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.

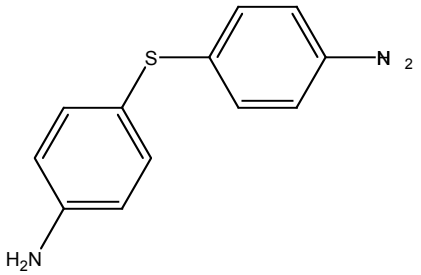

Figure 1-1. Structure of TDA

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.

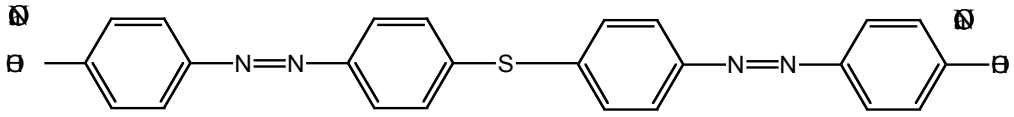

Figure 1-2. Structure of C.I. mordant yellow 16

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.

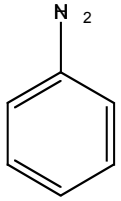

Figure 1-3. Structure of aniline

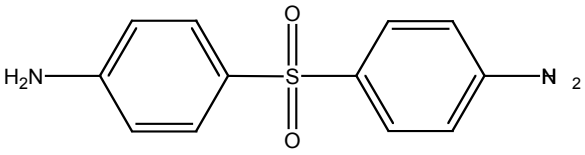

Figure 1-4. Structure of dapsone

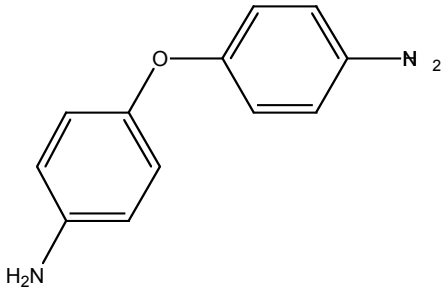

Figure 1-5. Structure of 4,4'-oxydianiline

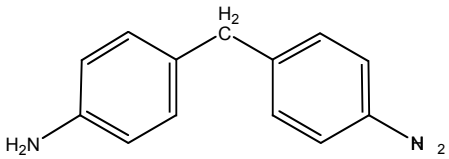

Figure 1-6a. Structure of 4,4'-methylenedianiline

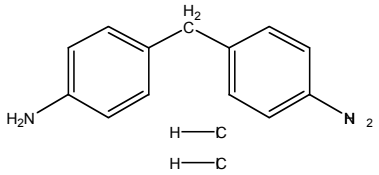

Figure 1-6b. Structure of 4,4'-methylenedianiline dihydrochloride

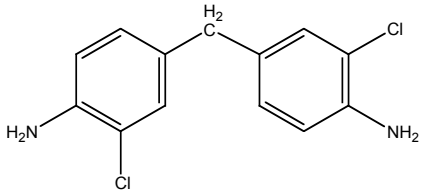

Figure 1-7. Structure of 4,4'-methylenebis(2-chloroaniline)

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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