1/33 (3%) low-dose males the neoplastic hepatocytes had invaded through the capsule, and numerous tumor transplantations were present throughout the mesentery. These tumors were frequently multiple in individual animals. Hepatocellular carcinomas had metastasized to the lungs in 7/33 (21%) low-dose and 1/33 (3%) high-dose males.

The colonic adenocarcinomas, present in low-dose and high-dose male rats, were polypoid-shaped masses comprised of large columnar epithelial cells, having a large, basophilic, elongated or flattened nucleus, and were attached to a fibrous connective

tissue stalk. The neoplastic glandular epithelium was devoid of mucous cells. The majority of the colonic masses were well differentiated and probably represented carcinoma in situ; however, in a few of the tumors the lymphatics of the lamina propria had been infiltrated by neoplastic epithelial cells.

In the majority of rats involved, the thyroid follicular-cell adenocarcinomas were bilateral in location. The neoplastic cells were arranged primarily into acini with the high cuboidal to columnar cells being aligned along a basement membrane. Many of the acini had papillary infoldings of epithelial cells projecting into the lumen, and the acini were separated by a fine fibrovascular stroma. The fibrous stroma was so abundant in some tumors that a sclerotic adenocarcinoma resulted. Neoplastic cell infiltration of blood vessels, lymphatics, and underlying tracheal and esophageal tissues was a frequent finding. Metastasis to the regional (mediastinal) lymph nodes was observed in three high-dose males and in one high-dose female. Pulmonary metastases were present in 17/33 (52%) high-dose and 15/33 (45%) low-dose males and in 11/32 (34%) high-dose females. Also, hepatic and splenic metastases were each observed in 1/33 (3%) high-dose males. Thyroid follicular-cell adenomas were present in 2/33 (6%) low-dose males.

Cells of the uterine adenocarcinomas had large, vesicular nuclei,

and prominent eosinophilic nucleoli, and were arranged into glands. The glands were usually separated by a fine fibrovascular stroma; however, the stroma became so abundant in some tumors that the result was a sclerotic adenocarcinoma. The neoplastic endometrial glandular tissues both projected into the uterine lumen and infiltrated the underlying muscle layers. In 12/33 (36%) low-dose and 2/33 (6%) high-dose rats the cells had infiltrated through the uterine wall and transplanted to the mesentery. Pulmonary metastasis was observed in 14/32 (44%) low-dose females, and metastasis to the mesenteric (abdominal) lymph nodes was observed in three low-dose females.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups. Many of these nonneoplastic lesions are commonly seen in aged rats; however, some were considered to be chemically induced. The incidences of the chemical-related lesions were as follows:

	RATS					
	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Lungs*	(15)	(33)	(33)	(15)	(32)	(32)
Epidermal inclusion cyst	0	5	0	0	2	0
Alveolar-cell hyperplasia	0	15	0	0	13	2
Squamous metaplasia (alveolar and bronchiolar)	0	12	0	0	4	0
Liver*	(15)	(33)	(33)	(15)	(32)	(33)
Hyperplasia, nodular or NOS	0	4	10	0	1	9
Bile Duct**	(15)	(33)	(33)	(15)	(33)	(33)
Hyperplasia, NOS, or cystic	0	8	25	0	6	12
Thyroid*	(15)	(33)	(33)	(14)	(33)	(32)
Follicular-cell hyperplasia	0	1	0	0	7	0

*Number of rats with tissue examined microscopically.
**Number of rats necropsied.

Squamous metaplasia of alveolar and bronchiolar epithelium was often observed with inclusion cysts. The metaplastic as well as the hyperplastic foci were often multiple or locally extensive, and their incidence was highest in those dosed groups in which the incidence of pulmonary squamous-cell carcinomas and alveolar-cell adenocarcinomas were the highest.

The incidence of nodular hyperplasias of the liver was greatest in the high-dose animals, whereas the incidence of liver tumors

was greatest in the low-dose rats. The incidence of bile duct hyperplasia paralleled that of nodular hepatocellular hyperplasia.

Feeding Fischer 344 rats 4,4′-thiodianiline for 18 months resulted in an increased incidence of tumors in all dosed groups. These tumors were all epithelial in origin, and included squamous-cell papillomas and a carcinoma of the skin; squamous-cell papillomas and carcinomas of the external ear canal; squamous-cell carcinomas, alveolar-cell carcinomas, and a bronchiolar adenoma of the lungs; hepatocellular adenomas and carcinomas; adenocarcinomas of the colon; follicular-cell adenomas and carcinomas of the thyroid; and adenocarcinomas of the uterus. Also, the high incidence of alveolar-cell hyperplasias and alveolar and bronchiolar squamous metaplasias with inclusion cyst formation in the lungs, hepatocellular nodular hyperplasias and bile duct hyperplasias and thyroid follicular-cell hyperplasias appeared to be chemically induced.

In the judgment of the pathologists, 4,4′-thiodianiline was carcinogenic for Fischer 344 rats under the conditions of this study.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses

of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In male rats, the Fisher exact comparisons of the incidences of hepatocellular carcinoma in dosed groups with that in the control group show that the incidences in the low- and high-dose groups are significantly higher than that in the control group ($P < 0.001$ and $P = 0.014$, respectively). These statistical tests indicate that the incidence of hepatocellular carcinoma in male rats is associated with 4,4′-thiodianiline at the doses of this experiment. Although the results of the Cochran-Armitage test for positive dose-related trend on these incidences are not significant, a significant departure from linearity is observed ($P < 0.001$), because the incidence in the low-dose group is higher than that in the high-dose group. This may reflect differences in early mortality in the low- and high- dose groups.

In each sex, follicular-cell carcinomas of the thyroid are found in high incidences in all the dosed groups (male rats: low-dose 28/33 [85%], high-dose 32/33 [97%]; female rats: low-dose 24/33 [73%], high-dose 32/32 [100%]). The results of the Cochran-Armitage test and of the Fisher exact test are all significant ($P < 0.001$). Because there is a sharp increase of incidences in the dosed groups, an indicated departure from linear trend is

observed (males: $P < 0.001$; females: $P = 0.025$). The statistical conclusion is that the incidence of follicular-cell carcinoma of the thyroid in rats is associated with administration of the chemical.

In male rats, the results of the Fisher exact test show that the incidence of squamous-cell papilloma or carcinoma of the ear canal in the low-dose group (15/33 [45%]) is significantly higher ($P = 0.001$) than that in the controls. The results of the Fisher exact comparison of incidences in the high-dose and control groups indicates a probability level of 0.037, which is above the 0.025 significance level required by the Bonferroni inequality criterion when multiple comparison is considered. No such tumors are seen in the controls. Although the results of the Cochran-Armitage test on the combined incidences of squamous-cell papilloma and carcinoma of the ear canal are not significant, an indicated departure from linear trend is observed ($P = 0.002$), because the incidence in the low-dose group is greater than that in the high-dose group. This difference may reflect the early mortality of the high-dose group when compared with that of the low-dose group. The first such tumor in the high-dose group was observed at 25 weeks.

In female rats, tumors of the ear canal appear in incidences of 0/15 in the controls, 6/33 (18%) in the low-dose, and 3/33 (9%)

in the high-dose groups. While these incidences are not statistically significant, each of the dosed groups has animals with tumors, and none appear in the controls.

Adenocarcinomas of the uterus are observed in the dosed females, but not in the controls. The results of the Cochran-Armitage test and of the Fisher exact test are all significant ($P < 0.001$), with a similarly high significant departure from linear trend ($P < 0.001$), due to the steep increase in incidence in the low-dose group. The statistical conclusion is that the incidence of these tumors of the uterus in female rats is dose related.

In summary, the administration of 4,4′-thiodianiline in Fischer 344 rats at the doses of this experiment is statistically associated with tumors of the liver and of the ear canal in males, of the thyroid in males and females, and of the uterus in females.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were markedly reduced (figure 3). High and low doses caused about the same reduction in weight. Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. No other clinical signs of toxicity were recorded.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed 4,4′-thiodianiline in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4.

In each sex, the result of the Tarone test for positive dose-related trend in mortality is significant ($P \leq 0.001$). In each sex, 29/35 (83%) of the high-dose animals and 34/35 (97%) of the low-dose animals lived at least as long as week 52 on study. In the control animals, all (14/14) of the males and 12/14 (86%) of the females lived beyond week 52 on study.

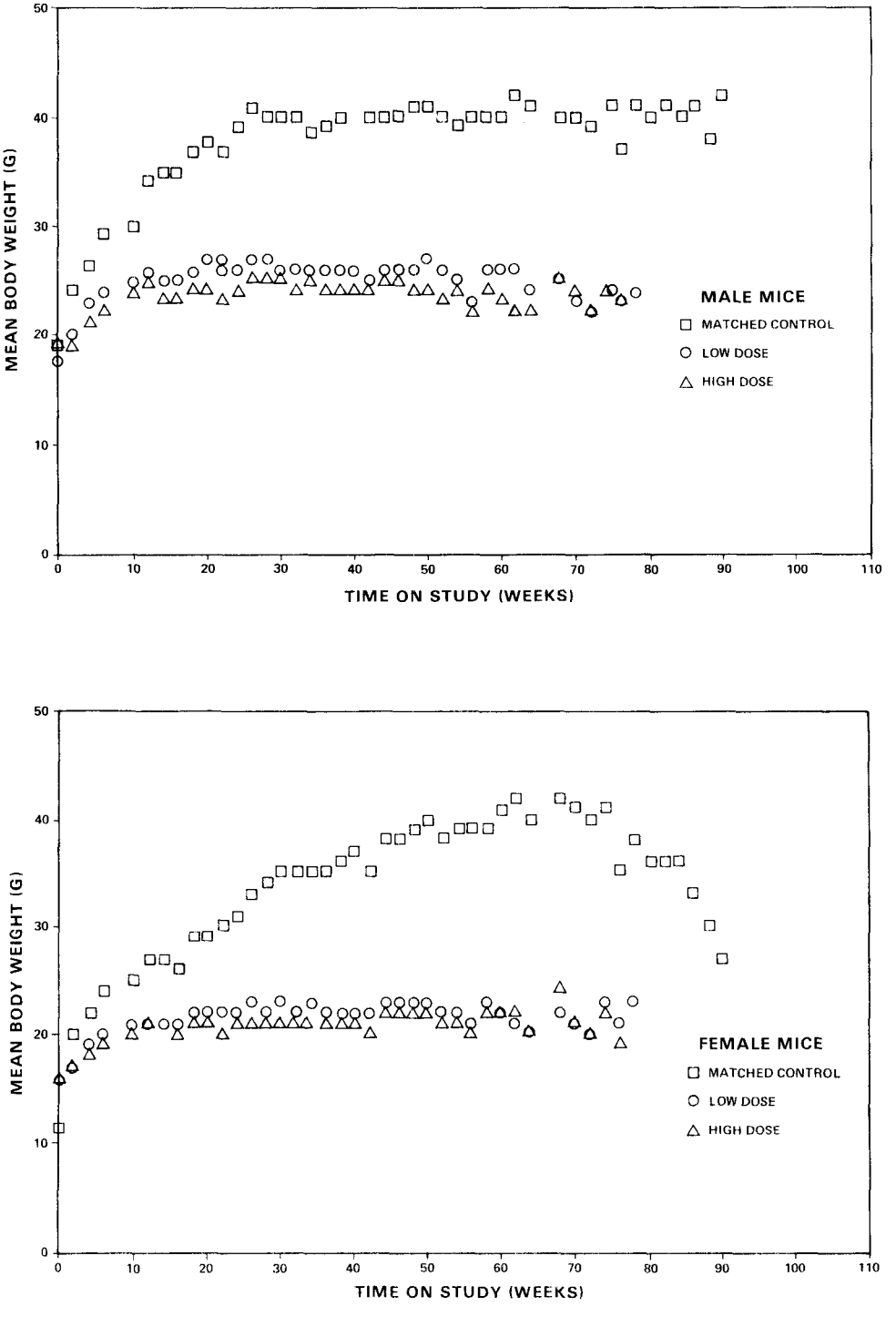


Figure 3. Growth Curves for Mice Fed 4, 4'-Thiodianiline in the Diet

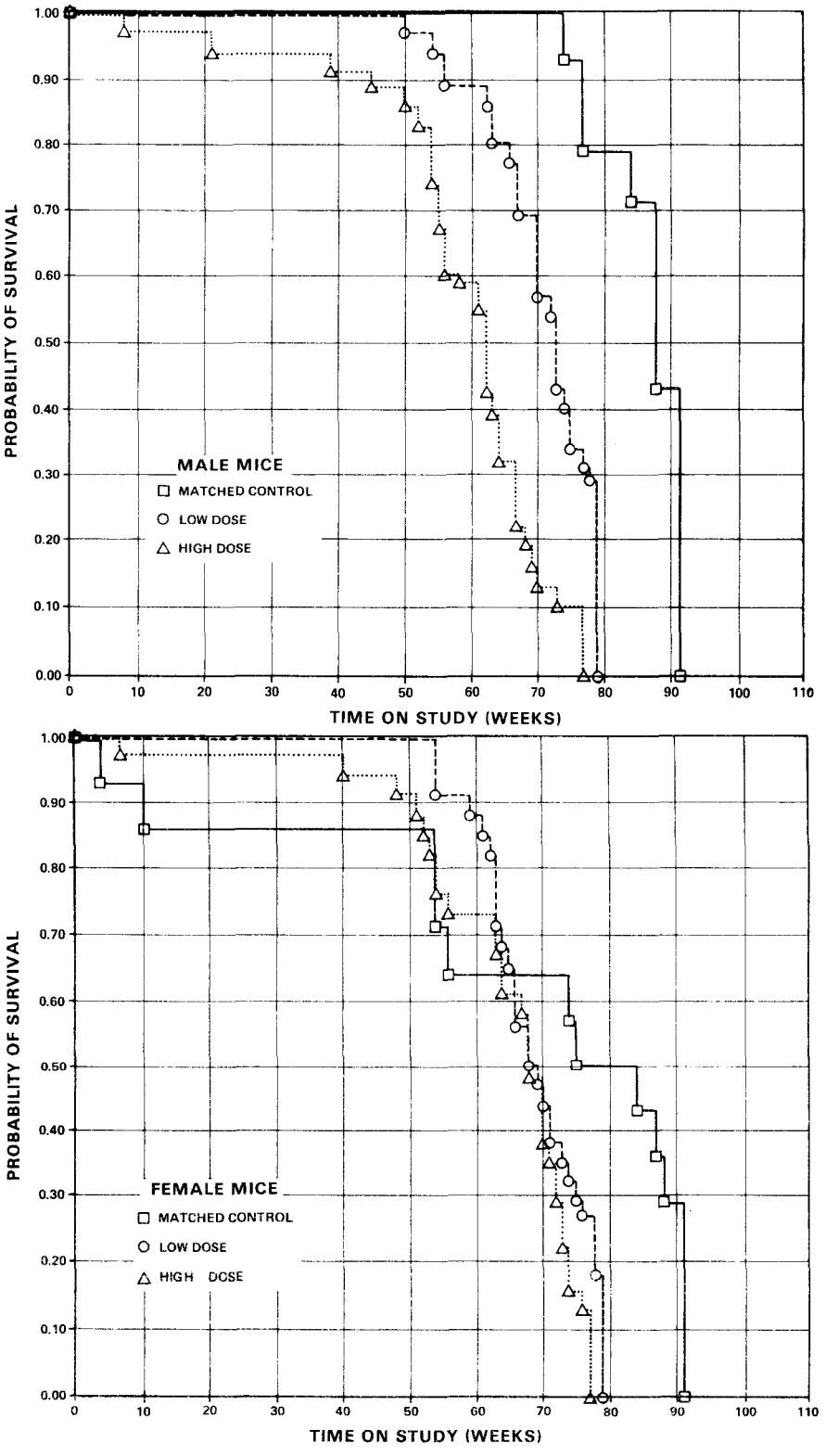


Figure 4. Survival Curves for Mice Fed 4, 4'-Thiodianiline in the Diet

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

A variety of neoplasms occurred in both the control and dosed groups. Some types of neoplasms occurred only, or with a greater frequency, in mice of dosed groups when compared with controls. These lesions are not uncommon in this strain of mouse independent of the administration of any chemical. However, many of the tumors observed in the mice appeared to be chemically induced. These tumors occurred at a high incidence in the dosed groups when compared with the controls; most were malignant, and some had metastasized to one or more locations. The incidences of these tumors were as follows:

	MICE					
	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Liver*	(13)	(34)	(24)	(12)	(34)	(31)
Hepatocellular carcinoma	1	32	22	0	32	30
Thyroid*	(14)	(33)	(23)	(11)	(33)	(30)
Follicular-cell adenoma	0	8	0	0	9	5
Follicular-cell carcinoma	0	15	20	0	3	15
Adenoma, NOS (small-cell)	0	0	0	0	0	2

*Number of mice with tissue examined microscopically.

The morphology of the hepatocellular carcinomas was similar to that described in the rats, with most carcinomas being trabecular in appearance. In 3/26 (12%) high-dose males, 3/32 (9%) high-dose females, and 2/34 (6%) low-dose females, the neoplastic cells had invaded through the hepatic capsule and had transplanted to the mesentery. Carcinomas were metastatic to the lungs in 4/28 (14%) high-dose and 3/34 (9%) low-dose females and to the kidneys in 1/34 (3%) low-dose males and 1/31 (3%) high-dose females.

The morphology of the thyroid follicular-cell adenocarcinomas was similar to that described in the rats. Invasion of blood vessels, lymphatics, and adjacent tracheal and esophageal tissues was also observed, but not as frequently as in the rats. Pulmonary metastases were observed in 2/23 (9%) high-dose and 1/34 (3%) low-dose males.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups. Most of these nonneoplastic lesions are commonly seen in aged mice; however, the follicular-cell hyperplasias involving the thyroid were often bilateral and observed in only the dosed mice.

Feeding B6C3F1 mice 4,4′-thiodianiline for 18 months resulted in

an increased incidence of tumors in all dosed groups. The tumors were all of epithelial origin, and included hepatocellular carcinomas and thyroid follicular-cell adenomas, carcinomas, and adenomas (small-cell type). Also, the high incidence of thyroid follicular-cell hyperplasia appeared to be chemically related.

In the judgment of the pathologists, 4,4′-thiodianiline was carcinogenic for B6C3F1 mice under the conditions of this study.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of hepatocellular carcinoma and of the Fisher exact comparisons of incidences in the dosed and control groups are all significant ($P < 0.001$). An indicated departure from linear trend ($P < 0.001$) is also observed in each sex, due to the sharp increase of incidences in the dosed group (males: matched controls 1/13 [8%], low-dose 32/34 [94%], high-dose 22/24 [92%]; females: matched controls 0/12, low-dose 32/34 [94%], high-dose 30/31 [97%]). The statistical conclusion is that the incidence of hepatocellular

carcinoma in mice is associated with 4,4′-thiodianiline at the doses of this experiment.

In both male and female mice, follicular-cell carcinomas of the thyroid were observed exclusively in the dosed animals, and the results of the Cochran-Armitage test are significant ($P < 0.001$). In the male mice, the results of the Fisher exact test show that the incidences in both the high- and low-dose groups are significantly higher than those in the controls ($P \leq 0.001$); in the females, the incidence in the high-dose group, but not the low-dose group, is significantly higher than that in the controls ($P = 0.002$). The statistical conclusion is that the incidence of follicular-cell carcinoma of the thyroid in mice is dose associated. When the incidences of follicular-cell adenoma and carcinoma are grouped for analyses, increased significance is observed in each sex over those of adenoma or carcinoma, taken separately.

V. DISCUSSION

In this bioassay, 4,4′-thiodianiline was toxic to both rats and mice, since there was a marked depression in mean weights in each of the dosed groups compared with corresponding control groups over the entire study, and all animals died prior to the end of the scheduled period of administration of the chemical. The low- and high-dose male rats and the high-dose female rats died by week 68 or 69, and the low-dose female rats by week 72. The low-dose male and female mice died by week 79, and the high-dose male and female mice by week 77.

Tumors of epithelial origin were found in many organs, and all dosed rats except one were affected at one or more sites (males: skin, ear canal, lungs, liver, colon, and thyroid; females: ear canal, lung, liver, thyroid, and uterus). These tumors were not found among any of the matched-control animals. Metastases were observed from many of these tumors. In addition, hyperplasias of the liver, of the follicular cells of the thyroid, and of the alveolar cells of the lungs were found in greater numbers in the dosed than in the control animals.

In male rats, several of these neoplastic lesions occurred with statistically significant incidences in one or both of the dosed groups. The incidences of hepatocellular carcinoma (controls

0/15, low-dose 21/33, high-dose 10/33) and of follicular-cell carcinoma of the thyroid (controls 0/15, low-dose 28/33, high-dose 32/33) were significant in each of the groups at $P \leq 0.014$. The combined incidences of squamous-cell carcinoma and squamous-cell papilloma of the ear canal in the low- and high-dose groups of males are both significantly higher (low-dose $P = 0.001$, high-dose $P = 0.037$) than that in the control group (controls 0/15, low-dose 15/33, high-dose 8/33). The first such tumor in the high-dose group was observed at 25 weeks.

Also in low-dose male rats, squamous-cell papilloma of the skin occurred in 4/33 animals, and squamous-cell carcinoma of the skin in 1/33, but no tumors of either type occurred in the controls. The incidences of these lesions were too low to have statistical significance. The majority of the squamous-cell tumors of the skin were located in one area near the commissure of the mouth, and only one such tumor occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the skin may be associated with administration of 4,4′-thiodianiline. Adenocarcinoma of the colon occurred in six low-dose rats and in one high-dose rat, but in no controls. This incidence is not statistically significant; however, no such tumors occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the colon are considered to be related to administration of the chemical.

In female rats, the incidences of hepatocellular adenoma or carcinoma in the dosed groups were greater than those in the controls, but not statistically significant (controls 0/15, low-dose 6/32, high-dose 3/33). Follicular-cell carcinoma of the thyroid and adenocarcinoma of the uterus occurred in the dosed females at statistically significant incidences ($P < 0.001$) in both dosed groups (follicular-cell carcinoma: controls 0/14, low-dose 24/33, high-dose 32/32; adenocarcinoma: controls 0/15, low-dose 31/33, high-dose 23/32). Squamous-cell papilloma or carcinoma of the ear canal occurred at increased, but not statistically significant, incidences in female rats (controls 0/15, low-dose 6/33, high-dose 3/33); however, no such tumors occurred among the 235 historical-control female rats at this laboratory. The tumors of the ear canal are therefore considered to be related to administration of the chemical.

In mice of each sex, the incidence of hepatocellular carcinoma was statistically significant ($P < 0.001$) in each of the dosed groups (males: controls 1/13, low-dose 32/34, high-dose 22/24, females: controls 0/12, low-dose 32/34, high-dose 30/31). In the males, follicular-cell carcinoma of the thyroid occurred at a statistically significant incidence ($P \leq 0.001$) in both the low- and high-dose groups (controls 0/14, low-dose 15/33, high-dose 20/23). In the females, the incidence was significant

(P = 0.002) only at the high dose (controls 0/11, high-dose 15/30); however, when follicular-cell adenoma and carcinoma were combined, the incidences in both the low- and high-dose groups of females were significantly higher (low-dose P = 0.025, high-dose P < 0.001) than that in the control group (controls 0/11, low-dose 11/33, high-dose 18/30).

No long-term studies with continuous administration of 4,4′-thiodianiline have been previously reported. The chemical was considered to be weakly active in a mammary tumor test system using young female Sprague-Dawley rats. In this system, the test chemicals were administered by gavage on 10 consecutive days, and the animals were necropsied 9 months later (Griswold et al., 1968). No evidence of mammary neoplasms was seen in the present bioassay with Fischer 344 rats.

It is concluded that under the conditions of this bioassay, 4,4′-thiodianiline was carcinogenic for Fisher 344 rats, inducing tumors in the liver, thyroid, colon, and ear canal of male rats, and the thyroid, uterus, and ear canal of female rats. 4,4′-Thiodianiline was carcinogenic for B6C3F1 mice, inducing tumors in the liver and thyroid of both males and females.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED 4,4′-THIODIANILINE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(33)	(33)
SQUAMOUS CELL PAPILLOMA		4 (12%)	
SQUAMOUS CELL CARCINOMA		1 (3%)	
BASAL-CELL CARCINOMA		1 (3%)	1 (3%)
*SUBCUT TISSUE	(15)	(33)	(33)
SEBACEOUS ADENOMA		1 (3%)	
SARCOMA, NOS	1 (7%)	1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(33)	(33)
SQUAMOUS CELL CARCINOMA		4 (12%)	
SQUAMOUS CELL CARCINOMA, METASTA		1 (3%)	1 (3%)
HEPATOCELLULAR CARCINOMA, METAST		7 (21%)	1 (3%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		3 (9%)	
FOLLICULAR-CELL CARCINOMA, METAS		15 (45%)	17 (52%)
SARCOMA, NOS, METASTATIC	1 (7%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(15)	(32)	(33)
FOLLICULAR-CELL CARCINOMA, METAS			1 (3%)
#MEDIASTINAL L.NODE			(3)
FOLLICULAR-CELL CARCINOMA, METAS			3 (100%)
CIRCULATORY SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(33)
HEPATOCELLULAR ADENOMA		2 (6%)	2 (6%)
HEPATOCELLULAR CARCINOMA		21 (64%)	10 (30%)
FOLLICULAR-CELL CARCINOMA, METAS			1 (3%)
SARCOMA, NOS, METASTATIC	1 (7%)		
#SMALL INTESTINE	(15)	(32)	(33)
MUCINOUS ADENOCARCINOMA			1 (3%)
#COLON	(15)	(32)	(33)
ADENOCARCINOMA, NOS		6 (19%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(15)	(32)	(33)
HAMARTOMA	1 (7%)		
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(24)	(27)
CHROMOPHOBE ADENOMA			1 (4%)
#THYROID	(15)	(33)	(33)
FOLLICULAR-CELL ADENOMA		2 (6%)	
FOLLICULAR-CELL CARCINOMA		28 (85%)	32 (97%)
C-CELL CARCINOMA	1 (7%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(15)	(33)	(33)
ADENOMA, NOS			1 (3%)
#TESTIS	(15)	(30)	(33)
INTERSTITIAL-CELL TUMOR	10 (67%)		
NERVOUS SYSTEM			
#BRAIN	(15)	(33)	(33)
ASTROCYTOMA	1 (7%)		1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(33)	(33)
SQUAMOUS CELL PAPILLOMA		10 (30%)	2 (6%)
SQUAMOUS CELL CARCINOMA		5 (15%)	6 (18%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(15)	(33)	(33)
SQUAMOUS CELL CARCINOMA, METASTA		1 (3%)	
*MESENTERY	(15)	(33)	(33)
HEPATOCELLULAR CARCINOMA, METAST		1 (3%)	
MUCINOUS ADENOCARCINOMA, METASTA			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@		7	3
MORIBUND SACRIFICE	9	28	32
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	6		
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	32	33
TOTAL PRIMARY TUMORS	14	90	58
TOTAL ANIMALS WITH BENIGN TUMORS	10	15	5
TOTAL BENIGN TUMORS	11	20	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	30	33
TOTAL MALIGNANT TUMORS	3	70	52
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	20	18
TOTAL SECONDARY TUMORS	2	25	25
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(33)	(33)
SARCOMA, NOS			1 (3%)
FIBROMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(32)	(32)
ADENOCARCINOMA, NOS, METASTATIC		14 (44%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (6%)	
FOLLICULAR-CELL CARCINOMA, METAS			11 (34%)
ADENOSQUAMOUS CARCINOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(33)	(33)
UNDIFFERENTIATED LEUKEMIA	1 (7%)		
#MEDIASTINAL L.NODE		(3)	(1)
FOLLICULAR-CELL CARCINOMA, METAS			1 (100%)
#ABDOMINAL LYMPH NODE		(3)	(1)
ADENOCARCINOMA, NOS, METASTATIC		1 (33%)	
#MESENTERIC L. NODE		(3)	(1)
ADENOCARCINOMA, NOS, METASTATIC		2 (67%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(32)	(33)
HEPATOCELLULAR ADENOMA		1 (3%)	2 (6%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA		5 (16%)	1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(22)	(27)
CHROMOPHOBE ADENOMA	3 (23%)		
#THYROID	(14)	(33)	(32)
FOLLICULAR-CELL CARCINOMA		24 (73%)	32 (100%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(33)
ADENOCARCINOMA, NOS		1 (3%)	
FIBROADENOMA	2 (13%)		
*PREPUTIAL GLAND	(15)	(33)	(33)
ADENOMA, NOS		2 (6%)	
*VAGINA	(15)	(33)	(33)
SQUAMOUS CELL CARCINOMA		1 (3%)	
#UTERUS	(15)	(33)	(32)
ADENOCARCINOMA, NOS		31 (94%)	23 (72%)
SARCOMA, NOS	1 (7%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(33)	(33)
SQUAMOUS CELL PAPILLOMA		5 (15%)	3 (9%)
SQUAMOUS CELL CARCINOMA		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(15)	(33)	(33)
ADENOCARCINOMA, NOS, METASTATIC		12 (36%)	2 (6%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	16	7
MORIBUND SACRIFICE	8	19	27
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	5		
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	32	33
TOTAL PRIMARY TUMORS	7	75	62
TOTAL ANIMALS WITH BENIGN TUMORS	4	7	4
TOTAL BENIGN TUMORS	5	9	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	32	33
TOTAL MALIGNANT TUMORS	2	66	57
TOTAL ANIMALS WITH SECONDARY TUMORS#		18	12
TOTAL SECONDARY TUMORS		29	14
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED 4,4′-THIODIANILINE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	14	34	26
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	26
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(14)	(34)	(23)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)	
FOLLICULAR-CELL CARCINOMA, METAS		1 (3%)	2 (9%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(34)	(24)
HEPATOCELLULAR ADENOMA	3 (23%)	1 (3%)	1 (4%)
HEPATOCELLULAR CARCINOMA	1 (8%)	32 (94%)	22 (92%)
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(25)
HEPATOCELLULAR CARCINOMA, METAST		1 (3%)	
TUBULAR-CELL ADENOMA		1 (3%)	
TUBULAR-CELL ADENOCARCINOMA		1 (3%)	
#URINARY BLADDER	(14)	(33)	(23)
TRANSITIONAL-CELL CARCINOMA		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(14)	(33)	(23)
FOLLICULAR-CELL ADENOMA		8 (24%)	
FOLLICULAR-CELL CARCINOMA		15 (45%)	20 (87%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(14)	(34)	(26)
HEPATOCELLULAR CARCINOMA, METAST			3 (12%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	14	35	35
NATURAL DEATH@	3	13	14
MORIBUND SACRIFICE	11	22	18
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE			
ANIMAL MISSING			3

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	34	24
TOTAL PRIMARY TUMORS	4	60	43
TOTAL ANIMALS WITH BENIGN TUMORS	3	10	1
TOTAL BENIGN TUMORS	3	10	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	34	23
TOTAL MALIGNANT TUMORS	1	50	42
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	5
TOTAL SECONDARY TUMORS		2	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	12	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(12)	(34)	(32)
BASAL-CELL CARCINOMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(12)	(34)	(28)
HEPATOCELLULAR CARCINOMA, METAST		3 (9%)	4 (14%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(12)	(34)	(32)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(12)	(34)	(31)
HEPATOCELLULAR ADENOMA			2 (6%)
HEPATOCELLULAR CARCINOMA		32 (94%)	30 (97%)
URINARY SYSTEM			
#KIDNEY	(12)	(34)	(31)
HEPATOCELLULAR CARCINOMA, METAST			1 (3%)
TUBULAR-CELL ADENOCARCINOMA		2 (6%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(12)	(34)	(30)
TRANSITIONAL-CELL PAPILLOMA		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(11)	(33)	(30)
ADENOMA, NOS			2 (7%)
FOLLICULAR-CELL ADENOMA		9 (27%)	5 (17%)
FOLLICULAR-CELL CARCINOMA		3 (9%)	15 (50%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(12)	(34)	(32)
FIBROADENOMA	1 (8%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(12)	(34)	(32)
HEPATOCELLULAR CARCINOMA, METAST		2 (6%)	3 (9%)
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	14	35	35
NATURAL DEATH@	2	20	11
MORIBUND SACRIFICE	12	14	21
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE			
ANIMAL MISSING			3
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	33	30
TOTAL PRIMARY TUMORS	2	49	54
TOTAL ANIMALS WITH BENIGN TUMORS	1	10	8
TOTAL BENIGN TUMORS	1	10	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	32	30
TOTAL MALIGNANT TUMORS	1	39	45
TOTAL ANIMALS WITH SECONDARY TUMORS#		5	8
TOTAL SECONDARY TUMORS		5	8
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED 4,4′-THIODIANILINE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(33)	(33)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	1 (3%)
RESPIRATCRY SYSTEM			
#LUNG	(15)	(33)	(33)
EMPHYSEMA, NOS			4 (12%)
EPIDERMAL INCLUSION CYST		5 (15%)	
ERONCHOPNEUMONIA SUPPURATIVE		5 (15%)	2 (6%)
ERONCHOPNEUMONIA CHRONIC SUPPURA		6 (18%)	3 (9%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		15 (45%)	
METAPLASIA, SQUAMOUS		7 (21%)	
#LUNG/ALVEOLI	(15)	(33)	(33)
METAPLASIA, SQUAMOUS		5 (15%)	
HEMATOPCIETIC SYSTEM			
#BONE MARROW	(12)	(33)	(32)
ATRCPHY, NOS	6 (50%)		
CIRCULATORY SYSTEM			
NCNE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(33)
INFLAMMATION, GRANULOMATOUS			1 (3%)
CYTOPLASMIC CHANGE, NOS		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR		4 (12%)	10 (30%)
*BILE DUCT	(15)	(33)	(33)
HYPERPLASIA, NOS		8 (24%)	25 (76%)
#PANCREAS	(15)	(32)	(33)
INFLAMMATION, CHRONIC		1 (3%)	
FIBROSIS		1 (3%)	
#PANCREATIC ACINUS	(15)	(32)	(33)
ATROPHY, NOS		1 (3%)	
#ESOPHAGUS	(15)	(33)	(33)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(32)	(33)
INFLAMMATION, CHRONIC	11 (73%)	4 (13%)	4 (12%)
#KIDNEY/PELVIS	(15)	(32)	(33)
HYPERPLASIA, EPITHELIAL		1 (3%)	4 (12%)
ENDOCRINE SYSTEM			
#THYROID	(15)	(33)	(33)
CYSTIC FOLLICLES	9 (60%)	4 (12%)	
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(15)	(33)	(33)
HYPERPLASIA, NODULAR			1 (3%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE		1 (3%)	
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN		1 (3%)	
*EAR CANAL	(15)	(33)	(33)
EPIDERMAL INCLUSION CYST		1 (3%)	
INFLAMMATICN, CHRONIC SUPPURATIV		2 (6%)	1 (3%)
HYPERKERATOSIS		2 (6%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(15)	(33)	(33)
INFLAMMATION, CHRONIC			1 (3%)
ALL CTHER SYSTEMS			
NONE			
SPECIAL MCRPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY		2	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(33)	(32)
INFLAMMATION, SUPPURATIVE	1 (7%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)	1 (3%)	
#LUNG/BRONCHUS	(15)	(32)	(32)
BRONCHIECTASIS	1 (7%)	1 (3%)	
#LUNG	(15)	(32)	(32)
EPIDERMAL INCLUSION CYST		2 (6%)	
EDEMA, NOS			1 (3%)
HEMORRHAGE			1 (3%)
INFLAMMATION, INTERSTITIAL		1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE			2 (6%)
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)	1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		13 (41%)	2 (6%)
METAPLASIA, SQUAMOUS		2 (6%)	
#LUNG/ALVEOLI	(15)	(32)	(32)
METAPLASIA, SQUAMOUS		2 (6%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(13)	(32)	(31)
ATROPHY, NOS	4 (31%)	1 (3%)	2 (6%)
#SPLEEN	(15)	(33)	(33)
HEMATOPOIESIS	1 (7%)	3 (9%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(15)	(32)	(33)
INFLAMMATION, INTERSTITIAL		1 (3%)	
HYPERPLASIA, NODULAR		1 (3%)	8 (24%)
HYPERPLASIA, NOS			1 (3%)
#LIVER/PERIPORTAL	(15)	(32)	(33)
FIBROSIS		1 (3%)	
*BILE DUCT	(15)	(33)	(33)
HYPERPLASIA, NOS		5 (15%)	12 (36%)
HYPERPLASIA, CYSTIC		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(32)	(33)
HYDRONEPHROSIS		1 (3%)	
INFLAMMATION, CHRONIC	5 (33%)		
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(22)	(27)
HYPERPLASIA, CHROMOPHOBE-CELL	2 (15%)		
#THYROID	(14)	(33)	(32)
CYSTIC FOLLICLES	4 (29%)	7 (21%)	
HYPERPLASIA, FOLLICULAR-CELL		7 (21%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(33)
CYST, NOS	1 (7%)		
#UTERUS	(15)	(33)	(32)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
#UTERUS/ENDOMETRIUM	(15)	(33)	(32)
INFLAMMATION, SUPPURATIVE		1 (3%)	4 (13%)
INFLAMMATION, CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)	5 (15%)	6 (19%)
HYPERPLASIA, NOS		2 (6%)	1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, CYSTIC	2 (13%)		3 (9%)
NERVOUS SYSTEM			
#BRAIN	(14)	(33)	(33)
INFLAMMATION, NECROTIZING			1 (3%)
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV		2 (6%)	1 (3%)
*LENS CAPSULE	(15)	(33)	(33)
MINERALIZATION		1 (3%)	
*EAR CANAL	(15)	(33)	(33)
EPIDERMAL INCLUSION CYST			1 (3%)
HYPERKERATOSIS			2 (6%)
*MIDDLE EAR	(15)	(33)	(33)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*EPICARDIUM	(15)	(33)	(33)
THROMBOSIS, NOS		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
ACCIDENTAL DEATH			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY		2	1

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED 4,4′-THIODIANILINE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	14	34	26
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	26
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(33)	(23)
INFLAMMATION, SUPPURATIVE		2 (6%)	1 (4%)
#LUNG	(14)	(34)	(23)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)	6 (18%)	2 (9%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	6 (43%)	1 (3%)	1 (4%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (7%)	1 (3%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(14)	(34)	(25)
HEMATOPOIESIS		1 (3%)	
#MESENTERIC L. NODE	(3)	(1)	(1)
INFLAMMATION, SUPPURATIVE		1 (100%)	
HYPERPLASIA, LYMPHOID	2 (67%)		1 (100%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(34)	(24)
HYPERPLASIA, NODULAR		1 (3%)	
*BILE DUCT	(14)	(34)	(26)
HYPERPLASIA, NOS		1 (3%)	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(25)
INFLAMMATION, CHRONIC		2 (6%)	
ENDOCRINE SYSTEM			
#THYROID	(14)	(33)	(23)
HYPERPLASIA, FOLLICULAR-CELL		29 (88%)	4 (17%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(14)	(34)	(26)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY			3
AUTO/NECROPSY/HISTO PERF			2
AUTOLYSIS/NO NECROPSY		1	6

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	12	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	31
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(12)	(33)	(30)
INFLAMMATION, SUPPURATIVE	2 (17%)		
#LUNG	(12)	(34)	(28)
BRONCHOPNEUMONIA SUPPURATIVE	1 (8%)	3 (9%)	
PNEUMONIA INTERSTITIAL CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA	10 (83%)	1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (8%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(12)	(33)	(30)
ANGIECTASIS		1 (3%)	
HEMATOPOIESIS	1 (8%)		1 (3%)
#MESENTERIC L. NODE	(3)	(1)	(2)
INFLAMMATION, SUPPURATIVE	1 (33%)	1 (100%)	2 (100%)
HYPERPLASIA, LYMPHOID	1 (33%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*BILE DUCT	(12)	(34)	(32)
HYPERPLASIA, NOS			4 (13%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*RECTUM	(12)	(34)	(32)
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
URINARY SYSTEM			
#URINARY BLADDER	(12)	(34)	(30)
HYPERPLASIA, EPITHELIAL		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(11)	(33)	(30)
CYSTIC FOLLICLES		4 (12%)	
HYPERPLASIA, FOLLICULAR-CELL		31 (94%)	26 (87%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(12)	(31)	(30)
INFLAMMATION, SUPPURATIVE	1 (8%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
HYPERPLASIA, CYSTIC	8 (67%)	2 (6%)	
#OVARY	(12)	(31)	(30)
CYST, NOS	1 (8%)		
HEMORRHAGE			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY			3
NECROPSY PERF/NO HISTO PERFORMED			1
AUTOLYSIS/NO NECROPSY	2	1	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED 4,4′-THIODIANILINE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4'-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Skin: Squamous-cell Papilloma[b]	0/15 (0)	4/33 (12)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.017		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.450	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	48	--
Skin: Squamous-cell Papilloma or Carcinoma[b]	0/15 (0)	5/33 (15)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure fom Linear Trend[e]	P = 0.007		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.613	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	48	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Squamous-cell Carcinoma[b]	0/15 (0)	4/33 (12)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from linear Trend[e]	P = 0.017		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.450	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	54	--
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/15 (0)	3/33 (9)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.039		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.291	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	50	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/15 (0)	4/33 (12)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.017		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.450	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	50	--
Liver: Hepatocellular Carcinoma[b]	0/15 (0)	21/33 (64)	10/33 (30)
P Values[c,d]	N.S.	P < 0.001	P = 0.014
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.352	1.450
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	53

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/15 (0)	23/33 (70)	12/33 (36)
P Values[c,d]	N.S.	P < 0.001	P = 0.005
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.713	1.790
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	53
Colon: Adenocarcinoma, NOS[b]	0/15 (0)	6/32 (19)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.010		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.803	0.026
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	65

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/15 (0)	28/33 (85)	32/33 (97)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.685	5.827
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	42	32
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/15 (0)	30/33 (91)	32/33 (97)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		5.156	5.827
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	42	32

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Ear Canal: Squamous-cell Carcinoma[b]	0/15 (0)	5/33 (15)	6/33 (18)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.613	0.778
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	25
Ear Canal: Squamous-cell Papilloma or Carcinoma[b]	0/15 (0)	15/33 (45)	8/33 (24)
P Values[c,d]	N.S.	P = 0.001	P = 0.037
Departure from Linear Trend[e]	P = 0.002		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		2.305	1.112
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	35	25

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

[a]Treated groups received doses of 1,500 or 3,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/15 (0)	2/32 (6)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.147	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	63	--
Liver: Hepatocellular Carcinoma[b]	0/15 (0)	5/32 (16)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.023		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.632	0.025
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	61	69

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/15 (0)	6/32 (19)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.803	0.291
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	61	65
Thyroid: Follicular-cell Carcinoma[b]	0/14 (0)	24/33 (73)	32/32 (100)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.025		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.660	6.059
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	46	44

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Ear Canal: Squamous-cell Carcinoma[b]	0/15 (0)	1/33 (3)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.026	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	58	--
Ear Canal: Squamous-cell Papilloma or Carcinoma[b]	0/15 (0)	6/33 (18)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.778	0.291
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	46	60

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Preputial Gland: Adenoma, NOS[b]	0/15 (0)	2/33 (6)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.142	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	63	--
Uterus: Adenocarcinoma, NOS[b]	0/15 (0)	31/33 (94)	23/32 (72)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		5.446	3.840
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	56	44

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

---

[a]Treated groups received doses of 1,500 or 3,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS

IN MICE FED 4,4′-THIODIANILINE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed 4,4′-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	1/13 (8)	32/34 (94)	22/24 (92)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		12.235	11.917
Lower Limit		2.762	2.601
Upper Limit		260.907	257.538
Weeks to First Observed Tumor	88	54	54
Liver: Hepatocellular Adenoma or Carcinoma[b]	4/13 (31)	33/34 (97)	23/24 (96)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		3.154	3.115
Lower Limit		1.633	1.555
Upper Limit		4.281	4.280
Weeks to First Observed Tumor	88	54	50

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Kidney: Tubular-cell Adenoma or Adenocarcinoma[b]	0/14 (0)	2/34 (6)	0/25 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.130	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	70	--
Thyroid: Follicular-cell Carcinoma[b]	0/14 (0)	15/33 (45)	20/23 (87)
P Values[c,d]	P < 0.001	P = 0.001	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		2.163	4.504
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	63	54

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/14 (0)	22/33 (67)	20/23 (87)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.041		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.316	4.504
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	63	54

[a]Treated groups received doses of 2,500 or 5,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P < 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when P < 0.05 for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed 4,4′-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/12 (0)	32/34 (94)	30/31 (97)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		4.451	4.712
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	54	40
Kidney: Tubular-cell Adenocarcinoma[b]	0/12 (0)	2/34 (6)	0/31 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.113	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	78	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/11 (0)	3/33 (9)	15/30 (50)
P Values[c,d]	P < 0.001	N.S.	P = 0.002
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.220	1.926
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	63	40
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/11 (0)	11/33 (33)	18/30 (60)
p Values[c,d]	P < 0.001	P = 0.025	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		1.228	2.363
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	59	40

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Adenoma, NOS[b]	0/11 (0)	0/33 (0)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite
Lower Limit		--	0.119
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	56

[a]Treated groups received doses of 2,500 or 5,000 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Review of the Bioassay of 4,4'-Thiodianiline* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

January 18, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which 4,4'-Thiodianiline was reviewed.

The primary reviewer agreed with the conclusion in the report that 4,4'-Thiodianiline was carcinogenic in both rats and mice, under the conditions of test. He noted that the subchronic study was performed in a different species of rat and mouse than used in the chronic phase. From the appearance of the weight curves, the primary reviewer said that it was likely that the MTD was exceeded in both species. Despite this deficiency, 4,4'-Thiodianiline was clearly carcinogenic in the treated animals. He concluded that his only reservation concerning the probability of a human carcinogenic risk from 4,4-Thiodianiline would be that its effect was elicited at highly toxic levels in the treated animals.

The secondary reviewer noted the inadequate number of animals used in the study. Irrespective of the deficiency, he said that it was clear that 4,4'-Thiodianiline was carcinogenic in the treated animals.

It was moved that the report on 4,4'-Thiodianiline be accepted as written and that it be presumed to pose a carcinogenic risk to humans. The motion was seconded and approved unanimously.

Members Present Were:

Arnold Brown (Acting Chairman), Mayo Clinic
Lawrence Garfinkel, American Cancer Society
Joseph Highland, Environmental Defense Fund
Charles Kensler, Arthur D. Little Company
Verald K. Rowe, Dow Chemical, U.S.A.
Sheldon Samuels, Industrial Union Department, AFL-CIO
Louise Strong, University of Texas Health Sciences Center
Sidney Wolfe, Health Research Group

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

*U.S. GOVERNMENT PRINTING OFFICE: 1978 260-899/3039 1-3